Opioid Maintained Subjects

And The Effects Of High Dose Morphine

And Adjuvant Analgesics

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Abstract

Research has shown that maintenance on methadone and buprenorphine for the treatment of opioid addiction can produce the effects of hyperalgesia. This presents difficulties in the management of moderate to severe acute pain in this population. The situation is complicated by a dearth of evidence-based guidelines for pain management.

The main aims of the four studies described in this thesis were to examine whether very high intravenous morphine doses alone (55.2 mg)(targeting plasma morphine concentrations of 180 ng/ml), or in combination with ketorolac (185.4 mg)(targeting plasma ketorolac concentrations of 4000 ng/ml), tramadol (229 mg)(targeting plasma tramadol concentrations of 1000 ng/ml) or S(+)-Ketamine (S-ketamine) (14.5 mg)(targeting plasma S-ketamine concentrations of 60 ng/ml) (opioid adjuvants) produced antinociception or respiratory effects in methadone maintained subjects (methadone subjects) and buprenorphine maintained subjects (buprenorphine subjects). The antinociceptive tests of the cold pressor and electrical stimulation were utilised. The effects of different maintenance doses of methadone and buprenorphine were also examined. Methadone maintained subjects were stratified into once daily dose groups of 11-45 (n=6), 46-80 (n=6) and 81-115 (n=6) mg per day. Buprenorphine maintained subjects were stratified into once daily dose groups of 2 to 8 (n=4), 9 to 15 (n=4) and 16-22 (n=4) mg per day.

A healthy control group was administered lower doses of morphine alone (11.95 mg), and with adjuvants. The same doses of adjuvants were used in each instance.

In the first study high dose morphine failed to provide antinociception for the methadone subjects. High dose morphine significantly decreased respiration rate, but only by an average of 2 breaths per minute. Methadone subjects were hyperalgesic in the cold pressor test. There were no differences in the antinociceptive responses of the different stratified methadone groups to the high dose morphine. Methadone subjects maintained on the highest doses had the highest respiratory depression.

In the second study buprenorphine subjects performed similarly to methadone subjects in at least three respects: firstly, high dose morphine had little antinociceptive effect; secondly, this dose significantly decreased respiration rate; and thirdly, buprenorphine and methadone subjects were similarly hyperalgesic in the cold pressor test. There were also no differences in the antinociceptive responses of the different buprenorphine groups to the high dose morphine.

In the third study tramadol and ketorolac, when combined with high dose morphine, failed to provide antinociception in either the cold pressor or electrical stimulation tests to methadone subjects. The combination of S-ketamine and high dose morphine provided statistically but not clinically significant improvement in antinociception in the cold pressor test.

In the fourth study ketorolac and high dose morphine did not provide antinociception in buprenorphine maintained subjects. While the combinations of S-ketamine or tramadol and high dose morphine provided statistically significant antinociception for buprenorphine maintained subjects in the cold pressor test, it was not clear whether this change represented a clinically significant improvement.

High dose morphine alone, or combined with opioid adjuvants at these concentrations is unlikely to provide pain relief in this population. The use of higher concentrations of adjuvants in combination with high dose morphine needs to be further evaluated. Other strategies should also be explored that may provide effective pain relief in patients maintained on opioids for the treatment of opioid dependence.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Peter Athanasos, May 2013

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Publications and Presentations in Support of This Thesis

Publications

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Conference Presentations

Athanasos P, Neild R. Clinical implications of the expanding understanding of hyperalgesia in chronic opioid administration. Australian Professional Society for Alcohol and Other Drugs (APSAD) (2012) Melbourne

McCarthur J, Kennedy T, Semple T, Brougham L, Compton P de Crespigny C and Athanasos P. Postoperative recovery of opioid tolerant patients. Australian Professional Society for Alcohol and Other Drugs (APSAD) (2008) Sydney

McCarthur J, Kennedy T, Semple T, Brougham L, Compton P and Athanasos P. Postoperative opioid loading requirements following major surgery for opioid tolerant and other drug dependent patients. Australian Professional Society for Alcohol and Other Drugs (APSAD)/ Cutting Edge. National Alcohol, Drug and Addiction Treatment Conference (2007) Auckland, New Zealand.

Athanasos P. Pain: The new comorbidity. Drug and Alcohol Nurses of Australasia National Conference (2007) Whyalla. (Oral Presentation)

Athanasos P and de Crespigny C. Opioid dependent patients: Specific nursing strategies for their pain management. Cutting Edge. National Alcohol, Drug and Addiction Treatment Conference (2006) Wellington, New Zealand. (Oral Presentation)

Compton P, Athanasos P and de Crespigny C. Opioid tolerance and effective management of acute pain. Drug and Alcohol Nurses of Australasia National Conference (2006) Sydney. Pre-conference keynote workshop.

Athanasos P and de Crespigny C. Specific nursing strategies for the pain management of opioid dependent patients. Drug and Alcohol Nurses of Australasia National Conference (2006) Sydney. (Oral Presentation).

Athanasos P, Smith C, Ling W, Bochner F, Somogyi A and White J. Morphine plus S (+) ketamine or tramadol elicit antinociception in opioid non-tolerant and buprenorphine maintained but not in methadone maintained subjects. International Association for the Study of Pain 11th World Congress (2005) Sydney, Australia.

Athanasos P, Smith C, Ling W, Bochner F, Somogyi A and White J. High dose morphine plus S (+) ketamine or tramadol elicits antinociception in buprenorphine maintained patients. 67th Annual Scientific Meeting of the College on Problems of Drug and Alcohol Dependence (2005) Orlando, Florida, USA (Oral presentation).

Athanasos P, Smith C, Hay J, White J, Somogyi A, Bochner F and Ling W. Opioid dependent patients are cross-tolerant to the antinociceptive effects of S (+) ketamine, ketorolac or tramadol and high dose morphine. 66th Annual Scientific Meeting of the College on Problems of Drug and Alcohol Dependence (2004) San Juan, Puerto Rico (Oral presentation).

Athanasos P, Smith C, White J, Somogyi A, Bochner F, Menelaou A, Edwards S and Ling W. High morphine concentrations do not provide antinociception to methadone maintenance patients. 64th Annual Meeting of the College on Problems of Drug and Alcohol Dependence (2002) Quebec City, Quebec, Canada (Oral presentation).

Athanasos P, Smith C, White J, Somogyi A, Bochner F, Menelaou A, Edwards S and Ling W. Methadone maintenance patients are cross-tolerant to the antinociceptive effects of very high morphine concentrations. Australian Professional Society for Alcohol and Other Drugs (APSAD) (2002) Adelaide, South Australia, Australia (Oral presentation).

Abbreviations, prefixes and symbols

(Morphine 1) (M1)

(Morphine 2) (M2)

5 hydroxytryptamine (5HT)

Analysis of variance (ANOVA)

Australian Professional Society for Alcohol and Other Drugs (APSAD)

Buprenorphine maintained subjects (buprenorphine subjects)

Calcitonin gene-related peptide (CGRP)

Electrospray (ESI)

High-performance liquid chromatography (HPLC)

Hydrochloric acid (HCl)

Liquid chromatograph mass spectrometer (LCMS)

Methadone maintained subjects (methadone subjects)

Post methadone dose (2 hours)

Pre methadone dose (0 hours)

Quality control (QC)

Residual standard deviation of the mean (RSD)

S(+)-Ketamine (S-ketamine)

Standard error of the mean (SEM)

1. Introduction

The management of acute pain in people maintained on opioids, either for the treatment of addiction or chronic pain is problematic. While research continues to emphasise the lack of optimal pain management in patients suffering from cancer (de Leon-Casasola 2008), there is an even greater risk of underprescribing for patients maintained on opioids for the treatment of opioid addiction. Acute pain presentations in this population include general trauma, medical illness, surgery (cancer and non-cancerous conditions) and the complications of drug use including infections and trauma. There are a number of reasons for underprescribing in this population. There is often a lack of knowledge among prescribers about the impact of opioid prescribing for pain management in the presence of an opioid addiction (Scimeca et al. 2000). Legislation exists that may interfere with the provision of adequate opioid medication to this population (Gilson and Joranson 2008). Negative attitudes persist among the medical profession that regards requests by these patients for analgesia as not reflective of true suffering (Scimeca et al. 2000, Mitra and Sinatra 2004, Alford et al. 2006). The problem may be compounded by the patients themselves. There may be a reluctance on the part of patients to give accurate histories for fear of having medications withheld by their physicians (Rich 2000).

Most importantly, there is substantial evidence that the pain experience for current and former opioid addicts is altered and that their response to the provision of more opioid analgesics is limited (Compton 1994, Doverty et al. 2001b, Carroll et al. 2004, Mitra and Sinatra 2004, Athanasos et al. 2006, Bourne 2010, Huxtable et al. 2011). Antinociceptive tolerance to opioids is a known consequence of opioid use. Hyperalgesia, an increased response to a stimulus which is normally painful, is another factor which may or may not underpin opioid antinociceptive tolerance and contributes to the altered pain experience in this population. Whether this altered pain experience is in response to the initial use of opioids for euphorogenic purposes or a consequence of maintenance on opioids for addiction treatment is not known.

The situation is further complicated by a lack of empirically derived guidelines. Most studies of the pain management requirements of opioid dependent people have been limited to retrospective case studies (Rubenstein et al. 1976, Kantor et al. 1980, Tucker 1990, Manfredi et al. 2001, Gordon et al. 2008) and two larger reviews from more than 15 years ago (de Leon-Casasola et al. 1993, Rapp et al. 1995). While the problems of managing acute severe pain in opioid tolerant individuals is becoming increasingly recognised (Mitra and Sinatra 2004, Peles et al. 2005, Alford et al. 2006, Macintyre et al. 2010, Huxtable et al. 2011), there have been few experimental studies in clinical populations examining the relationship between opioid maintenance treatment and analgesic response.

As a consequence, the challenge for the physician willing to treat an opioid maintained individual who has a concurrent pain issue is considerable. The opioid dependent person administered opioids for the treatment of their addiction, who then experiences acute or chronic pain, may suffer considerably.

The aim of this thesis is to provide evidence to support the development of effective guidelines to manage the treatment of acute pain in the opioid tolerant population. The thesis begins with a discussion of the nature of pain and a consideration of the nature of pain transmission, pain modulation and the treatment of pain.

Opioids have been used throughout human history. They remain one of the central pharmacological interventions for the treatment of acute pain. However, there are well recognised problematic side effects from the use of opioids. It is the management of this aspect of opioid use that is the focus of the thesis. A consideration of the history of opioid use reveals that many of our present concerns have centuries old antecedents.

The general pharmacology of opioids is explored with a focus on the intracellular events following mu opioid reception. General opioid pharmacodynamics and opioid pharmacokinetics are described. Methadone and buprenorphine pharmacology is then discussed and compared to opioid pharmacology in general.

The experimental evidence concerning tolerance and hyperalgesia in opioid maintained subjects is examined and critiqued. A variety of mechanisms have been proposed to explain the effect of chronic opioid use on the development of tolerance and hyperalgesia. Examples of these are described.

Tolerance and hyperalgesia are observed pharmacodynamic effects of opioids. They complicate the management of acute pain in the opioid maintained population. Three pharmacological agents have been selected to ascertain if they are able to provide antinociception to this population either alone or in the combination with opioids in experimental pain conditions to this. The pharmacology of each of the agents utilised in this series of studies is examined.

Finally, one of the central themes of the thesis is a consideration of the means in which it may be possible to provide relief from acute severe pain to people maintained on opioids for the treatment of opioid addiction. The history of pain management guidelines has, at times, been inconsistent and even contradictory. These guidelines are examined.

The worldwide use of opioids for the treatment of opioid addiction and chronic pain continues to increase (Ballantyne and LaForge 2007, Tetrault and Fiellin 2012). As the number of people

maintained on opioids continues to increase, the need for effective strategies to manage acute pain becomes more pressing. This thesis is a contribution to that body of knowledge.

1.1. Pain

There have been many proposed definitions to describe pain. The International Association for the Study of Pain describes pain as 'An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (Pain 2007). Others have suggested that such a definition does not take into account the different physiological, cognitive, affective, behavioural and sociocultural aspects. McCaffrey (Swanson and Klein 2005) defines pain more broadly as 'whatever the experiencing person says it is, existing whenever they say that it does'.

1.2. Classification of pain

Pain can be classified as either acute or chronic and by its pathology as either nociceptive or neuropathic. Complex conditions such as cancer can produce pain that is both nociceptive and neuropathic, and both acute and chronic simultaneously (Mitchell and Condon 2005). Acute or nociceptive pain is pain of sudden onset that occurs when there is activation of nociceptors (pain neurons) in response to mechanical (e.g. pinch), thermal (hot or cold), chemical (e.g. acid or bee sting) or artificial stimuli (electric shock) that impacts on body tissue. Acute pain occurs as a result of the stimulation of peripheral receptors and is often indicative of sudden tissue injury (Schumacher et al. 2007).

An example of acute pain is post-operative pain. The act of surgery results in tissue damage and cell destruction which causes the release of such pain producing substances as prostaglandins, 5-hydroxytryptamine and lactic acid. The release of these substances has a two-fold effect. They initiate the nociceptive impulses and also lower the pain threshold by sensitising the pain receptors causing hyperalgesia (Ready et al. 1988).

Chronic pain is the state where pain is present even after a cure for the injury or disease state would usually have been achieved. Another interpretation is that chronic pain occurs when the body is unable to prevent the interpretation of pain signals and symptoms after the injury has been resolved (Loeser and Melzack 1999). Neuropathic pain is a form of chronic pain. It may result from direct injury to or dysfunction occurring in the sensory axons in the central or peripheral nervous system (Schumacher et al. 2007).

Different fibres conduct different types of pain. Fast, sharp and localised pain is conducted by A delta fibres which are small and myelinated. C fibres are non myelinated and conduct slow dull pain. Although the signals travel along different ascending pathways, they converge in the thalamus where the sensation of pain is recognised (Cooper et al. 2006).

1.3. Pain transmission

As stated, acute pain (nociceptive pain) occurs when a strong noxious stimulation of skin or deep tissues occurs. This stimulates a firing of nociceptors (primary sensory nerve fibres or first order neurons). These nociceptors fire impulses along the peripheral nerve and past the sensory cell bodies in the dorsal root ganglion of the spinal vertebrae. These signals then pass along the dorsal roots and into the spinal cord (or brainstem) (Basbaum et al. 2008). In this location they activate the second or third order neurons in the central nervous system and this activity is then interpreted by the mind as pain.

1.4. Pain modulation mechanisms

1.4.1. Spinal mechanisms of pain modulation

1.4.1.1. Large fibre inhibition

The substantia gelatinosa is a v-shaped mass of gelatinous neuroglia which is located at the apex of the posterior/dorsal horn of the grey matter of the spinal cord. The grey matter of the spinal cord is made up of cytoarchitecturally distinct laminae or layers numbered I to VI. The Gate Control theory proposes that the interneurons of the substantia gelatinosa regulate the excitatory input of the large and small fibres to the laminae V cells in the spinal cord (See section 1.4.4) (Wall 1978). This action serves as a 'gating mechanism' (Dickenson 2002, Basbaum et al. 2008). The Gate Control theory was able to account for the fact that analgesia can be produced by low intensity stimulation of the skin. When thin (pain) and large (touch, etc.) fibres are activated by a noxious event, they produce dual effects. They excite a spinal cord nociceptive transmission of signal. They also effect the spinal cord inhibition of signal. The thin fibres inhibit the inhibitory mechanism (tending to 'leave the gate open') while the large diameter fibres excite the inhibitory mechanism (tending to 'close the gate'). As a consequence, the more large fibre activity relative to thin fibre activity acting on the inhibitory signal the less pain is felt.

1.4.1.2. Opioid inhibition

Opioids can be injected locally into the epidural space or intrathecally into the spinal cerebrospinal fluid to produce a profound analgesia. The injection of opioids causes a postsynaptic inhibition of laminae I and V neurons and also form a presynaptic block of the release of neurotransmitters such as substance P and calcitonin gene-related peptide (CGRP) (Basbaum et al. 2008).

1.4.2. Supraspinal mechanisms of pain modulation

Inhibitory mechanisms of pain also exist in the brain. The periaqueductal grey (PAG) is the grey matter located around the cerebral aqueduct within the tegmentum of the midbrain. When the PAG is stimulated, there is activation of 5 hydroxytryptamine (5HT) neurons that project to the

nucleus raphe magnus of the medulla. The 5HT axons from the nucleus raphe magnus inhibit the firing of neurons in the laminae 1 and V of the dorsal horn (Basbaum et al. 2008).

Parallel to these neurons are descending noradrenergic inhibitory controls as well. It is the combination of these circuits from the PAG to the spinal cord that constitute the brain's endogenous pain control system (Basbaum et al. 2008).

One of the functions of systemic morphine is to bind to opioid receptors in the PAG and initiate the PAG descending inhibitory control of spinal cord pain transmission neurons. Opioids also act in the thalamus and parts of the cerebral cortex. Specifically there is a high density of opioid receptors in the anterior cingulate cortex.

1.4.3. Other forms of pain modulation

There are also many studies showing how the attention of the subject, anticipation of pain, mood and anxiety all contribute to the processing and perception of pain in the brain. The placebo effect is of particular interest (Scott 2008). The most recent evidence suggests that the placebo effect is mediated by expectations and anticipatory processes which initiate endogenous opioid activation of the mu opioid receptors. The placebo effect can be reversed by the administration of pharmacological agents such as naloxone. (Levine et al. 1978, Colloca and Benedetti 2006, Petrovic et al. 2010).

1.4.4. Developments in our understanding of pain

One of the most important developments in our understanding of the nature of pain was the development of the Gate Control theory in the mid-1960s (Wall 1978). The theory continues to provide grounds for much debate among pain researchers and remains influential (Price et al. 2009). By drawing attention to hitherto relatively unexplored aspects of pain perception, most notably those of pathological states, the theory has contributed to a considerable surge in pain research, including the development of many models of pathological pain. It also emphasised the role that central modulation, both excitatory and inhibitory, has in pain perception (Price et al. 2009). The Gate Control theory was also important because it was one of the first theories to suggest that psychological factors play a role in the perception of pain. (Farrell 2005).

1.4.4.1. Neuroimaging and pain

More recent developments in the understanding of the physiological responses to pain have been facilitated by the process of neuroimaging. Research on the central mechanisms of the sensory-discriminative dimensions of pain have suggested there exists a complex network of cortical and subcortical brain structures involved in the transmission and integration of pain. This

has been described as the 'neuromatrix theory of pain' or the 'pain matrix' (Melzack 2005, May 2007).

Functional and structural Magnetic Resonance Imaging (MRI) is used as a means to explore the brain's response to noxious stimuli and specific pain-related forebrain responses. It also is used to ascertain pain modulatory effects in the brain. MRI has confirmed that the brain is capable of a high degree of plasticity with regards to structure and function in response to repeated and ongoing pain (Yilmaz et al. 2010, Davis 2011).

Advances in our understanding of the nature of pain have been accompanied by advances in our treatment approaches to pain. While there has been a growing emphasis on the psychological approaches to pain treatment, much research continues on developing more effective pharmacological interventions.

1.5. Treatment of pain

The sensation of pain is essential. It has adaptive value. By taking appropriate action to avoid or lower pain (e.g. resting a strained muscle, removing a hand from heat) one improves one's chances for survival. However, the sensation of pain can be extremely unpleasant. Throughout history, humans have laboured to produce more effective means of managing pain. Treatment approaches have included physical therapy such as massage and thermal agents, psychological therapies such as cognitive and behavioural therapies, and pharmacological interventions.

It is important to recognize that pain is a subjective experience that has a variable relationship with tissue damage. Many factors contribute to the individual's experience of pain. They include somatic (physical) factors, mood (e.g. depression and anxiety), cultural factors and the context in which the pain is occurring (Macintyre et al. 2010). The context in which trauma occurs may impact on the experience of pain. Similarly, cultural factors should always be considered. Interventions in these areas by skilled clinicians to allay anxiety and fear may be very effective in decreasing the pain experience.

A central approach to treating pain is pharmacological intervention. Opioids have been a central means of providing analgesia from ancient times and in spite of the development of other classes of pharmacological agents (N-methyl-D-aspartate (NMDA) receptor antagonists, non-steroidal anti-inflammatories, gabapentanoids and mixed action analgesics (e.g. tramadol with opioid agonism and monoaminergic inhibition), opioids continue to predominate. A description of the use of opioids in history and recognition that problematic side-effects of opioids have been observed for over three hundred years is helpful for our understanding of the present day complications of pain management in the opioid maintained client.

1.6. A brief history of opioids

Opium is the white milky sap from the partially ripe seedpod of the opium poppy Papaver Somniferum. The term opioid describes all drugs, natural and synthetic, with morphine like actions. These include the endogenous 'morphine like substances' (enkephalins, dynorphins and beta-endorphins) that bind to the same receptors as opioids and antagonists of opioid drugs (Martin 1967, Reisine and Pasternak 1996). As stated, opioids have an extensive history in human cultures. The first recorded opioid used in human civilisation was opium.

1.6.1. The Ebers Papyrus and Theophrastus

It has been suggested that as early as 3500 BC, opium was used in Egyptian religious rituals (Inverarity et al. 1983). The most extensive record of ancient Egyptian medicine known, The Ebers Papyrus (approximately 1550 BC), lists opium as a pain reliever (Burkholz 1987). At the beginning of the third century BC, the Greek philosopher Theophrastus described treatment of pain by use of meconium which is derived from the stems, leaves and fruit of Papaver Somniferum (Macht 1915).

1.6.2. Paracelsus and Laudanum, Coleridge and De Quincy

Philippus Aureolus Theophrastus Bombastus von Hohenheim, also known as Paracelsus (1493-1541) was a well-known physician of the 16th century and experimented with the use of tincture of opium (powdered opium dissolved in alcohol). He concluded that its medical value for a range of conditions was of such magnitude that he called it laudanum, from the Latin 'laudare' to praise (O'Brien and Cohen 1984). By the nineteenth century, the popularity of laudanum could be compared to the popularity of paracetamol today. To explain the popularity of opium, it has been argued that up until the beginning of the nineteenth century, the chief function of medicine was to relieve pain. Therapeutic agents were not directed at the cause, but rather at the symptoms of the illness. The wide popularity of opium, either on its own, or in combination with other medicines was due to its ability to relieve the symptoms of a range of medical conditions (Terry and Pellens 1928, Abadinsky 2008). It was used for the treatment of mental illness, diarrhoea, dysentery, asthma, rheumatism, diabetes, malaria, cholera, fevers, bronchitis, insomnia and pain of any kind, including menstrual pain (Fay 1975).

While the medical profession extolled the virtues of laudanum for treating a range of illnesses, famous literary figures drew inspiration from the ability of the opioid compounds to alter consciousness, most notably Samuel Taylor Coleridge (1772-1834) (Kublai Khan) and Thomas De Quincy (1785-1859) (Confessions of an English Opium Eater) (Abadinsky 2008).

Laudanum is still available in such countries as the United States and Germany as Tincture of Opium (also known as deodorised opium tincture which is equivalent to 10 mg per ml of anhydrous morphine) or paregoric (also known as camphorated tincture of opium which is

equivalent to 0.4 mg of morphine per ml). It is approved for the treatment of severe diarrhoea that does not respond to conventional therapy and is used in some countries for the treatment of neonatal abstinence syndrome (Langenfeld et al. 2005, Pasricha 2006).

1.6.3. John Jones, George Young, addiction and withdrawal

While the beneficial effects of opioids were well described many centuries ago, it was also apparent by the eighteenth century that opioid use carried negative consequences. One of the earliest descriptions of the nature of opioid withdrawal following rapid decrease or cessation of chronic opioid use was in 1700 AD by Dr. John Jones. Jones advocated the use of opium unstintingly for a range of maladies, but in moderate doses. He also recognised the dangers of addiction. In his book 'The Mysteries of Opium Revealed' he wrote "A return of all diseases, pains and disasters, must happen generally, because the opium takes them off by a bare diversion of the sense thereof by pleasure" (Jones 1700, Kramer 1979). Upon discontinuation or decrease, particularly after 'leaving off after long and lavish use thereof' symptoms such as diarrhea, sweating, itching and melancholy would result. Interestingly, he also recommends a gradual decrease in dose to manage withdrawal symptoms. Withdrawal could be managed by a decrease in dose by one-hundredth of a part each day until the drug was withdrawn.

In 1753, the physician George Young wrote 'A Treatise on Opium' and described the indications and contraindications for the use of opium in various diseases (Young 1753, Kramer 1979). He described a woman who started using opium as a medication and continued after the precipitating illness resolved itself. He reported that the woman's friends recommended she cease taking the opium before she became addicted, but that she responded by saying that she would rather cease spending time with these friends. Young described the use of opium for depression ('lowness of mood'). He reported that the effectiveness of opium was only intermittently good. He described another female patient who took 300 to 400 drops of laudanum per day in order to prevent the occurrence of depression ('without which she was depressed'). Interestingly he also referred to the Turkish population which took opium habitually and made them fearless. He said that, to his surprise, it had a stimulatory rather than a sedatory effect with this race of people. Finally he described a sailor for whom he prescribed opium to manage the pain of a lanced abscess. The sailor returned from his travels and praised the medication for both its pain relieving properties and the euphoria it produced. The sailor explained that he was resolved to carry the opium wherever he went in his travels (Young 1753, Kramer 1979).

Young's text described habituation, physical dependence, tolerance and euphoria. Young did not believe the drug to be completely benign. He considered it a slow poison, but only when misused and applied inappropriately in certain diseases (Young 1753, Kramer 1979).

This dilemma challenges the use of opioids to this day. The beneficial effects of opioids are counterbalanced by negative effects. Two of the most important are the withdrawal syndrome associated with abrupt cessation of opioid use and the need to increase use to maintain similar effect, also known as tolerance. Both withdrawal and tolerance are factors associated with the development of opioid addiction. It is important to note that tolerance develops differently depending on the effect. Opioid maintained patients readily become tolerant to the respiratory depressant effects of opioids (though this may still be problematic) but may continue to show sedation, miosis and constipation for months if not years after commencing maintenance (Martin et al. 1973). The need to increase use which is associated with the development of tolerance to the euphorogenic effects of opioids often leads to a small dose interval between what is effective euphorigenically and what is lethal in terms of respiratory depression.

1.6.4. Discovery of morphine and cures for 'morphinism'

Late in the eighteenth century (Latimer and Goldberg 1981) or early in the nineteenth century (Bresler 1980), the German pharmacist Friederich W. Sertürner, added liquid ammonia to opium and created an alkaloid that was many times more powerful than opium. He named it morphine after the Greek god of sleep and dreams, Morpheus.

By 1817 a range of articles had been published in scientific journals popularising the new drug. The medical profession at the time incorrectly viewed morphine as an opiate without addictive and other negative effects (Abadinsky 2008). Within a few years the medical profession changed their opinion, became aware of the addictive properties of morphine and sought possible cures. This was one of the first examples of a drug that was initially seen as having little addictive properties and a treatment for opioid addiction, becoming well known for causing dependence. Similarly, both diacetyl morphine (heroin) (see below) and cocaine were considered as treatments for opioid addiction when they were first introduced (Jennings 1901, Freud 1961, Freud 1974, Van Dyke and Byck 1982).

1.6.5. The hypodermic needle

Either Alexander Wood, a Scottish physician from Edinburgh and Charles Pravaz, a French surgeon, or the Irishman Francis Rynd, invented the hypodermic needle. It was introduced to medicine in the United States in 1856, and by direct injection of morphine into the bloodstream, revolutionised severe acute pain management. It was first widely used during the American Civil War (Terry and Pellens 1928, Abadinsky 2008).

1.6.6. Diacetyl morphine

In 1874 CRA Wright, an English chemist, working at St Mary's Hospital London, was experimenting by combining morphine with various acids. By boiling anhydrous morphine alkaloid with acetic anhydride for several hours he produced a more potent, acetylated form of morphine

now known as diacetyl morphine, or heroin (Wright 1874). Like many compounds, there were few further developments until Felix Hoffman, working at Bayer pharmaceutical company in Elberfield, Germany, in the process of trying to formulate a different means of producing codeine, also acetylated morphine and produced heroin. Bayer gave the substance the brand name 'heroin' from the German word 'heroisch' because in field studies people using the medicine felt 'heroic'. From 1898 to 1910 heroin was marketed as a non-addictive morphine substitute and cough suppressant (Bresler 1980, Bowden 2002).

1.6.7. The Narcotics Clinics 1918 to 1922

One of the landmark events of drug prohibition in the United States, and with impact felt internationally, was the Harrison Act of 1914. It required that any person having any dealings with opioids and cocaine had to be registered (Abadinsky 2008). Subsequently, the price of heroin in the United States increased by 900 percent and was sold in adulterated form. Opioid dependent people were no longer able to access it legally, were often unable to afford the high price of it illegally and many were forced to apply for treatment. Beginning in 1918, narcotic maintenance treatment clinics opened up in many cities in the United States. There is limited information about them and controversy exists about their operations. However, it is generally thought that most of them were well run with medical supervision. They enabled opioid dependent people to continue normal lives without having to access the black market (Duster 1970, Abadinsky 2008). Prohibition (of alcohol) began in the United States in 1920. In 1922 US federal narcotics agents closed the drug clinics and began to arrest physicians and pharmacists who provided drugs for maintenance treatment. This contributed to the development of an enormous illegal market in opioids (White 1998). The clinical intervention of opioid maintenance for opioid dependency was effectively closed down in the United States until methadone maintenance research began in the mid 1960's (Abadinsky 2008).

In 1955 The New York Academy of Medicine reviewed the operation of the early maintenance treatment clinics and affirmed that the clinics were not shut down because they had failed, but because their goals were not in accordance with the prevailing philosophy of a punitive approach to the problem of opioid dependence (Ball and Ross 1991).

In the early 1960s the idea of substitute opioid administration (also known as opioid maintenance) to manage opioid withdrawal became an established approach to the problem.

1.7. Brief history of methadone, buprenorphine and LAAM maintenance

In 1962, in response to concerns about rising numbers of opioid dependent people in New York City, the New York Health Research Council recommended research into alternative approaches to abstinence for the treatment of these patients. In 1964 Vincent Dole and Marie Nyswander admitted to the hospital at the Rockefeller Institute (now Rockefeller University) six heroin

dependent patients between 20 and 40 years old with addiction histories of at least 5 years. The patients were placed on a daily maintenance dose of between 80 and 120 mg of methadone (Ausubel 1966, Dole et al. 1966, Dole et al. 1966, Lowinson et al. 2003). The programme continued to expand over the years throughout New York and the rest of the United States. By 1969 there were 2,000 patients enrolled in methadone maintenance programs in New York City and 10,000 applicants awaiting admission (New York Academy of Science Committee on Public Health 1990). Since then methadone maintenance therapy has been introduced into many Western countries, including Australia.

In 1993 LAAM (levo-alpha-acetylmethadol), a longer acting methadone analogue was approved for use as a maintenance agent in the United States. However, because of indications that it was associated with prolongation of the QTc interval and the sometimes fatal Torsade de Pointes, it was subsequently removed from the market in Europe and production ceased in the United States (Lowinson et al. 2003).

Buprenorphine is a highly lipophilic opioid derived from thebaine. It was originally developed in the Reckitt and Colman laboratories in the early 1970s and marketed as an analgesic in the 1980s (Rosenthal and Bayait 1988). In France it was used as treatment for opioid dependence from the mid-1990s onwards (Fatseas and Auriacombe 2007). It was available in Australia from 2000 and from 2002 in the United States for opioid dependence treatment. Its popularity continues to grow as an alternative maintenance agent to methadone.

1.8. Opioid pharmacology

In the following section of the thesis general opioid pharmacology, pharmacokinetics and pharmacodynamics will be discussed with particular reference to the pharmacology of methadone, buprenorphine and morphine. Later in the thesis the specific pharmacology of methadone, buprenorphine, morphine, ketorolac, S-ketamine and tramadol will be discussed.

1.8.1. Mu, kappa and delta receptors

Opioids produce effects by binding to receptors on the cell membranes of neurons. Three major types of opioid receptors have been identified. These are the mu, delta and kappa receptors. These receptors subserve different physiological effects (Jaffe and Martin 1990, Jaffe 1995, von Zastrow 2010). While the physiological roles of the three receptor types have not been fully elucidated, it is broadly considered that the mu and delta receptors are involved in systems that influence mood, the addictive effects of opioids, respiration, pain, blood pressure, endocrine and gastrointestinal functions. Kappa receptors produce endocrine changes, analgesia and dysphoria. In animal studies, under experimental conditions, mu and delta agonists are self-administered. In contrast, Kappa agonists are not. Kappa agonists have been found to produce aversive effects in

animals and dysphoria, rather than euphoria, as found in human subjects (Woods and Winger 1987, Musacchio 1990, Knapp et al. 2003, Wang et al. 2010) .

Mu receptors are found primarily in the brainstem and medial thalamus. They are also found in the peri-aqueductal grey region and the superficial dorsal horn of the spinal cord. Mu receptors in the intestinal tract produce constipation by inhibiting peristaltic action. Kappa receptors are found in the limbic and other diencephalic areas and brain stem. They are also expressed in the dorsal root ganglia and dorsal spinal cord. While the location and effects of delta receptors have been less extensively studied, it has been suggested that delta opioid receptors have a prominent role in attenuating persistent pain but not acute pain perception (Trescot et al. 2008, Wang et al. 2010, Gaveriaux-Ruff et al. 2011).

Three opioid receptor genes that encode for the mu, kappa and delta receptors respectively have been identified. Each of these has a specific name and common variants have been associated with variable pain sensitivity in the general population. The OPRM1 receptor gene, encodes for the mu opioid receptor (Tremblay and Hamet 2010). The OPRK1 gene encodes for the kappa opioid receptor. The OPRD1 gene encodes for the delta opioid receptor (Tremblay and Hamet 2010).

While there is some pharmacological evidence suggesting that subtypes of the three basic opioid receptors exist, the significance of these subtypes is still unclear. The three basic subtypes and the Orphanin FQ/Nociceptin receptors were cloned by 1994. As Dietis (2011) discussed in a recent review, since the cloning of the basic opioid receptors, and the development of knockout animals through the use of this information, there has not been a successful attribution of ligand activity (pharmacological subtypes) to subclassifications of the primary types of opioid receptors. Dietis suggests that putative opioid receptor subtypes have been suggested based on three areas of research (Dietis et al. 2011). These are firstly, different modulations by pharmacological agents of functional responses, secondly, incomplete cross-tolerance profiles between different receptor agonists and thirdly, a range of complex binding characteristics including shallow ligand displacement curves and differential irreversibility of ligand binding (Dietis et al. 2011).

Against these findings suggesting subclassifications of opioid receptors are the findings that according to molecular evidence available, only single receptors are encoded by single genes and genetic knockouts of the single receptor genes produce an overall loss of ligand binding and function of the whole receptor (Dietis et al. 2011).

Dietis et al (2011) end their review by suggesting that the putative pharmacological subtypes can be reconciled with the molecular data by considering firstly, the impact of alternative splicing, secondly, receptor dimerization, thirdly, interaction with other proteins and biased agonism and fourthly, combination of the first three factors listed. There is clear evidence that

variations in ligand activity along with the major types of opioid receptors exist. However, there is not sufficient evidence at this stage that subtype function can be established. (Dietis et al. 2011, Wei and Loh 2011). Some of the factors are further discussed below.

1.8.2. mRNA splicing and allelic variants

There is evidence to suggest that variations in opioid receptor proteins may arise from two sources. These are alternative pathways in the splicing of opioid receptor messenger ribonucleic acids (mRNAs) and allelic variants. Allelic variants are variations in the nucleotide sequences in certain opioid receptor genes (Cadet et al. 2003, Pan et al. 2003).

Several splice variants of the human mu opioid receptor have been suggested and identified (Cadet et al. 2003, Pan et al. 2003). One of these is a variant that has been described as the mu three receptor. This is expressed in vascular tissue and leukocytes. It was reported to be sensitive to morphine but not the opioid peptide metenkephalin (Cadet et al. 2003).

An often cited example of a polymorphism of the human mu opioid receptor gene is one that involves substitution of aspartate for asparagine in the amino acid sequence of the receptor protein at position 40. This is also known as the A_{+118} G polymorphism. There is accumulating evidence that the different variants of this biallelic polymorphism have different pharmacological properties. For instance, plasma cortisol levels were significantly greater following challenge with the opioid antagonist naloxone in subjects with A/G or G/G variants than in those with the A/A variant. The cortisol response to naloxone has been studied as a marker of alcoholism risk among individuals who are offspring of alcoholic parents. These studies have shown a greater cortisol response to opioid blockade among individuals with a family history of the disorder compared to individuals without a family history (Hernandez-Avila et al. 2002, Hernandez-Avila et al. 2003, Uhart and Wand 2009). More recent work has demonstrated the involvement of A118G polymorphism of exon 1 with heroin and alcohol addiction in a population in eastern India. It was found that the association of A118G polymorphism with heroin and alcohol addiction may be because of altered regulation of protein kinase A and phosphorylated extracellular signal-regulated kinase (pERK 1/2) during opioid and alcohol exposures (Deb et al. 2010). However, a meta-analysis by Arias et al found no role for A118G in determining risk for substance use disorders (Arias et al. 2006). A recent study by Coller et al (2011) found that those subjects with the homozygous or heterozygous OPRM1 A118G genotype had no increased success when treated with naltrexone for alcohol dependence.

1.8.3. Intracellular events following mu opioid reception

Opioid receptors are part of a family of proteins that couple to the heterotrimeric G proteins (G alpha beta gamma Gαβγ) which are defined in terms of the Gα subunit (Lamberts et al. 2011). The mu opioid receptor couples to the Gα proteins of the pertussis toxin-sensitive $G\alpha_{i/o}$

family. The $G\alpha_{i/o}$ family includes G alpha o ($G\alpha_o$) (which includes the splice variants of this protein $G\alpha_{o1}$ and $G\alpha_{o2}$) and the G alpha I 1, 2 and 3 ($G\alpha i_1$, $G\alpha_2$ and $G\alpha_3$) (Laugwitz et al. 1993, Chakrabarti et al. 1995, Lamberts et al. 2011). The mu opioid receptor also couples to the pertussis toxin-insensitive G alpha z ($G\alpha_z$) protein (Garzon et al. 1997, Lamberts et al. 2011).

Following the interaction of the mu opioid receptor with the G protein, a number of effects occur including ion channel gating, modulation of calcium 2^+ levels and protein phosphorylation (Katzung 2007). There are two major effects of opioids as a result of G protein coupling. Firstly they close voltage gated calcium 2^+ channels on presynaptic nerve terminals. This produces a reduction in neurotransmitter release. Secondly they hyperpolarise, cause the opening of K^+ channels and by doing so inhibit the post-synaptic neuron (Katzung 2007). Additionally, the activation of opioid receptors promotes their phosphorylation. The phosphorylation of opioid receptors occurs via G protein coupled kinases and is a prelude to its forming a complex with beta-arrestin. The binding of arrestin to G protein coupled receptors (GPCR) sterically hinders interaction with G proteins resulting in the uncoupling of opioid receptors from them. This blunts opioid receptor signaling, which manifests as opioid receptor desensitization. Beta-arrestin also targets GPCRs to clathrin-coated pits, thereby initiating opioid receptor internalization and trafficking to other subcellular compartments such as lysozomes, where receptor degradation can occur (Zhang et al. 1996, Johnson et al. 2005, Gintzler and Chakrabarti 2006, Christie 2008).

1.8.4. Endogenous opioid peptides

The term endorphin refers to the opioid sub-class of endogenous opioid peptides. There are three families: the enkephalins, the dynorphins and the beta-endorphins. Each of these families of peptides is derived from a distinct precursor polypeptide and possesses a characteristic anatomical distribution (Hollt 1986). These precursors are designated as proopiomelanocortin (POMC), proenkephalin, and prodynorphin. The distribution of peptides from POMC is relatively limited in comparison to the distribution of the other families. POMC is distributed in the human brain (in areas where electrical stimulation can produce pain relief) and an example of this is in the arcuate nucleus. The arcuate nucleus projects its fibres widely to the limbic and brain stem areas (Pilcher et al. 1988). Some POMC containing fibres descend to the spinal cord. Other POMC peptides are also found in the endocrine organs such as the pituitary and the pancreas (Lewis et al. 1987, Dores and Baron 2011).

In contrast, the peptides from prodynorphin and proenkephalin are distributed widely throughout the CNS with a more complex pattern. Endogenous opioid peptides have been implicated in the development of opioid addiction, specifically proenkephalin (Gianoulakis 2004, Nikoshkov et al. 2008). Gieryk et al (2010) have suggested that forebrain prodynorphin expression may protect against drug addiction by limiting drug produced reward. This may be due to

dynorphin-mediated modulation of dopamine release in the nucleus accumbens (Gieryk et al. 2010).

This section has discussed opioid receptors, the possibility of opioid receptor subtypes, factors impacting on variations in ligand activity and endogenous opioid ligands. The thesis will now examine general opioid pharmacodynamics and pharmacokinetics with reference to the opioid maintenance agents methadone and buprenorphine

1.9. Opioid Pharmacodynamics

Opioid receptors are found predominantly in the brain, spinal cord and in the gastrointestinal tract. They are also found in lower densities in other parts of the autonomic nervous system. The effects for which morphine are most commonly prescribed are exerted on the central nervous system and the gastrointestinal tract.

Methadone and buprenorphine act mostly through the mu opioid receptor. They share with the other common opioids, the major effects in the central nervous system of analgesia, a sense of tranquility, decreased sense of apprehension and a suppression of the cough reflex (Knapp et al. 2003). Unfortunately like the other opioids, they may also produce depression of respiration, nausea, vomiting, constriction of pupils, alterations in temperature regulation and a variety of changes in the neuroendocrine system (Rhodin et al. 2010).

Respiration is depressed by morphine-like opioids as a result of a direct effect on the brainstem respiratory centres and in particular, the reduction in the responsiveness of the brainstem respiratory centres to carbon dioxide. Opioids also directly depress the pontine and medullary centres involved in regulating respiratory rhythm and the responsiveness of medullary centres to electrical stimulation (Martin 1983). In humans, death from opioids is almost always due to hypoxia (White and Irvine 1999). There is a particular risk of hypoxia during the induction period onto methadone and when there is polydrug use (Modesto-Lowe et al. 2010). In contrast buprenorphine, while behaving as a mu opioid agonist for analgesia in clinical practice, has a unique maximum ‘ceiling’ for respiratory depression (Pergolizzi et al. 2010).

Nausea and vomiting produced by opioids are caused by the direct stimulation of the chemoreceptor trigger zone for emesis in the area postrema of the medulla (Gutstein and Akil 2006). It has been suggested that opioid effects on the human pupil (miosis) are brought about by a direct excitatory action on the pupilloconstrictor nucleus (Knaggs et al. 2004). The evidence for this is not clear however. While Fontana first described the phenomenon in 1765, the exact mechanism remains unknown (Larson 2008). Most of the effects of opioids on the neuroendocrine system are unwanted effects. These include inhibition of gonadotropin-releasing hormone. With long term use this may result in decreased testosterone levels in males and disturbed menstrual

function in females (Jaffe and Martin 1990). One of the most prominent effects of mu opioid agonists on the gastrointestinal system is the slowing of the passage of food. (Knapp et al. 2003).

Another effect of some opioids is histamine release, particularly in the case of morphine. This causes vasodilation of cutaneous blood vessels. The skin of the face, neck and upper thorax often becomes flushed. Urticaria, often found at the site of opioid injection, is also likely to be a result of histamine release. It is not mediated by opioid receptors and is not blocked by naloxone (Gutstein and Akil 2006). Pruritis is another complication of opioid use. Opioids administered both systemically and intraspinally can cause the condition. It is particularly intense following intraspinal administration (Ballantyne et al. 1988). It appears to be mediated largely by dorsal horn neurons and is reversed by naloxone (Thomas et al. 1992).

The effects of most opioids on the cardiovascular system are not prominent, except under particular circumstances. For patients with a decreased blood volume, the effect of morphine-like opioids to produce peripheral vasodilation, reduced peripheral resistance and an inhibition of baroreceptor reflexes can aggravate hypovolaemic shock (Gutstein and Akil 2006). At high doses, some opioids such as LAAM and methadone may produce prolongation of the QT interval and cardiac arrhythmias such as Torsade de pointes (Peles et al. 2005).However, the prolongation of the QT interval produced by methadone maintenance is not clinically significant (Athanasos et al. 2008, Mayet et al. 2011)

The urinary voiding reflex is inhibited by morphine and the tone of the external sphincter and the volume of the bladder are increased. Urinary catheterisation is sometimes required following the administration of therapeutic doses of morphine. Bladder motility is affected from stimulation of opioid receptors peripherally (Moss and Rosow 2008).

1.10. Opioid pharmacokinetics

1.10.1. Absorption and distribution

When administered orally, opioids are absorbed from the gastrointestinal tract. The more lipophilic opioids are readily absorbed through the nasal mucosa. Buprenorphine has extensive first pass metabolism and therefore low oral bioavailability at less than 20% (Iribarne et al. 1997). An effective route of administration for buprenorphine is through the buccal mucosa and not via the gastro-intestinal tract. In comparison, methadone has a high oral bioavailability at between 79 and 95% (Lugo et al. 2005). For this reason it is administered effectively via oral administration.

Opioids are effectively absorbed following subcutaneous or intramuscular injection and can also penetrate the spinal cord following epidural or intrathecal administration (Stine and Kosten 1999). Lipid solubility is an important factor in transdermal absorption of opioids and distribution to the central nervous system. The more lipid soluble opioids (e.g. fentanyl) also act

more rapidly on the central nervous system than morphine after intravenous administration (Trescot et al. 2008).

1.10.2. Metabolism

Most opioids are converted to more polar metabolites by hepatic metabolism. This occurs primarily by cytochrome P450 enzymes or UDP-glucuronosyltransferase (UGT) (that catalyses a glucuronidation reaction). For a number of opioids, the primary metabolites that are formed undergo further metabolism. This occurs via secondary pathways. Phase 1 (functionalisation) is then followed by Phase 2 (conjugation). The polar metabolites are then excreted by the kidneys (Coller et al. 2009). Morphine, for example, is primarily (about 60%) conjugated to morphine-3-glucuronide. Approximately 10% of morphine is also metabolised to morphine-6-glucuronide (M6G). Morphine-6-glucuronide has been shown to have some analgesic activity clinically. It is unclear whether morphine-3-glucuronide has excitatory or 'antianalgesic' effect clinically (Penson et al. 2000, Andersen et al. 2003, Coller et al. 2009)

Heroin and remifentanil are esters and are rapidly hydrolysed by common tissue esterases. Heroin is hydrolysed to monoacetylmorphine and finally to morphine. Morphine is then conjugated with glucuronic acid.

Cytochrome P450 3A4 (CYP3A4) (among others) has been implicated as playing a major role in the metabolism of buprenorphine (Iribarne et al. 1997), methadone (Iribarne et al. 1997) and tramadol (Subrahmanyam et al. 2001). Codeine, hydrocodone and oxycodone undergo O-dealkylation by way of CYP2D6 as they are alkyl ethers at the 3-phenolic hydroxyl group. The metabolism of codeine, oxycodone, hydrocodone and tramadol, results in metabolites of greater potency (Schumacher et al. 2007). For example, codeine is converted to morphine and has analgesic action. Some researchers have suggested that codeine relies on metabolism to morphine for analgesic effect, while others suggest codeine itself or codeine and codeine-6-glucuronide are responsible for analgesic effects (Vree and Verwey-van Wissen 1992, Quiding et al. 1993, Sindrup and Brosen 1995, Vree et al. 2000, Coller et al. 2009).

1.10.3. Excretion

As stated, polar metabolites, including glucuronide conjugates of opioid analgesics are excreted mainly in the urine. The urine may also contain small amounts of the unchanged drug. Glucuronide conjugates have also been found in the bile, but enterohepatic circulation is responsible for only a small part of the excretory process (Gutstein and Akil 2006).

Methadone is primarily excreted into urine and faeces. Normally 20-50% is excreted in urine (Dean 2004, Lugo et al. 2005). Buprenorphine is primarily excreted in faeces with

approximately 10-30% excreted in the urine (Brewster et al. 1981, Cone et al. 1984, Walter and Inturrissi 1995, Elkader and Sproule 2005).

As a consequence of hepatic metabolism, there may be increased bioavailability or cumulative effects following oral administration of opioids in patients with hepatic disease. For example codeine should be avoided because it is generally considered to require hepatic transformation to morphine for analgesia its efficacy could be impaired (Vree and Verwey-van Wissen 1992, Quiding et al. 1993, Sindrup and Brosen 1995, Vree et al. 2000, Coller et al. 2009, Gandhi et al. 2011). In comparison, hepatic impairment does not have a clinically relevant effect on the pharmacokinetics of heroin and its metabolites (Rook et al. 2006). Similarly, renal disease can significantly alter the pharmacokinetics of some opioids such as morphine and produce toxic effects, but has less effect on opioids such as methadone (Coller et al. 2009, Niscola et al. 2010).

M3G is a metabolite of morphine and results from animal studies suggested that it may have an opposite effect to morphine and the morphine metabolite M6G (i.e. M3G was antianalgesic and stimulatory) clinically (Gong et al. 1992, Bartlett et al. 1994, Halliday et al. 1999, Smith 2000, Coller et al. 2009). It was also suggested that during renal failure, the accumulation of M3G would counteract the effect of morphine. This proposition has not been substantiated in clinical studies (Penson et al. 2000, Andersen et al. 2003, Coller et al. 2009). During renal failure the accumulation of morphine and morphine metabolites leads to increased sedation and respiratory depression.

1.11. Methadone and buprenorphine pharmacology

Methadone and buprenorphine are currently the primary pharmacological agents for the maintenance treatment of opioid addiction. For this reason, the thesis will examine the pharmacology of these drugs in detail.

1.11.1. Methadone pharmacology

Methadone is a long acting mu receptor agonist. It has a pKa of 9.0 and a molecular weight of 309. It contains a chiral carbon atom giving rise to levorotatory R-(-) methadone and dextrorotatory S-(+)- methadone (Gorman et al. 1997, Gutstein and Akil 2006). The levrotatory R-(-) isomer possesses analgesic activity while the dextrorotatory S-(+)- isomer is inactive or weak as an opioid (Davis and Inturrisi 1999, Inturrisi 2005). The dextrorotatory S-(+)- isomer has been shown to have NMDA receptor antagonist activity (Inturrisi 2005).

1.11.1.1. Methadone pharmacodynamics

Methadone acts mostly through the mu opioid receptor and produces analgesia, euphoria, respiratory depression and physical dependence. It is a non-competitive antagonist at the N-methyl-D-aspartate (NMDA) receptor complex and an uptake inhibitor of 5-hydroxytryptamine and noradrenaline (Codd et al. 1995, Gorman et al. 1997, Dyer et al. 1999).

1.11.1.2. Methadone pharmacokinetics

Methadone has an oral bioavailability of between 79 and 95%. The volume of distribution is between 3 to 5 L/kg. Ninety percent of methadone is plasma bound and it is primarily bound to alpha 1- acid glycoprotein. It has a half-life of between 22 and 52 hours. The total body clearance is approximately 115 mL/minute. Methadone is primarily metabolised through cytochrome P450 3A4 and cytochrome P450 2B6 (Lugo et al. 2005). Other authors have suggested there is contribution from P450 2C18, 2C19 and 2D6 (Anggard et al. 1975, Verebely et al. 1975, Meresaar et al. 1981, Nilsson et al. 1982, Inturrisi et al. 1987, Eap et al. 1990, Gorman et al. 1997, Dyer et al. 1999, Foster et al. 2000, Gutstein and Akil 2006, Kreek et al. 2010). More recently Shiran et al found that while variability in cytochrome P450 3A4 has statistically significant but modest influence on the oral clearance of methadone and its enantiomers, CYPs 1A2 and 2D6 have no impact at all (Shiran et al. 2009).

1.11.2. Buprenorphine pharmacology

Buprenorphine is a partial opioid agonist that has been shown to be effective in the treatment of opioid addiction (Johnson et al. 1992, Ling et al. 1998). It is derived from the morphine alkaloid thebaine (Heel et al. 1979) and belongs to the 6,14-endo-ethanotetrahydro-oripavine class of compounds that includes the opioids diprenorphine and etorphine (Cone et al. 1984).

1.11.2.1. Buprenorphine pharmacodynamics

The predominant activity of buprenorphine in humans is at the mu opioid receptor and is responsible for the manifestation of such features as supraspinal analgesia, respiratory depression and miosis. It has been suggested that the partial agonism of buprenorphine at the mu-opioid receptor may be responsible for its wider safety profile (particularly with regards to respiratory depression) in comparison with the safety profile of the full mu-agonists (Lewis 1985, Walsh et al. 1995). In addition, the slow dissociation of buprenorphine from the mu opioid receptor results in a long duration of action in the treatment of opioid dependency (Jones 2004). This may result in fewer clinical signs of opioid withdrawal when buprenorphine therapy is discontinued compared with mu-agonists such as morphine, methadone or heroin (Eissenberg et al. 1997).

Buprenorphine's high affinity for the mu receptor results in it not being easily displaced by antagonists such as naloxone which have relatively lower affinity for the mu receptor (Gutstein and Akil 2006). It has variable effect at the kappa receptors and antagonistic effect at the delta receptor. It also has lower affinity at the delta receptor (Johnson et al. 2005, Andresen et al. 2011). Increases in the dose of buprenorphine cause an increase in the physiological and subjective effects of the drug to a specific level. After this, increases in dose produce no further effects (Walsh et al. 1995). This feature of buprenorphine, also described as partial agonism, along with its long duration of

action means that extended intervals between doses of buprenorphine are equally effective as daily intervals. A series of studies have shown that effective and safe administration of buprenorphine can be achieved with 48 (Amass et al. 1994), 72 (Bickel et al. 1999), or even 96 hour dosing intervals (Petry et al. 1999, Petry et al. 2000) with proportionate increases in dose.

Constipation, miosis, headaches, sedation, nausea with or without vomiting, and changes in blood pressure and heart rate are some common side-effects of buprenorphine (Pickworth et al. 1993, Ling et al. 1996, Ling et al. 1998, Montoya et al. 2004). While there are dose-dependent sedative and respiratory depressant effects of the drug, there is a reported maximum level of effect that is considered non-significant clinically (when given sublingually) (Walsh et al. 1995, Zacny et al. 1997) and contributes to a favourable safety profile compared to a full agonist mu opioid. Overdosage may occur when the drug is administered in combination with other central nervous system depressants (e.g. benzodiazepines) (Johnson et al. 2005).

As a result of buprenorphine's partial agonism, there is the possibility of precipitation of the opioid abstinence syndrome when buprenorphine is administered to individuals with a high dependence on other opioids (Walsh et al. 1995). Care should be given when administering buprenorphine to opioid dependent individuals taking more than 25 mg of methadone orally or more than 115 mg of morphine parenterally (Johnson et al. 2005). In summary, buprenorphine is considered a safe and well-tolerated drug for the treatment of pain and opioid addiction.

1.11.2.2. Buprenorphine pharmacokinetics

Buprenorphine's slow rate of disassociation from the mu receptor contributes to its long duration of action, both as an analgesic and as a maintenance opioid for drug addiction. Some authors have suggested that plasma elimination in humans appears to follow a tri-exponential curve (Bullingham et al. 1980, Yassen et al. 2006, Jensen et al. 2007). Another study has suggested that a 2 compartment model adequately described buprenophine pharmacokinetics (Escher et al. 2007). Half-life has been measured at between 2.75 hours and 3.21 hours (Escher et al. 2007). There is considerable variation in the values reported for the terminal half-life of buprenorphine. Mean values range from 3-44 hours (Bullingham et al. 1980, Kuhlman et al. 1996, Harris et al. 2000, McAleer et al. 2003, Elkader and Sproule 2005). Some studies have shown that gender-related differences exist in the pharmacokinetics of buprenorphine (Bullingham et al. 1980, Kuhlman et al. 1996, Moody et al. 2011). Females had significantly higher area under the plasma concentration curve (AUC) and maximum plasma concentrations for buprenorphine and norbuprenorphine (Moody et al. 2011). Some studies have suggested that the degree of binding to plasma proteins is high (96%) and this is predominantly to the alpha and beta-globulin fractions (Bullingham et al. 1980, Cone et al. 1984, Kuhlman et al. 1996, Iribarne et al. 1997). Iribarne et al (1997) and Kobayashi (1998) found in human liver microsomal studies that cytochrome P450 3A4 is the major

enzyme involved in the metabolism of buprenorphine by N-dealkylation to norbuprenorphine. Conjugates with glucuronic acid were formed for both buprenorphine and norbuprenorphine (Mistry and Houston 1987, Ohtani et al. 1994, Ohtani et al. 1995). Norbuprenorphine has been shown to have approximately 25% of the intrinsic analgesic activity of buprenorphine in rat studies (Ohtani et al. 1995).

Buprenorphine has extensive first pass metabolism and therefore has low oral bioavailability, less than 20% (Iribarne et al. 1997). Clearance is high and has been estimated to be between 1.04-1.45 Litre/minute (Kuhlman et al. 1996, Mendelson et al. 1997, Yassen et al. 2006) Sublingual, intravenous, transdermal and intranasal delivery methods have all been utilised to provide efficient means of buprenorphine delivery. Mean systemic bioavailability following the sublingual route had large intersubject variability with an average of 51% (Kuhlman et al. 1996). The time to maximum plasma concentrations following a single sublingual administration of a 0.4 or 0.8 mg buprenorphine tablet ranged from 90 to 360 minutes (Bullingham et al. 1981, Bullingham et al. 1982). In contrast to single dose administration of the tablet, administration of buprenorphine over a number of days produces average peak concentrations two hours after dosing (Nath et al. 1999).

Buprenorphine is indicated for the treatment of moderate to severe pain. Common dosages are 0.2 to 0.4 mg sublingually or 0.3 to 0.6 parenterally six hourly. Transdermal systems release from 35 to 70 ug/hour continuously (Johnson et al. 2005). Other authors have suggested that transdermal buprenorphine of up to 20 micrograms per hour may be effective initial opioid therapy for patients with chronic low back pain (Gordon et al. 2010).

1.12. Tolerance and hyperalgesia

There is substantial evidence that the pain experience and response to pain management strategies of people maintained on opioids is limited by the processes of tolerance and hyperalgesia. One of the aims of this thesis is to explore effective means of providing antinociception in the presence of these factors. This section begins with an examination of the processes of tolerance and hyperalgesia.

1.12.1. Tolerance

Tolerance is the decreased response to a drug with repeated administration or the requirement for larger doses of the drug to produce the same effect. Tolerance develops to the analgesic, euphorogenic, sedative and other central nervous system depressant effects of opioids. Tolerance also develops to the potentially lethal effects of respiratory depression (Koob and Le Moal 2006). Some opioid tolerant patients are able to take up to 2000 mg of morphine over 2 to 3 hours with negligible changes in heart rate or blood pressure. In an non-opioid tolerant (healthy

control) person, morphine doses of 30 mg parenterally and 120 mg orally may be lethal (Ellenhorn and Barceloux 1988).

There are also differential effects of opioids. While subjects may become tolerant to the respiratory depressant effects of opioids, they may still continue to show sedation, miosis and constipation (Martin et al. 1973). For example, constipation may continue to be a problem in methadone maintained subjects up to 8 months after entering the program. Insomnia continues to be a problem in 10 to 20 percent of patients and excessive sweating occurs in half of all patients many months after stabilisation (White and Hay 2007). The insomnia and sweating may be a consequence of withdrawal from methadone at the end of the dosing period (Dyer et al. 2001).

Tolerance to the analgesic effects of opioids continues to be a significant hindrance to adequate pain management. Early research found that morphine administration to mice over 20 days produced significant rightward shifts in the antinociceptive effect of morphine challenge in two tests of nociception, the hot-plate test, and acetic acid-induced writhing (Fernandes et al. 1977). Opioid analgesic tolerance is well-recognised and has been found to occur over a period of days to weeks (Way et al. 1969, Foley 1993, Foley 1995). In spite of a large amount of research in the area, the mechanisms that underlie the development of tolerance to the analgesic effects of opioids remain unclear. However, research into this area continues (See (Ossipov et al. 2004, Christie 2008, Chu et al. 2012).

1.12.2. Hyperalgesia

Exposure to opioids either acutely or for extended periods of time can elicit a paradoxical greater sensitivity to pain or hyperalgesia. This is defined by the International Association for the Study of Pain as an increased pain from a stimulus that normally provokes pain (International Association for the Study of Pain 2011). This hyperalgesia, along with the other neurobiological adaptations that accompany this state, may play a significant role in the increasing need for opioids to produce antinociception. This may be interpreted as tolerance.

1.13. Tolerance in the absence of hyperalgesia

A recent large scale, randomised, placebo controlled clinical trial by Chu et al (2012) examined subjects with lower back pain after one month of morphine treatment and concluded that tolerance to opioids could occur in the absence of hyperalgesia. These subjects were taking not more than 30 mg of oral morphine equivalents per day prior to the study and had moderate to severe chronic nonmalignant lower back pain. They were assessed prior to treatment before and during remifentanil infusion. The subjects were then titrated to comfort or dose limiting side effects using sustained release morphine (to a mean of 78mg/day) or placebo capsules for one month. They then returned for a second pain evaluation before and during remifentanil infusion. The study

found no difference in pain threshold and pain tolerance between the morphine and placebo treated groups prior to remifentanil infusion and concluded that hyperalgesia had not developed. However, during the remifentanil infusion, on the second pain evaluation day, there was a significant degree of opioid analgesic tolerance in patients exposed to chronic morphine therapy compared to control subjects. The morphine exposed subjects had a 43% decrease in analgesic potency compared with 3% increase in placebo subjects. Absolute values were not given.

In the same edition of the journal, Richebe et al (2012) made the following observations regarding the study. The study was limited to a specific population of patients with chronic lower back pain and healthy controls. The mean titrated dose of sustained release morphine was 78 mg per day. This dose may have been too low a dose to observe opioid induced hyperalgesia. In addition, the subjects were not opioid naïve prior to the study but taking not more than 30 mg of oral morphine equivalents. However, the study by Chu et al (2012) does contribute important findings to our understanding of the development of tolerance and hyperalgesia. To date, no study has shown the development of tolerance without hyperalgesia in subjects maintained on opioids for the treatment of opioid addiction.

1.14. Tolerance, hyperalgesia and the opioid maintained patient

There is a body of experimental clinical evidence describing the existence of chronic opioid induced hyperalgesia and tolerance in people currently addicted to opioids and former opioid addicts (Table 1).

1.14.1. Pain responses of opioid dependent subjects

Author	Subjects	Test	Results
Martin and Inglis (1965)	24 former opioid dependent subjects and 24 controls	Cold pressor pain tolerance (maximum amount of time subjects could tolerate pain)	Former opioid dependent subjects were found to be hyperalgesic compared to controls. The temperature of the cold pressor pain was 5^{O} C with a range of 4.5^{O} C to 6.5^{O} C. Former opioid dependent subjects were able to keep their arms in the water for a mean of 73 seconds compared to 404 seconds for control subjects.
Ho and Dole (1979)	10 drug-free ex-opioid dependent subjects, methadone maintained ex-opioid subjects and their 10 non-opioid dependent siblings	Cold pressor pain threshold (when subjects first felt pain) and pain tolerance	Ten subjects were tested in each group. The cold-pressor test was used. This consisted of the subject placing their hand in water of 30^{O} C and then 1^{O} C. The subjects' pain threshold (the amount of time that the subject could keep their arm in the cold water before which the subject first feels pain) and pain tolerance (the maximum amount of time that the subject could keep their arm in the cold water) were measured. Pain threshold of drug-free ex-opioid dependent subjects and methadone maintained ex-opioid subjects was significantly lower than their non-opioid dependent siblings. Absolute values not given.
Compton (1994)	26 abstinent opioid users, 43 current opioid users (methadone maintenance), 32 abstinent cocaine users and 21 current	Cold pressor pain tolerance	Subjects currently using opioids or cocaine (including methadone maintenance for opioid dependent subjects) (70±74.8 SD seconds) were not able to tolerate pain for as long as cocaine and opioid users currently abstinent (145±114 SD seconds $p<0.001$).

	cocaine users.		
Compton (2000)	60 methadone maintained subjects and 60 non opioid dependent subjects	Cold pressor pain tolerance	Methadone maintained subjects (44±61 SD seconds) were not able to tolerate as much pain as non-opioid dependent subjects (94±104 SD seconds p=0.002).
Schall (1996)	42 levomethadone maintained patients and controls	Mechanical pressure stimulation tolerance	Antinociceptive effect was observed by changes in plasma methadone concentrations. However, no significant differences between methadone maintained subjects and controls under steady state conditions Absolute values not given.
Dyer (1999)	18 methadone maintained subjects (9 non holders (experienced withdrawal symptoms between methadone dose administration))	Electrical stimulation of the earlobe. Pain threshold	There were no differences in plasma concentrations between those who reported significant withdrawal and those that did not. The mean pain threshold of all methadone subjects was significantly increased following dosing. Absolute values not given.
Doverty (2001a)	16 methadone subjects and 16 control subjects	Cold pressor and electrical stimulation of the earlobe. Pain threshold and pain tolerance	Prior to their normal daily methadone dose, in the electrical stimulation test, methadone subjects had lower pain tolerance values but not pain threshold values in comparison with control subjects. Prior to dosing, in the cold pressor test, methadone subjects had lower pain threshold and pain tolerance scores compared to controls. They were hyperalgesic. Subsequent to dosing, in the electrical stimulation test,

			methadone subjects had significantly higher values for both pain threshold and pain tolerance compared to controls. In contrast, in the cold pressor test following dosing, methadone subjects had significantly lower values in the pain tolerance measure but not the pain threshold measure in comparison to controls. Absolute values were not given

Table 1 Pain responses of opioid dependent subjects

1.14.1.1. Martin and Inglis

In 1964 Martin and Inglis examined 24 former opioid dependent female prisoners and 24 control subjects (Martin and Inglis 1965). They examined their tolerance to cold pressor pain (immersion of the subject's hand into cold water). The temperature of the cold pressor pain was 5^{O} C with a range of 4.5^{O} C to 6.5^{O} C. Former opioid dependent subjects were able to keep their arms in the water for a mean of 73 seconds compared to 404 seconds for control subjects. Compared to later studies, the nature of the cold pressor induced pain was substantially different. More recent cold pressor methods have temperatures between 0.5 and 1.5^{O} Celsius (Doverty et al. 2001a, Compton et al. 2010). Despite this, former opioid dependent subjects were similarly found to be hyperalgesic compared to a control group using the same experimental paradigm (Martin and Inglis 1965).

1.14.1.2. Ho and Dole

Ho and Dole (1979) examined drug-free ex-opioid dependent subjects, methadone maintained ex-opioid dependent subjects and their non-opioid dependent siblings. Ten subjects were tested in each group. The cold-pressor test was used. This consisted of the subject placing their hand in water of 30^{O} C and then 1^{O} C. The subjects' pain threshold (the amount of time that the subject could keep their arm in the cold water before which the subject first feels pain) and pain tolerance (the maximum amount of time that the subject could keep their arm in the cold water) were measured. Ho and Dole found that the pain threshold of the drug-free ex-opioid dependent subjects and the methadone maintained ex-opioid dependent subjects was significantly lower that their non-opioid dependent siblings (Ho and Dole 1979). In contrast, they found no significant difference in pain tolerance between the groups. Absolute values were not given.

1.14.2. Opioid dependent subjects on maintenance treatment

1.14.2.1. Compton

Compton et al (1994) examined pain tolerance in the cold pressor test of 26 abstinent opioid users, 43 current opioid users (methadone maintenance), 32 abstinent cocaine users and 21 current cocaine users. The cohort was a group of people who were using heroin and/or cocaine and seeking treatment. Compton et al (1994) found that subjects currently using opioids or cocaine (including methadone maintenance for opioid dependent subjects) (70±74.8 SD seconds) were not able to tolerate pain for as long as cocaine and opioid users currently abstinent (145±114 SD seconds $p<0.001$).

In 2000 Compton and coworkers examined 60 methadone maintained subjects and 60 non opioid dependent subjects and found that methadone subjects were significantly less tolerant in the cold pressor test than control subjects. This supported their previous work. Methadone maintained

subjects (44±61 SD seconds) were not able to tolerate as much pain as non-opioid dependent subjects (94±104 SD seconds p=0.002).

1.14.2.2. Schall

In 1996 Schall et al also examined the pain experience of methadone subjects and compared them to controls (Schall et al. 1996). The experimental nociceptive test used in this study consisted of pressure stimulation of the middle finger and the measurement of pain threshold and pain tolerance. Testing occurred prior to and 1, 2 and 4 hours after methadone dosing. An antinociceptive effect was observed from the administration of methadone. However, there were no significant differences observed between the methadone maintained and the control subjects. Absolute values were not given.

1.14.2.3. Dyer

Dyer et al (1999) examined 18 methadone subjects. Half of the methadone subjects experienced withdrawal symptoms between methadone dose administrations. These subjects were classified as 'non-holders'. The administered methadone dose did not 'hold' their methadone withdrawal symptoms for the entire 24 hours. The experimental nociceptive test was electrical stimulation of the ear lobe (electrical stimulation test).

Dyer et al (1999) confirmed the findings of Schall et al (1996). There were no differences in plasma concentrations between those who reported significant withdrawal and those that did not. The mean pain threshold of all methadone subjects was significantly increased following dosing. Pain threshold reached a peak between 1 and 2 hours after dosing and lasted approximately 6 hours after dosing. Absolute values were not given.

1.14.2.4. Doverty

Doverty et al (2001a) attempted to reconcile the disparate results found in earlier studies. They examined 16 methadone subjects and compared them to 16 control subjects. Doverty et al utilised the cold pressor test and the electrical stimulation test. Subjects were tested just before and three hours after their methadone dosing. The antinociceptive markers used were pain threshold (called pain detection by Doverty and co-workers) (when subjects first felt pain) and pain tolerance (maximum amount of pain that could be tolerated by subjects).

Prior to their normal daily methadone dose, in the electrical stimulation test, methadone subjects had lower pain tolerance values but not pain threshold values in comparison with control subjects. Prior to dosing, in the cold pressor test, methadone subjects had lower pain threshold and pain tolerance scores compared to controls. They were hyperalgesic.

Subsequent to dosing, in the electrical stimulation test, methadone subjects had significantly higher values for both pain threshold and pain tolerance compared to controls. In contrast, in the cold pressor test following dosing, methadone subjects had significantly lower values in the pain tolerance measure but not the pain threshold measure in comparison to controls.

The findings of Doverty and co-workers supported those of Ho and Dole (1979). Both groups found that, in the cold pressor test, pain threshold was lower in methadone subjects both prior to and following dosing. These findings also supported the work of Compton et al (1994) who found that methadone subjects had lower pain tolerance in the cold pressor test.

The findings of Doverty and co-workers also supported those of Dyer et al (1999). Both groups found that prior to dosing, in the electrical stimulation test there was no significant difference between methadone subjects and controls in pain threshold. Following dosing, both Doverty et al (2001a) and Dyer et al (1999) found that methadone subjects had higher pain tolerance than controls.

The pain sensations produced by electrical stimulation test (phasic stimuli) differ quantitatively, neurologically and functionally from the pain sensations derived from the cold pressor (tonic pain) test (Doverty et al. 2001a). Doverty et al (2001a), citing Chen et al (1989), suggested that phasic pain and tonic pain may be subserved by different neurophysiological pathways and therefore be differentially affected by opioids. Doverty et al (2001a) suggested that this could explain why the methadone subjects were considerably less tolerant of pain in the cold pressor test compared to the electrical stimulation test. The study demonstrated that maintenance on opioids for the treatment of opioid addiction is associated with hyperalgesia in the cold pressor test. In addition, the study highlighted the importance of using different nociceptive stimuli.

1.14.3. Other opioid maintenance and hyperalgesia

The studies described above suggest that subjects with long term exposure to methadone develop a greater sensitivity to pain. There are also several studies that suggest that subjects exposed to other opioids for a substantial period of time also develop a greater sensitivity to pain.

Compton et al (2001) examined subjects maintained on methadone and buprenorphine. They found that methadone subjects (56 seconds) and buprenorphine subjects (62 seconds) were hyperalgesic in the cold pressor test in comparison with controls (138 seconds).

Mitchell et al (2006) examined the impact of switching from methadone maintenance to slow release morphine maintenance. Subjects remained hyperalgesic on both maintenance agents.

Compton et al (2012) recently examined the pain experiences of opioid dependent individuals entering treatment. The subjects were examined prior to opioid maintenance treatment,

once they had been stabilised and when they had been dosing chronically on methadone and buprenorphine. Compton et al (2012) found that subjects chosen to be selected to be inducted onto methadone (16±6 SD seconds) and subjects selected to be inducted onto buprenorphine (23±16 SD seconds) were hyperalgesic compared to controls (42±31SD seconds p=0.01) in the cold pressor test.

1.14.4. Opioid abstinence and the restoration of normal pain sensitivity

Pud and co-workers (2006) examined whether abstinence from opioids following a period of addiction could 'reset' pain sensitivity. The study group compared the pain sensitivity of 60 opioid addicted subjects entering a 28 day detoxification program with 70 healthy controls. The opioid addicted subjects (opioid subjects) consisted of those subjects who presented a positive opioid urine result at entry to the program and had a past history of either heroin or methadone abuse. The following measurements were made: pain threshold time, pain tolerance time and resulting pain intensity from the cold pressor test (using a visual analogue scale).

In comparison with control subjects, opioid subjects had longer pain threshold times (11±8 SD seconds) compared to controls (7±4 SD seconds $p<0.0001$)), shorter pain tolerance times (opioid subjects 29±37 SD seconds compared to control subjects 56±51 SD $P=0.001$) and lower VAS pain intensity scores (opioid subjects 57±21 SD compared to control subjects 73±16 SD $P<0.0001$). The authors stated that the reason for the disagreement with previous studies is not clear. They suggest it may be related to methodological differences between the trials. At the end of 28 days opioid subjects experienced no significant improvement in pain sensitivity (Pud et al. 2006). It should be noted that pain tolerance times for opioid subjects were significantly shorter than for control subjects.

Several of the studies above suggest that even with abstinence from opioids, subjects who have developed hyperalgesia with chronic opioid use remain hyperalgesic (Martin and Inglis 1965, Ho and Dole 1979, Pud et al. 2006). However, other studies have found that with abstinence, pain sensitivity may return to levels comparable with healthy control subjects. These are the studies of Liebmann and co-workers (Liebman et al. 1994, Liebmann et al. 1997) (The surname of Leibman P.M. is spelt with one 'n' in the 1994 letter to the Lancet and with two 'n' in the 1997 study published in Biology Psychiatry)

Liebmann and co-workers examined former opioid addicts who had undergone opioid detoxification with the cold pressor test using the markers of pain threshold and pain tolerance (Liebmann et al. 1994). The former opioid addicts were defined as a cohort of subjects who had not used opioid for at least a month. They had a mean abstinence period of 8±5 months. Liebmann and co-workers found that while there was no difference in pain tolerance times (amount of time

subjects were able to tolerate pain) between the two groups, the former opioid addicts had significantly longer pain threshold times (time at which they could first feel pain) (Liebmann et al. 1994).

Liebmann and co-workers found similar results in a study published in 1997 (Liebmann et al. 1997). They examined the effect of naltrexone on a group of detoxified opioid users undergoing rehabilitation and compared them to a group of drug-free subjects undergoing the cold pressor test. They found that the ex-opioid users had significantly increased pain thresholds independent of the administration of naltrexone (Liebmann et al. 1997).

A limitation of the work of Liebmann et al (1997) is that absolute values were not supplied as part of the results. The study states that pain sensitivity was determined with a cold pressor test and that times were recorded at first pain sensation (threshold) and when pain was no longer tolerable (tolerance). Time intervals were not given. 'Pain and tolerance threshold levels were normalized by logarithmisation' (Liebmann et al. 1997). Log pain thresholds of controls given were 1.4±0.3 (n=31), ex-addicts placebo were 1.6±0.2 (n=31) and ex-addicts following naltrexone was 1.6±0.2 (n=29) (Liebmann et al. 1997).

It is not easy to reconcile the findings of Liebmann et al (1997) with other pain management research in the opioid maintained population. It may be argued that Liebmann et al (1997) were studying a population of opioid maintained patients that was not representative of the whole opioid maintained population in some way. Perhaps the fact that this subgroup was successful in residential treatment may have some bearing on the results. In addition, Liebmann et al (1997) utilized a variation on the cold pressor test. Doverty et al (2001a) and Athanasos et al (2006) utilized a cold pressor test with the temperature of 0.5 to 1.5^{O} C. Liebmann et al (1997) utilized a cold pressor test with a water bath temperature of 4 to 6^{O} C. Nonetheless, the findings of Liebmann et al (1997) with respect to opioid induced hyperalgesia in former opioid addicts should be noted as an important exception to other findings in this area.

Most recently, Treister et al (2012), in an observational study, examined the pain experience of male subjects in the cold pressor test. These were active opioid addicts on heroin or methadone (n=50), former opioid addicts (at least 5 months of abstinence from drug use) (n=43) and healthy controls (n=50). They found significant differences in pain tolerance between the groups. Opioid addicts had mean pain tolerance of 30 seconds, former opioid addicts 64 seconds and healthy controls 56 seconds. They suggested that abstinence from opioids for at least 5 months 'reset' pain sensitivity and suggested that hyperalgesia may be a reversible phenomenon. The research group included D. Pud who was lead author of the 2006 study which showed that pain

sensitivity did not return after 28 days abstinence. The work of Treister et al (2012) provided support for the work of Liebmann (Leibman) et al (1994).

The majority of these studies found that the process of hyperalgesia accompanied maintenance on opioids in human subjects. The mechanisms purported to produce the hyperalgesia and tolerance found in opioid tolerant subjects are many and varied. Opioid effect occurs when the binding of an agonist to a G-protein coupled opioid receptor leads to the activation of K^+ channels and inhibition of voltage gated Ca^{2+} channels. This binding also results in inhibition of adenylyl cyclase and the cAMP-protein phosphorylation cascade. Electrical excitability is inhibited and there is a decrease in neurotransmitter release (Connor and Christie 1999). The following section describes two examples of the variety of mechanisms postulated to produce hyperalgesia and tolerance.

1.15. Cellular and synaptic adaptations following chronic opioid use

1.15.1. NMDA receptor cascade

Work by Mayer, Mao and others (Mao et al. 1995, Mao 1999, Mayer et al. 1999, Chang et al. 2007) suggests that both chronic pain and chronic administration of morphine result in the activation of the NMDA receptor and the initiation of a series of intracellular cascades including protein kinase C translocation and activation, nitric oxide (NO) production and NO-activated poly (ADP ribose) synthetase (PARS) activation. It is also postulated that excessive PARS activity induces the programmed cell death of inhibitory interneurons (dark neurons) in the superficial laminae of the spinal cord dorsal horn. These actions culminate in a neuropathic-like hyperalgesic state and morphine tolerance.

The postulated sequence is as follows. Chronic pain or chronic morphine administration causes a direct NMDA-receptor activation. The increase of Ca^{2+} influx into the neurons causes a second messenger sequence of events. This causes firstly, the translocation/activation of protein kinase C (PKC) from cytosol to cell membrane. Secondly, there is increased intracellular nitric oxide production and NO-activated poly (ADP ribose) synthetase (PARS) activation. Thirdly, mu-opioid receptor hypo-responsiveness develops.

1.15.2. Spinal cord glial cells

Traditionally, models of hyperalgesia have focused almost exclusively on the roles taken by neurons. Over the past fifteen years evidence has been accruing that the immune cells of the central nervous system, glia, are key players in pain facilitation and may contribute importantly to the development of opioid tolerance.

Glia (astrocytes and microglia) have an important role in the central nervous system as structural supports for neurons and for maintaining central nervous system homeostasis. They provide neurochemical precursors and energy sources to neurons, regulate extracellular ion concentrations and remove debris among many other functions (Watkins et al. 2007).

It has also been demonstrated that the administration of opioids activates glial cells and that this glial activation plays an important role in compromising the ability of opioids to suppress pain. In particular, repeated morphine administration induces the release of the proinflammatory cytokine interleukin-1β (Hutchinson 2004). It has been argued that this action contributes to the development of morphine induced hyperalgesia (Beilin et al. 2003, Johnstone et al. 2004). A number of studies have shown that inhibiting glial activation enhances the analgesic effects of acute and chronic morphine. These include the administration of minocycline (Song and Zhao 2001, Ge et al. 2007), administration of inhibitors of proinflammatory cytokines (Beilin et al. 2003, Johnstone et al. 2004) and disruption of the signalling of the proinflammatory cytokines on a genetic level (Beilin et al. 2003). In contrast, it has also been shown that direct activation of glial cells decreases the ability of morphine to suppress pain (Raghavendra et al. 2003, Hutchinson 2004, Johnstone et al. 2004).

There is evidence to suggest that glial based strategies may be developed in the future to potentiate morphine analgesia, decrease hyperalgesia and modify opioid dependence and withdrawal (Watkins et al. 2007).

1.15.3. Strategies to overcome hyperalgesia and tolerance

In a second paper published in 2001, Doverty and co-workers examined the antinociceptive effects of clinically used intravenous morphine doses in methadone subjects (14.8 mg morphine) and control subjects (11.95 mg) using the cold pressor and electrical stimulation tests (2001b). The aim of the second study was to examine whether higher plasma morphine doses would provide antinociception in the cold pressor test compared to lower doses administered to controls. In spite of significantly greater plasma morphine concentrations, methadone subjects experienced minimal antinociception in the cold pressor test compared with control subjects. The study also confirmed that methadone subjects were hyperalgesic in the cold pressor but not electrical stimulation test.

1.15.3.1. Recent work

Compton et al (2008, 2010) have examined two other pharmacological interventions for the provision of antinociception to methadone maintained subjects. These are dextromethorphan and gabapentin.

Dextromethorphan is an antagonist at the excitatory ionotropic N-methyl-D-aspartate (NMDA) receptor on dorsal horn neurons. These receptors have been implicated in the

development of opioid induced hyperalgesia (see section previously on the NMDA receptor cascade) (Mao et al. 1995, Mao 1999, Mayer et al. 1999, Chang et al. 2007).

Compton et al suggested that as the potent NMDA receptor antagonist ketamine was shown to counteract opioid induced hyperalgesia in animals, the 'well-tolerated but weaker' NMDA receptor antagonist dextromethorphan was chosen for evaluation in the clinical setting (Compton et al. 2008).

In 2008 Compton et al examined the effect of chronic dextromethorphan administration (titrated to 480 mg per day) on both cold pressor and electrical stimulation evoked pain (Compton et al. 2008). The authors did not find that dextromethorphan, chronically administered, provided antinociception in either test. The finding did suggest that with larger numbers of subjects, an effect with women may become significant. Unexpectedly, this effect would be negative. Women would experience a decrease in pain tolerance. There was no control group and therefore hyperalgesia was not established, although the average cold pressor scores were consistent with previous work published in this area (Doverty et al. 2001a, Doverty et al. 2001b, Athanasos et al. 2006).

Gabapentin is a gamma-aminobutyric acid (GABA) agonist anticonvulsant. Van Elstraete and others have shown that fentanyl-induced hyperalgesia can be prevented in rats by the administration of gabapentin in a dose dependent manner (Van Elstraete et al. 2008). Other authors have suggested that preoperative gabapentin administration results in decreased post-operative opioid requirements and that gabapentin (and pregabalin) has antihyperalgesic properties (Tiippana et al. 2007).

In 2010 Compton et al examined the effect of gabapentin (titrated to 2400 mg/day) on cold pressor responses in methadone maintained subjects (2010). Gabapentin and pregabalin are similar in structure (Sills 2006). They appear to interact with the alpha 2-delta subunit of the presynaptic N-type voltage-dependent calcium channels located in the peripheral and central nervous systems (Sills 2006, Durkin et al. 2010). They are one of the classes of drugs used as opioid adjuvants for postoperative pain and may provide antinociception in people maintained on opioids for the treatment of opioid dependency (Compton et al. 2010, Weinbroum 2012).

The Compton et al (2010) gabapentin study in methadone maintained patients was similar in one important aspect to the Compton (2008) dextromethorphan study in methadone maintained patients. There was no control group in each and therefore hyperalgesia was not established. However, the average cold pressor scores of the methadone maintained subjects were consistent with the findings of hyperalgesia in previous studies (Doverty et al. 2001a, Doverty et al. 2001b, Athanasos et al. 2006). There was significant improvement in both cold pressor threshold and tolerance found with this pharmacological intervention in the study. A major limitation of this

study is that the absolute improvement was small, in the range of 2-3 seconds. At baseline pain responses ranged between 7-21 seconds. Since there was no control group, it is not possible to ascertain what the normal value was. A second limitation is that of the cohort. The subjects that showed a significant change in response were those who were able to remain abstinent from illicit drug use over the course of the five week study. Such a cohort may not be representative of the majority of people maintained on opioids for the treatment of drug dependence. Nonetheless, the study suggested that gabapentin, in clinically tolerated doses, might be a useful adjuvant for the significant number of methadone clients who experience acute severe pain.

1.16. Adjuvant analgesia pharmacology

Morphine is an important pharmacological intervention in the provision of acute pain management. It is also the opioid to which many other opioids are compared for discussion of pharmacological and pharmacokinetic effects. As stated above, Doverty et al (2001b) examined the antinociceptive effects of clinically used intravenous morphine doses (11.95) in methadone and control subjects. There were limited antinociceptive and respiratory depressant effects from these doses. High doses of morphine, in contrast, might provide antinociception either alone or in combination with commonly used opioid adjuvants in the methadone and buprenorphine maintained population.

Other opioids rather than morphine such as oxycodone or fentanyl may provide antinociception in this population. A recent review failed to find evidence to support morphine as the drug of choice for treating severe chronic pain (Bekkering et al. 2011). However, morphine remains the drug of choice for physicians managing severe chronic pain and the reference against which strategic decisions in pain therapy are made (Wiffen and McQuay 2007, Bekkering et al. 2011). For this reason high dose morphine, with or without opioid adjuvants, was examined in the series of studies that comprise this thesis, for its antinociceptive properties in the opioid maintained population. The pharmacodynamics and pharmacokinetics of morphine have been discussed in sections describing opioid pharmacology.

Other important analgesics used alone and in combination with morphine include N-methyl-d-aspartate (NMDA) receptor antagonists i.e. ketamine (Elvir-Lazo and White 2010), non-steroidal anti-inflammatory drugs (NSAIDS) (e.g. ketorolac) (Gwirtz et al. 1995) and mixed action drugs (e.g. tramadol) (Webb et al. 2002, Kocabas et al. 2005). It is becoming increasingly popular to use a combination of opioid and non-opioid analgesics that act at different sites within the central and peripheral nervous systems as a means to improve pain control and decrease opioid related side effects (Elvir-Lazo and White 2010).

Some authors contend that NSAIDS and paracetamol are the foundations of multimodal analgesia and are well established as the standard of care for acute postoperative pain (Pergolizzi and Will 2006, Christensen et al. 2011). As stated, one of the goals of multimodal analgesia is to reduce opioid requirements so as to reduce opioid side effects. Paracetamol, on average, reduces postoperative opioid consumption by 20% and is not considered as effective as other opioid adjuvants (Elia et al. 2005, Remy et al. 2005, Christensen et al. 2011). Ketorolac, is a frequently used adjunct in multimodal postoperative regimens and there is strong evidence for its effectiveness (De Oliveira et al. 2012, White et al. 2012). The use of NSAIDs and opioids concurrently is common practice. Cepeda et al (2005) examined over one thousand patients following surgery and found that adding NSAIDs to the opioid treatment reduced morphine requirements and opioid-related side effects. In addition, ketorolac has been shown to decrease both opioid dependence and withdrawal in animal studies (Trang et al. 2002).

It has been hypothesised that ketamine, when added to opioids, may block the NMDA receptors, prevent the development of tolerance and have an opioid sparing effect. The effectiveness of combining ketamine and opioids has been well demonstrated in animal models (Celerier et al. 2000, Kissin et al. 2000, Laulin et al. 2002, Rivat et al. 2002). Subramaniam et al (2004) performed a systematic review of randomized, double blind clinical trials of ketamine added to opioid analgesia. Both systemic and epidural ketamine were shown to have beneficial opioid sparing effects. In addition, ketamine has been shown to provide superior pain relief in opioid tolerant patients (Bell 1999, Eilers et al. 2001, Sator-Katzenschlager et al. 2001, Haller et al. 2002, Mitra and Sinatra 2004, Bell et al. 2005, Loftus et al. 2010, Laskowski et al. 2011, Weinbroum 2012).

In 2007 Marinangeli et al, in a prospective study, examined the addition of tramadol to transdermal fentanyl in 70 patients with intractable cancer (Marinangeli et al. 2007). The addition of tramadol allowed for a more gradual increase of analgesic delivery than was possible by using fentanyl alone. Fentanyl dose escalation was slowed. Webb et al (2002) examined the effect of adding tramadol to morphine for patient controlled analgesia in 69 patients following abdominal surgery. They found that the addition of tramadol improved analgesia and reduced morphine requirements. Unlugenc et al (2009) found the combination of tramadol and remifentanil to be effective in postoperative pain management.

As mentioned in the previous section, another group of opioid adjuncts are the gabapentanoids, gabapentin and pregabalin. The authors of a study and a recent review of gabapentanoids were cautious in expressing definitive conclusions about the efficacy of gabapentin and pregabalin for postoperative pain (Clarke et al. 2009, Dauri et al. 2009). Gabapentanoids are more effective than placebo in pain reduction and opioid sparing but evidence was lacking according to these authors

about the analgesic potentials of gabapentanoids in comparison with other standard postoperative regimens (Dauri et al. 2009, Weinbroum 2012). Compton (2010) found some effect with gabapentin titrated to 2400 mg per day in methadone maintained subjects but this effect was limited.

The studies described in chapters 3 and 4 will examine the antinociceptive effect of opioid adjuvants ketorolac, S-ketamine and tramadol alone, and in combination with high doses of morphine in methadone and buprenorphine maintained subjects. One or more of these drugs may be effective alone, or in combination with morphine in the provision of pain relief to this population. The following section will examine the pharmacological characteristics of these opioid adjuvants.

1.16.1. S (+) –ketamine pharmacology

Ketamine is a congener of phencyclidine (known colloquially as PCP or Angel Dust). It is used in anaesthesia and has antinociceptive properties. The mechanism most important and most frequently studied of ketamine is NMDA receptor antagonism (Petrenko et al. 2003, Niesters et al. 2011). It is generally available as a racemate but the S (+) -isomer (S-isomer) is three to four times more potent with fewer side-effects (White et al. 1982, Trevor and White 2007). The increased potency is attributable to the higher affinity of the S-isomer to the phencyclidine binding sites on the NMDA receptors (Sinner and Graf 2008). Ketamine is both water and lipid soluble (Sinner and Graf 2008). This property allows it to be administered conveniently via various routes and provides extensive distribution throughout the body.

1.16.1.1. S (+) -ketamine pharmacodynamics

Ketamine causes bronchodilation and stimulation of the sympathetic nervous system and cardiovascular system. Ketamine and particularly S-ketamine are used for premedication, sedation and induction, and maintenance of general anaesthesia. Ketamine produces a hypnotic state quite distinct from that of other anaesthetics. Patients have profound analgesia, unresponsiveness to commands, and amnesia, but may have their eyes open, move their limbs involuntarily and breathe spontaneously. The term dissociative anaesthesia has been given to this cataleptic state (Evers et al. 2006, Sinner and Graf 2008).

While subanaesthetic doses of ketamine have analgesic effects, psychotomimetic side-effects may still be present. Low-dose or subtherapeutic ketamine acts like an analgesic and is used in the treatment of pain following surgery and for chronic pain that is caused by peripheral and central sensitization (Petrenko et al. 2003, Sigtermans et al. 2009). Clinically, low-dose ketamine has been shown to enhance the acute antinociceptive effect of opioids and thereby reduce opioid use and opioid related side-effects (Schmid et al. 1999, Nesher et al. 2008, Zakine et al. 2008,

Sigtermans et al. 2009). The NMDA receptor antagonism is considered to be most important for its analgesic activity. However there is reported to be some weak agonism at the mu opioid receptors (Kalsi 2010).

1.16.1.2. S (+) -ketamine pharmacokinetics

CYP3A4 is the principal enzyme responsible for ketamine N-demethylation to nor-ketamine in human liver microsomes. CYP2B6 and CYP2C9 have a contribution to ketamine N-demethylation at therapeutic concentrations of the drug (Hijazi and Boulieu 2002). Ketamine is metabolised to norketamine, which has reduced CNS activity but has activity of about one third that of ketamine. Norketamine is further metabolised and excreted in urine and bile (Chang et al. 2007).

The mean half-life of racemic ketamine administered intravenously has been estimated as 2.1 hours (healthy volunteers) (Yanagihara et al. 2003) 3.1 hours (healthy volunteers) (Clements et al. 1982), 4.9 hours (intensive care patients) (Hijazi and Boulieu 2002) and 5.2 hours (chronic neuropathic pain patients) (Chong et al. 2009). Median clearance of racemic ketamine administered intravenously is between 0.9 L/h/kg (chronic neuropathic pain patients) (Chong et al. 2009), 1.1-1..2 L/h/kg (healthy volunteers) (Yanagihara et al. 2003) and 2.2 L/h/kg (intensive care patients) (Hijazi and Boulieu 2002).

1.16.2. Tramadol pharmacology

Tramadol hydrochloride is a synthetic opioid analgesic that has two chiral centres. The marketed drug is the racemate of the trans isomers. (Raffa et al. 1992, Ardakani and Rouini 2007)

1.16.2.1. Tramadol pharmacodynamics

Tramadol is both an opioid agonist with selectivity for the mu-receptor and an inhibitor of monoamine neurotransmitter (noradrenaline and serotonin) reuptake. The (+) enantiomer and the metabolite (+)-0-desmethyl-tramadol (M1) are agonists of the mu opioid receptor (Grond and Sablotzki 2004). Tramadol possesses only a modest affinity for mu opioid receptors and no affinity for delta or kappa receptors (Raffa et al. 1993, Grond and Sablotzki 2004). From in vitro studies, the metabolite M1 binds with about 300 fold higher affinity than the parent compound, but about one tenth lower than morphine (Hennies et al. 1988, Frink et al. 1996, Grond and Sablotzki 2004).

(+)-Tramadol inhibits serotonin reuptake and (-)-tramadol inhibits noradrenaline reuptake. This combination of effects enhances the inhibitory effects on pain transmission in the spinal cord. The complementary and synergistic inhibitory actions of the two enantiomers improve the analgesic efficacy and tolerability profile of the racemate. Common side effects of tramadol include nausea, vomiting, dizziness, dry mouth, sedation and headache. The degree of constipation is less than that seen after equivalent doses of codeine (Duthie 1998).

1.16.2.2. Tramadol pharmacokinetics

Tramadol is rapidly and almost completely absorbed after oral administration. As a result of first pass hepatic metabolism, its absolute bioavailability is 65 to 75% (Gibson 1996). Tramadol is rapidly distributed in the body and plasma protein binding is approximately 20%. The principal metabolic pathways, O and N-demethylation, involve cytochrome P-450 enzymes 2D6, 2B6 and 3A4. The O-demethylation of tramadol to M1, the main analgesic metabolite, is catalysed by cytochrome P4502D6. The N-desmethylation of tramadol to M2 is catalysed by CYP2B6 and 3A4 (Grond and Sablotzki 2004).

The primary metabolites O-desmethyltramadol (M1) and N-desmethyltramadol (M2) may be further metabolised to secondary metabolites namely, N,N-didesmethyltramadol (M3) and N,N,O-tridesmethyltramadol (M5). In phase 2 metabolism, the O-demethylated metabolites are conjugated with glucuronic acid and sulphate before excretion into urine. Approximately 10-30% of the parent drug is excreted unchanged in the urine. (Grond and Sablotzki 2004).

The mean elimination half-life has been calculated at approximately 5-6 hours (Raffa et al. 1995, Lintz et al. 1998, Lintz et al. 1998, Lintz et al. 1999, Lintz et al. 2000). Intravenous mean total clearance has been calculated at 467 mL per minute (Lintz et al. 1998).

1.16.3. Ketorolac pharmacology

Non-steroidal anti-inflammatory drugs (NSAIDS) are among the most commonly prescribed medications. NSAIDS can be classified as cyclooxygenase (COX) 1/2 inhibitors and selective COX-2 inhibitors (coxibs). Ketorolac is a chiral NSAID marketed as the racemic mixture and is a member of the group of COX 1/2 inhibitors. The major mechanism by which ketorolac and other NSAIDs exert their pharmacological effects is inhibition of prostaglandin synthesis by sterically hindering the entrance of arachidonic acid (Limongelli et al. 2010). NSAIDs are most active in the periphery to produce their effects (Gillis and Brogden 1997). The anti-inflammatory activity of the levorotatory isomer of the drug is twice that of the dextrorotatory isomer. Most of ketorolac's analgesic and COX inhibitory activity is retained within the S-isomer (Sinha et al. 2009). Ketorolac is a moderately effective anti-inflammatory drug and analgesic. It is one of the few NSAIDS available parenterally (Burke et al. 2006).

1.16.3.1. Ketorolac pharmacodynamics

Ketorolac has both systemic analgesic and anti-inflammatory activity. It also inhibits platelet aggregation. Common adverse effects include gastrointestinal disturbances (including gastrointestinal bleeding), perforation of the stomach and peptic ulceration. The gastrointestinal bleeding is particularly a problem with elderly patients (Sinha et al. 2009). It may also produce pain at the injection site, sweating and purpura (Rossi 2012).

1.16.3.2. Ketorolac pharmacokinetics

Ketorolac has an onset of action of 30 to 60 minutes after oral administration. The oral bioavailability of ketorolac is about 80 to 100% (Gillis and Brogden 1997). Over 99% of drug is bound to proteins. It has a half-life of 4 to 6 hours and more than 90% of the drug is metabolised to the glucuronide conjugate (Burke et al. 2006).

1.17. History of pain management guidelines in opioid tolerant patients

One of the central questions in this thesis focuses on the means by which it would be possible to provide relief from severe acute pain to people maintained on opioids for the treatment of opioid addiction. The findings may have implications for the treatment of acute pain in people maintained on opioids for the treatment of chronic pain and as substitution treatment for addiction.

The history of pain management guidelines in opioid dependent people is supported by little scientific evidence. In general, the guidelines are informed by expert opinion. Some are based on retrospective studies. They are often inconsistent and are outlined below.

Opioid maintenance for the treatment of opioid dependency was initially practised in the first two decades of the twentieth century. This therapeutic approach ceased in 1922 in the United States with the rise of Prohibition and the arrest of physicians and pharmacists who provided drugs for opioid maintenance (White 1998). In 1964 Dole and Nyswander resumed the practice by placing six heroin dependent patients on daily doses of methadone and the modern age of opioid maintenance pharmacotherapy was born (Lowinson et al. 2003). Dole has commented on the treatment of acute severe pain in opioid maintained patients and suggested that the analgesic use of opioids in this situation would have detrimental outcomes for their opioid addiction treatment (Dole et al. 1966). A quandary existed. If opioids are the preferred treatment for acute severe pain, but according to Dole et al (1966), were contraindicated in the opioid maintained population, then an alternative treatment was required. Many modern researchers such as Alford et al (2006) would not agree with Dole et al (1966). Unfortunately, as stated earlier, the guidelines for the treatment of acute severe pain in opioid maintained patients have often been inconsistent.

1.17.1. Conventional doses of analgesics

1.17.1.1. Cushman (1972), Rubenstein (1976)

Cushman (1972), on the basis of a review of 5 sets of case notes, suggested that continued administration of regular daily methadone doses may complicate patient recovery from major surgery (the reasons why continued administration may complicate recovery was not stated). As a result of these concerns, he suggested methadone should be tapered pre-operatively and discontinued during the first few post-operative days. During this interval analgesic needs and

avoidance of the withdrawal syndrome would be met by frequent use of conventional doses of analgesics. Following this, methadone therapy should be resumed by gradually increasing doses to full maintenance doses.

Rubenstein (1976) reviewed the case notes of 100 methadone patients admitted for surgery. He found that normal doses of analgesic (i.e. meperidine) in addition to their regular daily doses of methadone provided satisfactory postoperative analgesia.

1.17.1.2. Kantor (1980), Portenoy and Payne (1997)

Kantor et al (1980) reviewed the analgesic requirements of 25 methadone maintenance patients hospitalised for surgical procedures and traumatic episodes and compared them to 25 matched methadone clients who had not been hospitalised. His findings supported those of Rubenstein and colleagues (1976) and concluded that methadone patients required 'normal' doses of analgesics in addition to their regular maintenance dose. He also found that 20 months after surgery, there were no differences in maintenance doses from when they were first hospitalised. He concluded, in contrast to Dole and co-workers (1966), that treating methadone patients with opioids for acute severe pain did not exacerbate their opioid addiction.

Portenoy and Payne (1997) suggested that while morphine has an average duration of 3 to 4 hours in healthy subjects, it is likely to have an average duration of 1-2 hours in opioid tolerant patients and in consequence dosages should be adjusted accordingly.

1.17.2. Additional methadone approaches

1.17.2.1. Rogers (1989), Schulz (1997) and Savage (1998)

Rogers (1989), Schulz (1997), Savage (1998), Scimeca et al (2000) and Manfredi et al (2001) all made recommendations regarding the utilisation of methadone for pain relief in methadone maintained patients.

Rogers (1989), on the basis of one retrospective case study, suggested that methadone patients responded better to methadone. Schulz (1997) stated that increasing the methadone dose would not be effective in methadone maintained patients. A different opioid would be more effective. Savage (1998) had concerns similar to Dole et al (1966) and stated that use of the same drug for pain relief that was used to treat addiction may complicate both pain treatment and addiction treatment.

1.17.2.2. Scimeca et al (2000) and Manfredi et al (2001)

Scimeca et al (2000) suggested that, as a consequence of the slow onset of analgesic action of methadone, utilisation of this opioid for pain relief may be difficult to titrate. Manfredi et al (2001) examined 5 case studies of patients maintained on methadone for the treatment of cancer

pain. They found that additional methadone 3 to 4 times a day was better than hydromorphone, morphine or fentanyl.

The work of Rogers (1989), Schulz (1997), Savage (1998) Scimeca et al (2000) and Manfredi et al (2001) were mostly based on small scale case studies or expert opinion. They were limited in their methodology. There have been only two major case study reviews of the analgesic requirements of opioid tolerant patients following surgery. These are De Leon-Casasola et al (1993) and Rapp et al (1995).

1.17.3. Large retrospective case studies

1.17.3.1. De Leon-Casasola (1993)

De Leon-Casasola and colleagues (1993) reviewed the casenotes of 116 patients following surgery. There were 99 patients in the opioid naïve group and 17 patients in the opioid using group. The patients in the opioid using group had been using more than 50 mg of oral morphine daily for at least 3 months. Their daily morphine use ranged between 90 and 360 mg per day with a mean of 183 mg. They all received bupivacaine (0.1%) and morphine (0.01%) epidural anaesthesia. They found that the opioid using group following surgery required a mean total of 137 mg bupivacaine compared to 44 mg for the non-opioid using group. They also used a mean total of 48 mg of morphine compared to 10 mg for the non-opioid using group.

1.17.3.2. Rapp (1995)

Rapp and co-workers (1995) examined the post-operative opioid requirements of 202 patients who used opioids pre-operatively for pain or addiction treatment. They compared their requirements with 180 non-opioid tolerant control patients. They found that the patients who used opioids pre-operatively had significantly higher pain scores and needed 3 to 4 times the amount of opioid analgesics than the opioid naïve group. This corroborated the work of de Leon-Casasola.

1.17.4. Smaller studies

Since these studies were published, there have been a number of other smaller scale studies that have examined the post-operative requirements of patients with prior exposure to opioids. Patanwala et al (2008) compared opioid requirements in 9 opioid-tolerant and 20 opioid naïve patients after total knee arthroplasty. Opioid consumption (in intravenous morphine equivalents) was significantly greater in the opioid tolerant group (56 mg, P=0.0013) than the opioid-naïve group (8 mg) during the first 24 hours after discharge from the post anaesthesia care unit (PACU).

Urban et al (2008) examined the use of ketamine as an adjunct in 26 opioid tolerant patients after spinal fusions. They did not use a control group. Chazan et al (2008) also examined the use of ketamine for acute and subacute pain in 8 opioid tolerant patients with no control group. Davis et al (2003) presented a case study of a single patient challenged with a fentanyl infusion

prior to surgery. Davis et al (2005) also examined 20 opioid tolerant patients having elective multilevel spinal fusion and challenged them with fentanyl infusion until their respiration rate was less than 5 breaths per minute. Pharmacokinetic simulation was used to individualize the administration of analgesics. There was no control group in this study. As a result of the initial loading dose of fentanyl for the purposes of the fentanyl challenge, they suggested they were not able to calculate the intraoperative and postoperative fentanyl requirements with any clinical relevance.

De Leon Casasola and Lema (1994) examined the effect of bupivacaine/sufentanil therapy for post-operative pain control in 20 chronic cancer pain patient who all used large doses of opioids and were unresponsive to epidural bupivacaine/morphine. Belgrade and Hall (2010) presented a case series of 11hospitalised patients with opioid induced hyperalgesia who received dexmedetomidine to improve pain control and lower opioid doses while avoiding opioid withdrawal.

Two studies have found that patients with prior opioid exposure have not required more opioids perioperatively. Fanning et al (2012) examined 31 children with previous continuous opiate exposure for 10 or more days followed by weaning and without signs of withdrawal for at least 72 hours prior to the surgical procedure. This group was compared to a control group of 31 age and case matched opiate-naïve patients who underwent a surgical procedure during a similar time frame as the study patients. They found that perioperative opiate requirements in opiate exposed patients were not significantly different form opiate naïve patients. They concluded that special pain management for future procedures was not warranted with this group of paediatric patients who were successfully weaned after prolonged opiate use.

Hoflich et al (2011) investigated 40 deliveries of 37 opioid dependent women and compared them to a non-dependent comparison group of 80 pregnant women in a double-blind double-dummy randomized controlled trial. The purpose of the study was to examine the safety and efficacy of methadone (mean dose at time of delivery of 64 mg daily) and buprenorphine (mean dose at time of delivery of 14 mg daily). Following caesarean delivery opioid maintained women received significantly less opioid analgesics and received non-steroidal anti-inflammatory drugs more frequently than the healthy control group. The authors concluded that the differences might be at least partly due to the psychosocial consequences of opioid addiction and the lack of an interdisciplinary consensus on pain treatment protocols for opioid dependent patients.

A general finding is that opioid tolerant people require greater doses of opioids than healthy controls to manage acute severe pain. Some reviewers have suggested that very high doses of morphine or fentanyl will overcome opioid tolerance (Macintyre 2005). A number of reviewers

have suggested that one pain management strategy that may be effective in this population is the use of non-opioid agents alone or in combination with opioids (Carroll et al. 2004, Mitra and Sinatra 2004). Such approaches are used routinely for post-surgical management of pain in this population (Macintyre 2005, Richebe and Beaulieu 2009).

1.18. Summary

Opioids are used in the short term to treat moderate to severe acute pain. They can also be used for prolonged periods of time to manage chronic pain, and to suppress withdrawal symptoms in the treatment of opioid addiction. The two most commonly used maintenance agents in the treatment of opioid addiction are methadone and buprenorphine. In addition to analgesic actions and suppression of withdrawal symptoms, studies have shown that long term opioid administration can produce hyperalgesia and opioid tolerance. This presents difficulties in the management of moderate to severe acute pain in the opioid maintained population.

The situation is complicated by a lack of evidence based pain management guidelines for the methadone and buprenorphine maintained population. Some guidelines suggest that high doses of intravenous morphine alone or in combination with opioid adjuvants may provide antinociception in this population (Mitra and Sinatra 2004, Macintyre et al. 2010). The aim of these studies is to provide evidence that will support the development of effective guidelines to manage the treatment of acute pain in the opioid tolerant population.

1.19. Hypotheses

The overall hypothesis is that subjects maintained on methadone and buprenorphine need a higher therapeutic plasma concentration range of morphine with or without the addition of opioid adjuvants (ketorolac or S-ketamine or tramadol) for acute experimental antinociception in comparison with a group of non-opioid tolerant healthy controls.

Four subsidiary hypotheses follow.

1.19.1. Study 1

Subjects maintained on methadone need a higher therapeutic plasma concentration range of morphine for acute experimental antinociception in comparison with a group of non-opioid tolerant healthy controls.

1.19.2. Study 2

Subjects maintained on buprenorphine need a higher therapeutic plasma concentration range of morphine for acute experimental antinociception in comparison with a group of non-opioid tolerant healthy controls.

1.19.3. Study 3

Subjects maintained on methadone, and for whom high therapeutic plasma concentration ranges of morphine are not effective in providing antinociception, need a higher therapeutic plasma concentration range of morphine combined with opioid adjuvants (ketorolac, tramadol or S-ketamine) for acute experimental antinociception in comparison with those obtained from a group of healthy tolerant controls.

1.19.4. Study 4

Subjects maintained on buprenorphine, and for whom high therapeutic plasma concentration ranges of morphine are not effective in providing antinociception, need a higher therapeutic plasma concentration range of morphine combined with opioid adjuvants (ketorolac, tramadol or S-ketamine) for acute experimental antinociception in comparison with those obtained from a group of non-opioid tolerant healthy controls.

2. Methodology and subjects

2.1. Introduction and study design

The four studies described in this thesis examined the antinociceptive and respiratory effects of high dose morphine and adjuvant analgesics alone, and in combination, in subjects maintained on methadone (Methadone Maintenance Treatment) (MMT) and buprenorphine (Buprenorphine Maintenance Treatment) (BMT) for the purposes of treatment of opioid addiction, and in healthy control subjects.

Study 1 (methadone morphine study) examined the effects of high dose morphine in methadone maintained subjects (methadone subjects).

Study 2 (buprenorphine morphine study) examined the effects of high dose morphine in buprenorphine maintained subjects (buprenorphine subjects).

Study 3 (methadone adjuvant study) examined the effects of adjuvant analgesics (S-ketamine, tramadol, or ketorolac) alone and in combination with high dose morphine in methadone subjects.

Study 4 (buprenorphine adjuvant study) examined the effects of adjuvant analgesics (S-ketamine, tramadol, or ketorolac) alone and in combination with high dose morphine in buprenorphine subjects.

Each study compared the opioid tolerant group against a group of opioid non-tolerant (opioid - naïve) healthy control subjects (controls). The purpose of this chapter is to provide an outline of the study design with an emphasis on elements common to all four studies. Methodological details specific to individual studies will be discussed in greater detail in the relevant chapters.

Royal Adelaide Hospital Pharmacy Department provided prescribed regular maintenance doses of opioids on study days and administration of the maintenance drug to opioid dependent subjects and healthy controls was supervised by medical and nursing trained research personnel. Royal Adelaide Pharmacy also produced the solutions of morphine and adjuvant analgesics.

2.1.1. Studies 1 and 2.Methadone and buprenorphine subjects plus high dose morphine studies.

Study 1 (methadone subjects plus morphine study) examined four groups of subjects. They were controls and methadone maintained subjects in once daily dose groups of 11-45 (n=6), 46-80 (n=6) and 81-115 (n=6) mg per day.

Study 2 (buprenorphine subjects plus morphine study) also examined four groups of subjects. They were controls and buprenorphine maintained subjects in once daily dose groups of 2 to 8 (n=4), 9 to 15 (n=4) and 16-22 (n=4) mg per day.

As stated previously, one healthy control group served as comparison in both the methadone and buprenorphine morphine studies. The group was matched to the methadone and buprenorphine groups with regards to age, sex and weight. All subjects were tested on two occasions, at least five days apart; once with morphine, once with saline placebo. The order of administration was randomised.

2.1.2. Studies 3 and 4. Methadone and buprenorphine plus adjuvant and high dose morphine studies

Study 3 (methadone subjects plus adjuvants and morphine study) examined two groups of subjects. They were controls and methadone maintained subjects.

Study 4 (buprenorphine subjects plus adjuvants and morphine study) also examined two groups of subjects. They were controls and buprenorphine maintained subjects.

As stated earlier, one healthy control group served as comparison for both the methadone and buprenorphine adjuvant studies. The group was matched to the methadone and buprenorphine groups with regards to age, sex and weight. This group was different from the group of healthy controls in the methadone and buprenorphine plus morphine studies. All four testing occasions occurred at least five days apart. Methadone and buprenorphine subjects were tested once each with S-ketamine, ketorolac, tramadol (adjuvant analgesics) and saline placebo.

Controls were tested with the same drugs but the saline day was omitted. The control subjects did not have a day where they received morphine without an adjuvant. This is because control subjects had been tested with morphine without an adjuvant in the methadone and buprenorphine studies and it was considered unethical to test the control subjects again with this procedure.

The order of administration was randomised. On each occasion morphine was administered.

2.2. Ethical considerations

The Research Ethics Committee of the Royal Adelaide Hospital, Adelaide, South Australia, Australia (RAH Protocol no: 010222) and the Institutional Review Board, Friends Research Institute, Los Angeles, California, USA (FRI IRB no: 00-03-057-02) approved the studies. The study was supported by National Institutes of Drug Abuse (NIDA) grant R01 DA 13706-02. Approval was sought from two ethics committees because the study was supported by the United States of America Government NIDA grant and conducted by the University of Adelaide at the

Royal Adelaide Hospital, Australia. Subjects were opioid dependent individuals maintained on either methadone or buprenorphine for the purpose of treatment of opioid addiction.

All subjects provided written informed consent, were paid for their involvement in the study and were free to withdraw at any time.

2.3. Subject inclusion and exclusion criteria

- All subjects were aged between 18 and 45 years of age.
- Opioid dependent subjects were maintained on either methadone or buprenorphine for more than a month without a dose change.
- Exclusion criteria for all subjects included pregnancy or lactation, use of antiretroviral drugs, significant medical or psychiatric illness that required ongoing treatment (except opioid addiction for methadone and buprenorphine subjects), poor venous access, participation in another research project, unwillingness to comply with study protocol and alcohol consumption exceeding 40 g per day for men and 20 g per day for women.
- Subjects were excluded if they showed severe liver impairment (serum aspartate aminotransferase and alanine aminotransferase concentrations greater than 3 times the upper limit of normal range and albumin concentrations less than 33 grams per litre) or haemoglobin counts outside the normal range.

Subjects were asked not to use any analgesics or illicit substances for twenty-four hours prior to testing. Methadone and buprenorphine subjects were recruited only if they self-reported intravenous heroin use at least once in the previous month. It was considered more ethical to administer morphine to individuals who continued to use illicit heroin, rather than to those who used no opioids, apart from their prescribed opioid maintenance dose. Healthy control subjects were excluded if they had any personal or family history of addictive behaviours.

A urine sample was collected on each study day for the detection of opioids, benzodiazepines, sympathomimetic amines, cannabinoids and barbiturates. Analysis of these samples confirmed that control subjects had not taken any of these psychoactive substances.

During the studies, methadone and buprenorphine subjects commonly had positive urine drug screens for a variety of illegal substances. As stated above, one of the conditions of enrolment for these subjects in this series of studies was that they reported heroin use at least once in the previous month. While this is a distinct subgroup of methadone and buprenorphine subjects, they represent a substantial and representative proportion of the treatment population (Darke et al.

2007). Clinical staff emphasised to all subjects that they needed to abstain from any illegal substances for 24 hours prior to screening and testing days or risk having to repeat the testing day or be excluded from the study. While methadone and buprenorphine subjects commonly had positive urine drug screens, all subjects were excluded from testing on the day if they presented showing any signs of intoxication from any substance. Clinical staff involved in the series of experiments had extensive experience is the assessment of substance intoxication.

Opioid dependent subjects were recruited from 2001 until 2004 from public methadone and buprenorphine clinics of the Maintenance Pharmacotherapies Unit of the Drug and Alcohol Services Council of South Australia and private medical practitioners registered as methadone and buprenorphine prescribers in South Australia. Prospective subjects were given an information sheet and consent form describing the nature and purpose of the study by either the project manager or research assistant. The author of this thesis is a registered general nurse, registered mental health nurse and was the project manager. Prospective subjects were asked to take the information sheet and consent form home and discuss possible participation with family or friends before enrolling in the study.

2.3.1. Study 1. Methadone subjects plus high dose morphine

Eighteen methadone subjects (12 men and 6 women), ranging between 24 and 45 years, with a mean age of 33 years were recruited. They had a weight range between 48 and 101 kg with a mean weight of 70 kg. They had been receiving methadone with no dose change between 1 and 12 months with a mean period of 3 months. The total period they had been maintained on methadone was between 1.5 and 72 months in total with a mean of 20 months. The subjects were stratified according to dose, with six subjects in each of the dose ranges of 11-45 mg, 46-80 mg and 81-115 mg per day.

2.3.2. Study 2. Buprenorphine subjects plus high dose morphine

Twelve buprenorphine subjects (7 men and 5 women) ranging between 24 and 42 years with a mean age of 35 years were recruited. Their weight ranged between 49 and 97 kg with a mean weight of 71 kg. The group had been receiving buprenorphine for a period between 1.5 and 12 months with a mean of 4 months with no dose change. They had been enrolled in a buprenorphine maintenance program in total ranging between 2 and 22 months with a mean of 10 months. The group was stratified according to dose with four subjects in each of the dose ranges of 2 to 8 mg, 9 to 15 mg and 16 to 22 mg per day.

2.3.3. Study 3. Methadone subjects plus adjuvant analgesics and high dose morphine

Six methadone subjects (3 men and 3 women), ranging between 24 and 39 years with a mean age of 31 years were recruited. Their weight range was between 56 and 86 kg with a mean

weight of 70 kg. Their daily methadone dose ranged between 40 and 78 mg per day with a mean dose of 55 mg. The period that they had been maintained on methadone with no dose change ranged between 2 and 14 months with a mean of 4 months. The group of subjects had been in the methadone maintenance program in total for a period of between 3 and 28 months with a mean of 7 months.

2.3.4. Study 4. Buprenorphine subjects plus adjuvant analgesics and high dose morphine

Six buprenorphine subjects (3 men and 3 women), aged between 25 and 37 years with a mean age of 32 years were enrolled. Their weights ranged from 55 to 85 kg with a mean of 70 kg. They had been receiving a daily buprenorphine dose between 2 and 16 mg with a mean of 10 mg with no dose change for 2 to 9 months with a mean of 5 months. The group had been in buprenorphine maintenance treatment in total ranging between 2 to 18 months with a mean of 8 months.

2.3.5. Healthy controls plus morphine

Ten healthy control subjects (5 men and 5 women) were selected. They were aged between 21 and 41 years with a mean age of 31 years. The weight range of the group was between 59 and 102 kg with a mean weight of 80 kg. The ten member healthy control group served as the control group for both the methadone and buprenorphine subject groups in the high dose morphine studies. They were administered lower doses of morphine.

2.3.6. Healthy controls plus adjuvant analgesics and morphine

Six healthy control subjects (3 men and 3 women) were also recruited. They were aged between 24 and 39 years with a mean age of 31 years. The weight range of the group ranged from 56 to 86 kg with a mean of 68 kg. The six member healthy control group served as controls for both the methadone and buprenorphine maintained subject groups in the adjuvant analgesics and high dose morphine studies. They were administered lower doses of morphine. A summary of the demographics of the subjects is shown in Table 2.

Study	Number Subjects Males (m) Females (F)	Age (mean years and range)	Body Weight (mean kg and range)	Maintenance No dose change (mean months and range)	Maintenance (mean months and range)	Stratified (mg/day range Number)
Study 1 MMT Morphine	18 12 m 6 f	33 (24-45)	70 (48-101)	3 (1-12)	20 (1.5-72)	11-45 (6) 46-80 (6) 81-115 (6)
Study 2 BMT Morphine	12 7 m 5 f	35 (24-42)	71 (49-97)	4 (1.5-12)	10 (2-22)	2-8 (3) 9-15 (3) 16-22 (3)
Study 3 MMT Morphine Adjuvants	6 3 m 3 f	31 (24-39)	70 (56-86)	4 (2-14)	7 (3-28)	No stratification Mean dose mg 55 (40-78)
Study 4 BMT Morphine Adjuvants	6 3 m 3 f	32 (25-37)	70 (55-85)	5 (2-9)	8 (2-18)	No stratification Mean dose mg 10 (2-16)
Controls Morphine	10 5 m 5 f	31 (21-41)	80 (59-102)			
Controls Morphine Adjuvants	6 3 m 3 f	31 (24-39)	68 (56-86)			

Table 2 Subject demographics. MMT methadone maintenance treatment clients. BMT buprenorphine maintenance treatment clients.

2.4. Procedure

The studies were conducted at the Clinical Pharmacology human subject testing facility located on level 7 of the Emergency Wing of the Royal Adelaide Hospital, Adelaide, Australia under constant ambient temperature (24^0C) and constant illumination (70 lux). Each testing session commenced at approximately 8 am. Prior to testing, in the Recovery Department, Department of Surgery and Anesthetics, Royal Adelaide Hospital, two indwelling catheters (Insyte Autoguard, Becton Dickenson, Sandy, Utah, USA) were inserted into peripheral veins on opposite arms. This was performed by anaesthetic registrars. The catheter in the dominant arm served for drug infusion; the catheter in the non-dominant arm for blood sampling. The subject was then escorted back to the testing rooms and the experiments began. The morphine studies lasted eight hours and the adjuvant studies lasted ten and a half hours.

2.4.1. Drug administration

2.4.1.1. Methadone and buprenorphine morphine studies

Morphine sulphate (David Bull Laboratories, Melbourne, Australia) infusions of 1 mg/ml were administered intravenously in two sixty-minute stages to achieve two consecutive target pseudo steady-state plasma concentrations. This procedure has been previously described (Eckhardt et al. 1998, Doverty et al. 2001a) and utilised a syringe driver infusion pump (3100 Graseby Syringe Pump, Watford, Hertfordshire, UK). Methadone and buprenorphine subjects were administered an initial bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 8.3 mg/hr for one hour to achieve a target pseudo steady-state plasma concentration of 80 ng/ml (Morphine 1). They were then administered an additional bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 16.5 mg/hr for one hour to achieve the second target pseudo steady-state plasma concentration of 180 ng/ml (Morphine 2).

Control subjects were administered an initial bolus of 2.2 mg morphine sulphate followed by a constant infusion of 1.2 mg/hr for one hour to achieve a target pseudo steady-state plasma concentration of 11 ng/ml (Morphine 1). They were then administered 4.95 mg of morphine sulphate followed by a constant infusion of 3.6 mg/hr to achieve the second target pseudo steady-state plasma concentration of 33 ng/ml (Morphine 2).Table 3 shows loading and maintenance doses intended to achieve these target pseudo steady-state plasma concentrations. Figure 1 shows the experimental design.

Studies 1 and 2				
Morphine	Infusion	Loading Dose (mg)	Maintenance Dose (mg/hr)	Target Plasma Morphine Concentrations (ng/ml)
MMT/BMT Subjects	1	15.2	8.3	80
	2	15.2	16.5	180
Control Subjects	1	2.2	1.2	11
	2	4.95	3.6	33
Studies 3 and 4				
Adjuvants	Infusion	Loading Dose (mg)	Maintenance Dose (mg/hr)	Target Plasma Adjuvant (S-ketamine/Ketorolac/Tramadol) Concentrations (ng/ml)
S-ketamine	1 S-ketamine	1.6	0.9	15
	2 S-ketamine	4.8	3.6	60
	2 S-ketamine /Morphine		3.6	60
Ketorolac	1 Ketorolac	3	7.4	0.4
	2 Ketorolac	27	74	4.0
	2 Ketorolac /Morphine		74	4.0
Tramadol	1 Tramadol	55	6	288
	2 Tramadol	128	20	1000
	2Tramadol /Morphine		20	1000
Morphine (When Adjuvants Co-administered)	Infusion	Loading Dose (mg)	Maintenance Dose (mg/hr)	Target Plasma Morphine Concentrations (ng/ml)
MMT/BMT Subjects	2 Adjuvant /Morphine	34	16.5	180
Control Subjects	2 Adjuvant /Morphine	5	3.8	33

Table 3 Loading and maintenance doses of morphine and adjuvants to achieve target pseudo steady state plasma concentration. MMT methadone maintenance treatment clients. BMT buprenorphine maintenance treatment clients.

Studies 1 and 2 (High Dose Morphine Studies)

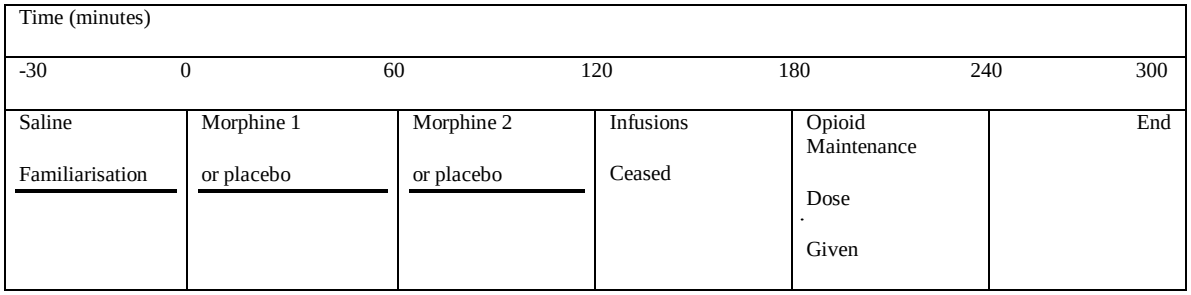

Figure 1 Schematic diagram of the experimental design for studies 1 and 2. Pain was tested, respiration rate was measured and blood samples were taken at time -30 minutes, 0 and hourly thereafter. Blood samples were also taken at 0.25, 0.5 and 0.75 hours after the end of the last infusion. These additional blood sample points are not shown.

Studies 3 and 4 (Adjuvant Analgesic and High Dose Morphine Studies)

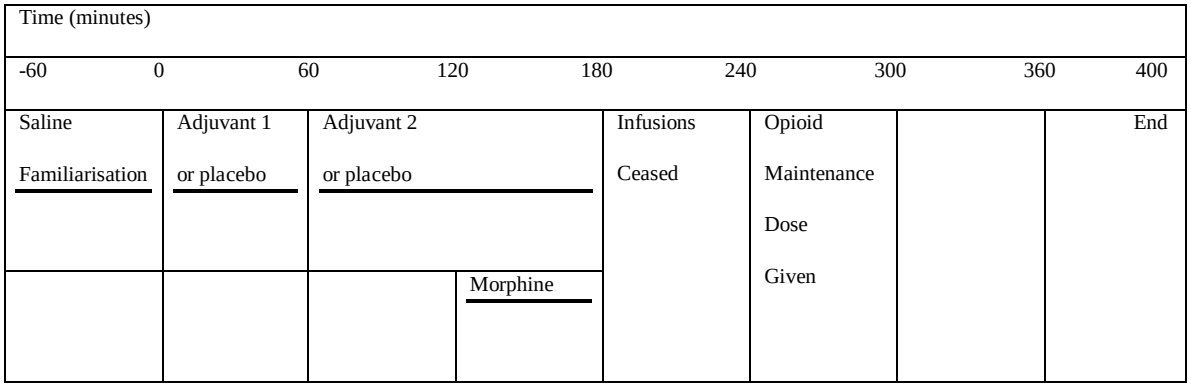

Figure 2 Schematic diagram of the experimental design for studies 3 and 4. Pain was tested, respiration rate was measured and blood samples were taken at times -60 minutes, 0 and hourly thereafter. Blood samples were also taken at 0.25, 0.5 and 0.75 hours after the end of the last infusion. These additional blood sample points are not shown.

2.4.1.2. Infusions of S-ketamine, tramadol and ketorolac.

Infusions of S-ketamine (Ketanest S, Pfizer (Parke-Davis), Karlsruhe, Germany), tramadol (Tramal, C.S.L., Stolberg, Germany) or ketorolac (Toradol, Roche, Switzerland) 1 mg/ml were administered intravenously in two sixty-minute stages to achieve two consecutive target pseudo steady-state plasma concentrations. Subjects were administered an initial bolus of the adjuvant analgesic (or saline placebo for methadone and buprenorphine subjects) followed by a constant infusion for one hour to achieve a target pseudo steady-state plasma concentration (Adjuvant 1). They were then administered a second bolus of the adjuvant analgesic (or saline placebo) followed by a second infusion for one hour to achieve a higher target pseudo steady-state plasma concentration (Adjuvant 2). At the end of the hour of the second infusion, the infusion was paused briefly while a loading dose of morphine was administered. An infusion of morphine was then commenced, the second infusion recommenced and the two infusions were maintained concurrently for one hour (Adjuvant 2/Morphine).

Loading and maintenance doses calculated to achieve target pseudo steady-state plasma concentrations of 15 and 60 ng/ml for S-ketamine, 0.4 and 4.0 mg/L of ketorolac and 288 and 1000 ng/ml of tramadol are shown in Table 3. While methadone, buprenorphine and control subjects received identical loading and maintenance doses of the adjuvant analgesics, they received different loading (control subjects 5 mg, methadone and buprenorphine subjects 34 mg) and maintenance doses of morphine (control subjects 3.8 mg, methadone and buprenorphine subjects 16.5 mg) to achieve different target pseudo steady-state plasma morphine concentrations (control subjects 33 ng/ml, methadone and buprenorphine subjects 180 ng/ml). Table 3 shows loading and maintenance doses intended to achieve these target pseudo steady-state plasma concentrations. Figure 2 shows the experimental design.

As stated in the introduction ketamine is generally available as a racemate but the S - isomer is three to four times more potent with fewer side-effects (White et al. 1982, Trevor and White 2007). For this reason, in this series of studies, the more active isomer S-ketamine was used in preference to the racemic drug. I wish to thank Pfizer Australia Pty Ltd for their kind supply of S-ketamine in these studies.

2.4.2. Blood sampling and assessment times

In studies 1 and 2 (morphine studies), seven ml blood samples were taken prior to the thirty minute saline familiarisation infusion, ten minutes prior to end of this infusion (this was designated as baseline) and ten minutes prior to the end of each of the two morphine or placebo saline infusions. Further blood samples were taken at 0.25, 0.5, 0.75, 1.0, 2.0, and 3 hours after the end of the last infusion.

In studies 3 and 4 (adjuvant studies), a seven ml blood sample was taken prior to the sixty minute saline familiarisation infusion, ten minutes prior to the end of this infusion (this was designated as baseline), ten minutes prior to the end of the two adjuvant/saline placebo infusions and ten minutes prior to the end of the adjuvant analgesic/saline placebo and morphine infusion. Further blood samples were taken 0.25, 0.50, 0.75, 1.0, 2.0, 3.0 and 4.0 hours after the last infusion.

The blood samples were centrifuged immediately and the plasma stored at –20^{o}C until assay.

2.5. Nociceptive tests and physiological responses

Two nociceptive tests were administered: the cold pressor using the non-dominant arm, and electrical stimulation using the earlobe. As stated, there is a body of evidence describing the existence of chronic opioid induced hyperalgesia and tolerance in people currently addicted to opioids and former opioid addicts. However, there are also contradictions in the literature as described in 1.14. Differences in the measurements of pain and means of inducing pain may contribute to these contradictions. There have been a number of recent reviews of opioid induced hyperalgesia that have described how pain responses vary with the type of pain stimulus used (Fishbain et al. 2009, Staahl et al. 2009, Lee et al. 2011). Phasic pain, which includes electrically induced pain, is neurologically and qualitatively different from the tonic pain induced by the cold pressor text. The use of different means of pain induction provides for a greater range of sensitivity to different pain responses. These tests have been described and used previously in a number of studies to investigate the provision of analgesia (Eckhardt et al. 1998, Dyer et al. 1999, Doverty et al. 2001a, Doverty et al. 2001b, Hay et al. 2009, Compton et al. 2012).

2.5.1. Cold pressor test

This method was adapted from the procedures of Eckhardt et al (1998). Two cylindrical plastic containers (380 cm in depth, 300 cm in diameter) were used. One contained warm water (34.5–35.5°C), which was controlled by a thermo-regulator (Unistat 110, Thermoline Scientific, Sydney, Australia). The other container was filled with crushed ice and cold water (temperature 0.5–1°C). Ice was added as required to ensure the temperature remained between 0.5 and 1°C. An aquatic pump (Brolga MV 1500, Brolga Australia Pty. Ltd., Haberfield, NSW, Australia) was used to circulate the cold water in order to prevent laminar warming around the subject's limb. Prior to testing, each subject was instructed to verbally indicate when they first felt pain (detection) and, when they could no longer tolerate the stimulus (tolerance) to remove their arm from the container. Each subject was then instructed to kneel on cushions in front of the two water containers and eye patches were placed over both eyes to exclude visual distractions including temporal cues. Subjects then placed the non-dominant hand and forearm, with fingers wide apart, in the warm water container for 2 min. A blood pressure cuff was placed on the non-dominant upper arm. One minute

and 45 s after immersion the cuff was inflated to 20 mmHg below diastolic pressure to minimize the role of vascular flow in determining reaction to the cold water. Fifteen seconds later subjects were instructed and assisted to transfer their arm from the warm water container into the cold water with fingers wide apart and not touching the container. Detection was recorded as the time from full immersion of the limb to verbal indication of pain; tolerance was recorded as the time from full immersion until withdrawal of the arm from the cold water container. Both indices were quantified in seconds. The blood pressure cuff was then deflated, eye patches were removed, and each subject was given a towel to dry their forearm.

2.5.2. Electrical stimulation

This was delivered via cutaneous electrodes attached to one earlobe. The electrical stimulator (Grass model S6C, Grass Instruments, Quincy, MA, USA) delivered square wave pulses of 14 ms duration (0.7 pulses/s). Electrode gel (Spectra 360, Parker Laboratories, Orange, NJ, USA) was used to provide conductance between the ear clip and the skin. Voltage, set to zero at baseline, was increased at a constant rate of 2 V every 1.4 s. Each subject sat in a comfortable chair for the duration of this method. As the voltage increased, the subjects verbally indicated when they first perceived pain (detection), and when they could no longer tolerate the stimulus intensity (tolerance). The stimulus was terminated immediately upon indication of the latter. Both indices were quantified in volts (Doverty et al. 2001a).

Respiration rate was measured over one minute by observation when the subject was at rest and without the subject's awareness. Safety was monitored and recorded throughout the study by means of continuous pulse oximetry, continuous ECG waveform, categorical nausea scale (Del Favero et al. 1992) and categorical sedation scale (Ready et al. 1988). Respiration rate was measured and nociceptive tests (see above) were administered immediately after the collection of each blood sample except at 0.25, 0.50 and 0.75 hours after the last infusion.

2.6. Drug Assays

The following assays were not performed by the author of this thesis. These assays were performed by other members of the Discipline of Pharmacology, University of Adelaide (Andrew Menelaou and Glynn Morrish).

2.6.1. Plasma morphine, S-ketamine, ketorolac and tramadol concentrations

The quantification of plasma morphine was by high-performance liquid chromatography (HPLC) with coulometric detection as previously described (Doverty et al. 2001b). The assay had a lower limit of quantification of 1 ng/ml and all variability in accuracies and coefficients of variation were below 7%. The quantification of plasma S-ketamine was by HPLC with ultra-violet

detection (Menelaou et al. 2001) and had a lower limit of quantification of 2 ng/ml. All variability in accuracies and coefficients of variation were below 8%. HPLC with ultra-violet detection and a lower limit of quantification of 100 ng/ml was used to quantify plasma ketorolac (Chaudhary et al. 1993). All variability in accuracy and coefficients of variation were below 9%. The quantification of tramadol was by HPLC with fluorescence detection. The assay had a lower limit of quantification of 50 ng/ml and all variability in accuracy and coefficients of variation were below 13% (Menelaou et al. 2002). These assays were not interfered with by the other drugs administered.

2.6.2. Plasma buprenorphine concentrations

2.6.2.1. Instrumentation

The liquid chromatograph mass spectrometer (LCMS) system consisted of a LC-10AD pump (Shimadzu, Kyoto, Japan), a DGU-12A solvent degasser (Shimadzu), a SIL-10AD autoinjector (Shimadzu), a SPD-10A UV-VIS detector (Shimadzu), and an LCMS-2010A liquid chromatograph mass spectrometer (Shimadzu) with an Electrospray (ESI) probe (Shimadzu) in positive ionisation mode. The system was controlled using a SCL-10A system controller (Shimadzu), and LCMS solutions software (v2.04-H3, Shimadzu). High purity (99.99%) nitrogen gas (BOC Gases, Salisbury, Australia) was used for the nebulisation and drying gas. The following ions were monitored in single ion monitoring mode: m/z 468.4 for buprenorphine; m/z 414.4 for nor-buprenorphine; m/z; 472.4 for the $^{2}H_{4}$-buprenorphine internal standard; and 417.4 $^{2}H_{3}$-nor-buprenorphine internal standard. Optimal ionisation conditions were: a curved desolation line voltage of 20V at 250 C, heating block of 200 C, Q-Array voltage of +25V, detector gain voltage of 2.0 kV, 1.5 L/min nebulisation gas and 2 L/min drying gas.

2.6.2.2. Liquid chromatography conditions

The analytical column was a C18 (2) LUNA 150 X 2.0 mm I.D. (150x2.0 mm, Phenomenex, USA), the mobile phase comprised 0.1% Formic Acid in 44% methanol at a flow-rate of 0.2 ml.min^{-1}. Injection volume was set at 40 µl, and run time was 14 min per sample, with retention times of 3.1 and 4.5 min for nor-buprenorphine and buprenorphine respectively.

2.6.2.3. Sample preparation

Briefly, plasma samples (1 ml) and internal standard (50 µL of 20 ng/ml d^{3}-norbuprenorphine and d^{4}-buprenorphine) were aliquoted into 10 ml tapered bottom plastic tubes, alkalinized (30 µl, 1 M NaOH pH 10) and extracted with 5 ml of 30:70 (v/v) diethyl ether:hexane for 20 min on a rotary mixer. Samples were then centrifuged (2000xg, 10 min) and the organic phase transferred to a clean 10 ml tapered bottom plastic tube containing 100 µl ml of 5 mM hydrochloric acid (HCl) and vortexed for 1 min. Samples were then centrifuged (2000xg, 10 min), the organic phase aspirated to waste and 40 µl of the 5mM HCl was injected onto the chromatography system. In the case of calibration standards and quality control (QC) samples, 100

μl of an appropriate stock solution containing buprenorphine and nor-buprenorphine was mixed with 900 μl of blank human plasma and extracted as outlined above.

2.6.2.4. Calibration curves

Calibration curves consisting of 8 standards were constructed in blank plasma over the concentration range 0.125-10 ng/ml of each analyte. Low (LQC), medium (MQC) and high (HQC) quality control samples were also prepared in duplicate, with final concentrations of 0.35 ng/ml, 2.5 ng/ml and 7 ng/ml achieved for each analyte respectively. The robustness of the analytical method was assessed by assaying 6 replicates of each QC sample and the lowest calibration standard (LLOQ) on a single day to determine the intra-assay accuracy and precision. Inter-assay accuracy and precision were determined by analysis of duplicates of each QC sample, and the LLOQ, on eight different assay days. Extraction recovery was approximately 80% for all analytes, without evidence of differences between the unlabelled and stable-labelled compounds or concentration dependency. Peak areas of each compound of interest were converted into peak area ratios using the peak area of the internal standard. Linear regression analysis (GraphPad Prism v4.03, GraphPad Software, CA, USA), weighted $1/y^2$, of peak area ratios against nominal concentrations provided an estimate of slope, intercept and coefficient of determination (r^2). Accuracy was calculated as the mean (calculated concentration/nominal concentration) x 100% for each individual sample, and the residual standard deviation of the mean (RSD) was taken as the precision.

There were no interfering peaks in the analysis of 6 blank plasma samples, or in the patients' samples. Calibration curves for all analytes were linear over the 0.125-10 ng/ml concentration range, with r^2 values greater than 0.99 for all assays with no evidence of time related changes in slope values. The assay demonstrated excellent precision and accuracy over the entire calibration range, both within and between days. Briefly, inter-assay accuracy and precision (accuracy±RSD %) were 101±5% (HQC), 105±6% (MQC), 102±8% (LQC), 101±1% (LLOQ, 0.125 ng/mL), for buprenorphine and 100±7% (HQC), 107±9% (MQC), 92±14% (LQC), 101±5% (LLOQ) for nor-buprenorphine. Similarly, intra-assay accuracy and precision were 103±2% (HQC), 107±2% (MQC), 91±2% (LQC), 101±3% (LLOQ, 0.125 ng/mL), for buprenorphine and 97±1% (HQC), 105±2% (MQC), 89±10% (LQC), 104±12% (LLOQ) for nor-buprenorphine.

2.7. Data collection and statistical analysis

The clinical data were initially recorded using case report forms with pen on paper. They were then transcribed onto the statistical program GraphPad Prism 4.03 for Windows. (GraphPad Software, San Diego, California, USA).

Data are presented as mean ± standard error of the mean (SEM) (with 95% confidence intervals (95% CI)) in all four studies. The alpha (level) of $P>0.05$ was used for all studies. This was

considered an appropriate alpha (level) to limit both type 1 errors (false positives) and type 2 errors (false negatives) (Tukey 1977, Perneger 1999).

In studies 1 and 2 (morphine studies), one-way analysis of variance (ANOVA) was used to compare outcome variables (cold pressor tolerance (seconds), electrical stimulation tolerance (volts), respiration rate (breaths per minute)) between the methadone dose groups and between the buprenorphine dose groups. One-way ANOVA was also used to compare each outcome variable across treatments for the methadone dose groups, buprenorphine dose groups, combined methadone group, combined buprenorphine group and the control group.

In studies 1 and 2 methadone and buprenorphine subjects were stratified according to daily dose of maintenance opioid. Post test for a linear trend analysis was performed to determine the role of increasing methadone and buprenorphine dose in cold pressor and electrical stimulation pain tolerance, and respiration rate. Post test for a linear trend determines whether the means increase (or decrease) systematically.

In study 1 linear regression analysis was used to examine the relationship of plasma R-(-) methadone concentrations and cold pressor pain tolerance values at baseline on the saline administration day.

Unrelated samples t-tests were used to compare baseline values between the combined methadone or combined buprenorphine group and the control group. Bonferroni's and Dunnet's tests were used for post-hoc analyses as appropriate.

In studies 3 and 4 (adjuvant studies) one-way ANOVA was used to analyse each outcome variable (cold pressor tolerance, electrical stimulation tolerance and respiration rate) across treatments for the methadone group, buprenorphine group and the control group. Unrelated samples t-tests were used to compare baseline values between the methadone group or buprenorphine group and the control group. Dunnet's test was used for post-hoc analyses.

2.8. Discussion

2.8.1. Design

The studies utilised a double blind placebo controlled design. The studies were three group (methadone, buprenorphine and healthy controls) quasi-experimental designs in which the main factor was opioid dependence (present versus absent). Opioid dependent groups were maintained on stable regimens of methadone and buprenorphine. Healthy control subjects were matched for age, gender, weight and ethnicity to the opioid dependent groups. Quasi-experimental designs are differentiated from experimental designs in that they lack the key ingredient of random assignment. The design used is a non-equivalent groups design. The groups (i.e. methadone, buprenorphine and

controls) are not created through random assignment. Although there is a control group matched for age, weight and sex, they are non-equivalent groups.

As discussed earlier in sections 2.4 and 2.5, the studies were double blind randomised controlled trials in strictly controlled experimental environments designed to minimise confounding variables.

Walter Ling and the supervisors of the PhD thesis Professors Jason White, Andrew Somogyi and Felix Bochner suggested the overall design and devised the target pseudo steady-state concentrations used in the series of studies. The rationale for these target concentrations are described below.

2.8.2. Target pseudo steady-state plasma drug concentrations

2.8.2.1. Morphine

As cited previously, the results from the work of Doverty et al (2001a), Compton et al (2000, 2001) and others suggested that methadone from the maintenance dose did not provide analgesic relief to people maintained on opioids for the treatment of opioid addiction. Doverty et al (2001b) examined the antinociceptive effects of clinically used intravenous morphine doses in methadone maintained subjects (14.8 mg morphine) and healthy control subjects (11.95 mg) using the cold pressor and electrical stimulation tests. The plasma morphine concentrations in the methadone subjects at pseudo steady-state reached 16±2 and 55±5 ng/mL and in the healthy controls 11±2 and 33±6 ng/mL. Using the cold pressor test, the methadone subjects did not achieve the same level of antinociception (tolerance: baseline 25±1 seconds; morphine 26±6 seconds) as the healthy controls (tolerance: baseline 55±12 seconds, morphine 116±24 seconds). This was despite the fact that the methadone subjects' plasma morphine concentrations were twice as high as the healthy controls', and were superimposed on plasma R(+)- and S(-)-methadone concentrations ranging from 148-1152 ng/mL. It was demonstrated that clinically used doses of morphine failed to provide analgesic relief to this opioid maintained population.

Strategies for analgesia in methadone subjects could therefore include very high doses of opioids such as morphine, non-opioid analgesics, or a combination of an opioid dose and a non-opioid. By extrapolation from the data of Doverty et al (2001b), it was estimated that for opioid maintained subjects, restoration of normal pain sensitivity in the cold pressor would require concentrations of morphine at 180 ng/ml. Loading and maintenance doses to achieve the target plasma morphine pseudo steady-state concentrations were described in Table 3.

As discussed in section 1.16 and based on previous studies, the opioid adjuvants ketorolac, S-ketamine and tramadol may be effective alone, or in combination with morphine in the provision

of pain relief to the population maintained on opioids for the treatment of opioid addiction. The rationale for target pseudo steady-state concentrations of these opioid adjuvants are given below and described in Table 3.

2.8.2.2. Ketorolac

Ketorolac is a parenterally administered non-steroidal anti-inflammatory drug, effective for moderate to severe pain. The target pseudo steady-state concentrations of ketorolac were 400 and 4000 ng/mL. These plasma concentrations were the approximate EC_{50} and EC_{80} values, respectively, obtained by Mandema & Stanski (1996). In that study, patients who were being treated for moderate to severe post-operative pain were given a single intra-muscular dose of ketorolac. Analgesia was reported as pain relief on a 5-category ordinal scale.

2.8.2.3. Ketamine

Tucker et al (1999) derived plasma concentration-effect relationships for racemic ketamine and fentanyl, each given alone, to healthy volunteers to whom electrical and pressure pain stimuli were applied. The concentration-effect response to fentanyl was repeated in the presence of a sub-hypnotic and sub-antinociceptive concentration (30 ng/mL) of racemic ketamine. This produced a shift of the plasma fentanyl concentration-effect relationship to the left, indicating potentiation of the analgesic effect by ketamine. Based on S(+)-concentration-effect relationship studies (Arendt-Nielsen et al. 1996, Tucker et al. 1999), it was estimated that plasma S(+)-ketamine concentrations of 15 and 60 ng/mL would achieve 20 and 80%, respectively, of maximum antinociception without unacceptable adverse effects.

2.8.2.4. Tramadol

After major orthopaedic or gynaecological surgery, the median effective concentration of intravenously infused tramadol was 288 ng/mL and the maximum effective concentration was approximately 1000 ng/mL (Lehmann et al. 1990). It was therefore estimated that 300 ng/mL and 1000 ng/ml would produce median and maximum effect in our population without unacceptable adverse effects.

2.8.3. Daily dose ranges and subject numbers

The three daily dose ranges of methadone (11-45 mg, 46-80 mg and 81-115 mg per day) and three daily dose ranges of buprenorphine (2 to 8 mg, 9 to 15 mg and 16 to 22 mg per day) represented three commonly used ranges of methadone and buprenorphine among patients on opioid maintenance therapy in Adelaide, South Australia.

The original design of the experiment included six methadone subjects in three daily dose ranges of methadone (11-45 mg, 46-80 mg and 81-115 mg per day) and six buprenorphine subjects

in three daily dose ranges of buprenorphine (2 to 8 mg, 9 to 15 mg and 16 to 22 mg per day) tested with high doses of morphine. After four subjects had been tested in each of the three daily dose ranges of buprenorphine, and no differences found in the cold pressor test ($p=0.93$), electrical stimulation test ($p=0.72$) or in terms of respiration rate ($p=0.67$), it was decided that it was unethical to expose subjects to more pain testing.

3. Study 1. Antinociceptive and respiratory effects of high dose morphine in methadone maintained subjects

3.1. Introduction

A complicating factor in the pain management of opioid dependent people is the development of opioid tolerance, as reflected by a rightward shift in the dose-response curve. Doverty et al (2001b) examined the antinociceptive effect of morphine in people maintained on methadone and found that a higher than therapeutically recommended intravenous dose of morphine (15 mg), which resulted in plasma morphine concentrations of 55 ng/ml, produced 'minimal antinociceptive effect', indicating substantial cross-tolerance between the two opioids. In the same study, controls showed an antinociceptive effect with lower doses (12 mg) and plasma concentrations (33 ng/ml) of morphine.

Some guidelines suggest that very high intravenous doses of morphine may provide antinociception in methadone maintained people. Based on a case review, Rapp et al. (1995) suggested that patients with a history of prior opioid consumption require three to four times the amount of morphine required by opioid naïve patients. Given that plasma morphine concentrations of 50 ng/ml are associated with relief of severe post-surgical pain (Berkowitz et al. 1975), concentrations required for methadone maintained people might therefore be in the range of 150-200 ng/ml. At these concentrations, consideration needs to be given to the respiratory depressant and other adverse effects of morphine.

The magnitude of cross-tolerance to morphine may be dependent on the magnitude of the daily methadone dose. The typical dose ranges from 40 to 100 mg per day, but a minority is maintained on a dose outside this range. To date, no studies have examined the effect of different daily maintenance doses on the response to morphine.

The aim of the study was to examine whether very high intravenous morphine doses produce antinociception and respiratory depressant effects in methadone subjects and to determine whether the magnitude of the daily methadone dose affects these responses. Lower doses of morphine were administered to healthy controls. .

3.2. Methods

The subjects, procedures and statistical analyses for the methadone high dose morphine study were as described in detail in chapter 2.

3.2.1. Subjects

In summary, eighteen methadone subjects (12 men and 6 women), ranging between 24 and 45 years, with a mean age of 33 years were recruited. They had a weight range between 48 and 101 kg with a mean weight of 70 kg. They had been receiving methadone with no dose change between

1 and 12 months with a mean period of 3 months. The total period they had been maintained on methadone was between 1.5 and 72 months in total with a mean of 20 months. The subjects were stratified according to dose, with six subjects in each of the dose ranges of 11-45 mg, 46-80 mg and 81-115 mg per day.

Ten healthy control subjects (5 men and 5 women) were selected. They were aged between 21 and 41 years with a mean age of 31 years. The weight range of the group was between 59 and 102 kg with a mean weight of 80 kg. The ten member healthy control group served as the control group for both the methadone and buprenorphine subject groups in the high dose morphine studies.

3.2.2. Drug administration

Methadone subjects were administered an initial bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 8.3 mg/hr for one hour to achieve a target pseudo steady-state plasma concentration of 80 ng/ml (Morphine 1). They were then administered an additional bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 16.5 mg/hr for one hour to achieve the second target pseudo steady-state plasma concentration of 180 ng/ml (Morphine 2).

Control subjects were administered an initial bolus of 2.2 mg morphine sulphate followed by a constant infusion of 1.2 mg/hr for one hour to achieve a target pseudo steady-state plasma concentration of 11 ng/ml (Morphine 1). They were then administered 4.95 mg of morphine sulphate followed by a constant infusion of 3.6 mg/hr to achieve the second target pseudo steady-state plasma concentration of 33 ng/ml (Morphine 2). Table 3 shows loading and maintenance doses intended to achieve these target pseudo steady-state plasma concentrations. Figure shows the experimental study design.

3.3. Results

3.3.1. Plasma morphine concentrations

Plasma morphine concentrations in control participants and the combined three methadone dose groups are shown in Figure 3 (upper panel). Combined methadone subjects achieved a mean first pseudo-steady-state plasma morphine concentration (Morphine 1) (M1) of 78±4 (range 49-126) ng/ml and control participants 7.0±0.4 (range 5-9) ng/ml. Combined methadone subjects achieved a mean second pseudo steady-state plasma morphine concentration (Morphine 2) (M2) of 173±11 (range 106-305) ng/ml and control participants 25±1 (range 19-32) ng/ml. The mean Morphine 1 plasma morphine concentrations for the individual daily methadone dose groups 11-45, 46-80 and 81-115 mg were 74±3 (range 62-84), 86±11 (range 58-126) and 77±6 (range 50-93) ng/ml, respectively. At Morphine 2, mean plasma morphine concentrations for the 11-45, 46-80 and 81-115 mg daily dose groups were 160±14 (106-200), 195±24 (143-305) and 170±15 (114-220) ng/ml, respectively. There were no differences in the plasma morphine concentrations between the three methadone dose groups at Morphine 1 (P=0.48) or Morphine 2 (P=0.40).

3.3.2. R-(-)-methadone (plasma methadone) concentrations

The combined methadone subjects' mean plasma R-(-)- methadone concentrations on saline and morphine administration days are shown in Figure 3 (lower panel). No significant differences could be detected between mean plasma R-(-)-methadone concentrations on morphine and saline placebo administration days at baseline (P=0.15), one (P=0.25) or two hours (P=0.67) post methadone dosing.

The mean plasma R-(-)-methadone concentrations at baseline (180 minutes prior to methadone administration) on morphine administration days for the individual daily dose groups 11-45, 46-80 and 81-115 mg were 75±15 (50-120), 123±20 (37-166) and 175±34 (92-305) ng/ml respectively. There was a significant difference (P=0.028) between mean plasma R-(-) methadone concentrations for the daily dose groups 11-45 and 81-115 (P<0.05; 95% CI -137 to -8) but not 11-45 and 45-80 (P>0.05) or 45-80 and 80-115 (P>0.05) on the saline administration days. There was also significant difference (P=0.033) between mean plasma R-(-) methadone concentrations for the daily dose groups 11-45 and 81-115 (P<0.05; 95% CI -192.6 to -8) but not 11-45 and 45-80 (P>0.05) or 45-80 and 80-115 (P>0.05) on the morphine administration days. At the time of methadone dosing, the mean plasma R-(-)- methadone concentrations in the dose groups were 69±15 (35-121), 103±16 (30-137) and 152±28 (range 79-261) ng/ml, respectively and these rose to 101±22 (range 42-175), 163±21 (range 101-233), and 243±47 (range 142-449) ng/ml respectively, 2 hours after daily methadone dose administration.

On saline administration days, the mean plasma R-(-)-methadone concentrations at baseline for the individual daily dose groups 11-45, 46-80 and 81-115 were 76±17 (27-135), 112±15 (45-152) and 150±20 (76-211) ng/ml, respectively. At the time of methadone dosing, the mean plasma R-(-)- methadone concentrations in the dose groups were 76±19 (range 27-145), 100±15 (range 35-129) and 152±23 (range 65-231) ng/ml respectively, and these rose to 105±22 (range 39-175), 166±20 (range 91-216) and 223±19 (range 161-285) ng/ml respectively, 2 hours after daily dose administration.

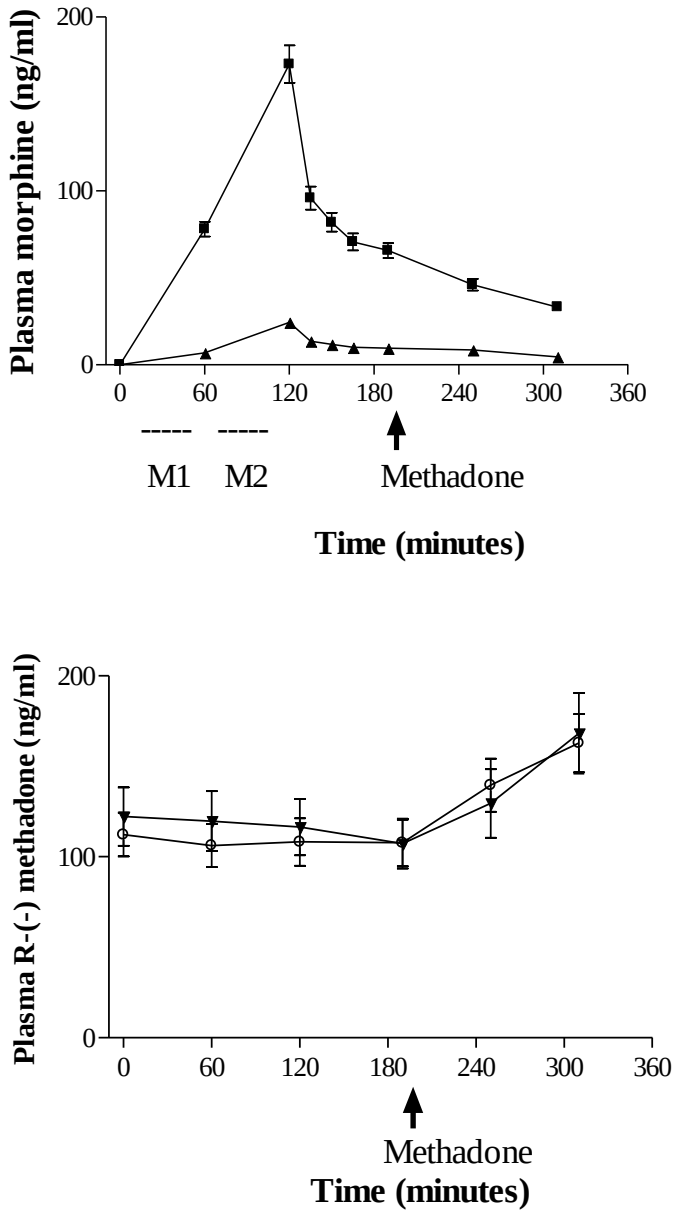

Figure 3 Plasma morphine concentrations (upper panel) in 18 methadone maintained (■) and 10 healthy control participants (▲). Pseudo steady-state plasma concentration 1 (M1), pseudo-steady-state plasma concentration 2 (M2) and the time of the methadone dose administration are indicated. Plasma R-(-)-methadone concentrations (lower panel) from 0 to 310 minutes on morphine administration (▼) and saline administration days (O) in methadone subjects are indicated. The time of the methadone dose administration is also indicated. Data are represented as mean ± SEM.

3.3.3. Cold pressor responses

Pain tolerance responses for control and combined methadone subjects in the cold pressor test at baseline and Morphine 2 are shown in Figure 4 (upper panel). Pain tolerance values for combined methadone subjects (15±2 range 5-25 seconds) were significantly lower than for control participants (34±6 range 4-73 seconds) at 0 h (P=0.0009; 95% CI 8 to 29). Within-group comparisons revealed that pain tolerance values for control participants increased significantly (P=0.04) from baseline to Morphine 2 (52±11 range 6.6-123 seconds) (P<0.05; 95% CI 2 to 34), but not baseline to Morphine 1 (38±7 range 4.6-64 seconds) (P>0.05).

There were no significant changes (P=0.24) in cold pressor pain tolerance values for combined methadone subjects from baseline to Morphine 1 (16±2 range 5-26 seconds) or Morphine 2 (17±2 range 5-31 seconds).

There were no significant differences in the cold pressor pain tolerance test from baseline to Morphine 2 for the specific dose groups of methadone subjects 11-45 (P=0.62), 46-80 (P=0.36) or 81-115 (P=0.41) mg per day. Subjects maintained on the lower doses of methadone did not react differently to the high dose morphine as compared to subjects maintained on the higher doses of methadone. There were also no significant differences (P>0.18) between the groups (11-45, 46-80 or 81-115) of methadone subjects at the baseline or Morphine 2 time point in the cold pressor pain tolerance test (Figure 5 upper panel).

As stated in the data analysis section, post test for a linear trend determines whether the column means increase (or decrease) systematically as the columns go from left to right. Trend analysis showed that there was no significant change in the linear component of trend for cold pressor pain tolerance at baseline (P=0.08) or Morphine 2 (P=0.12) between dose groups of methadone subjects in the 11-45 (P=0.62), 46-80 (P=0.36) or 81-115 (P=0.41) mg per day ranges.

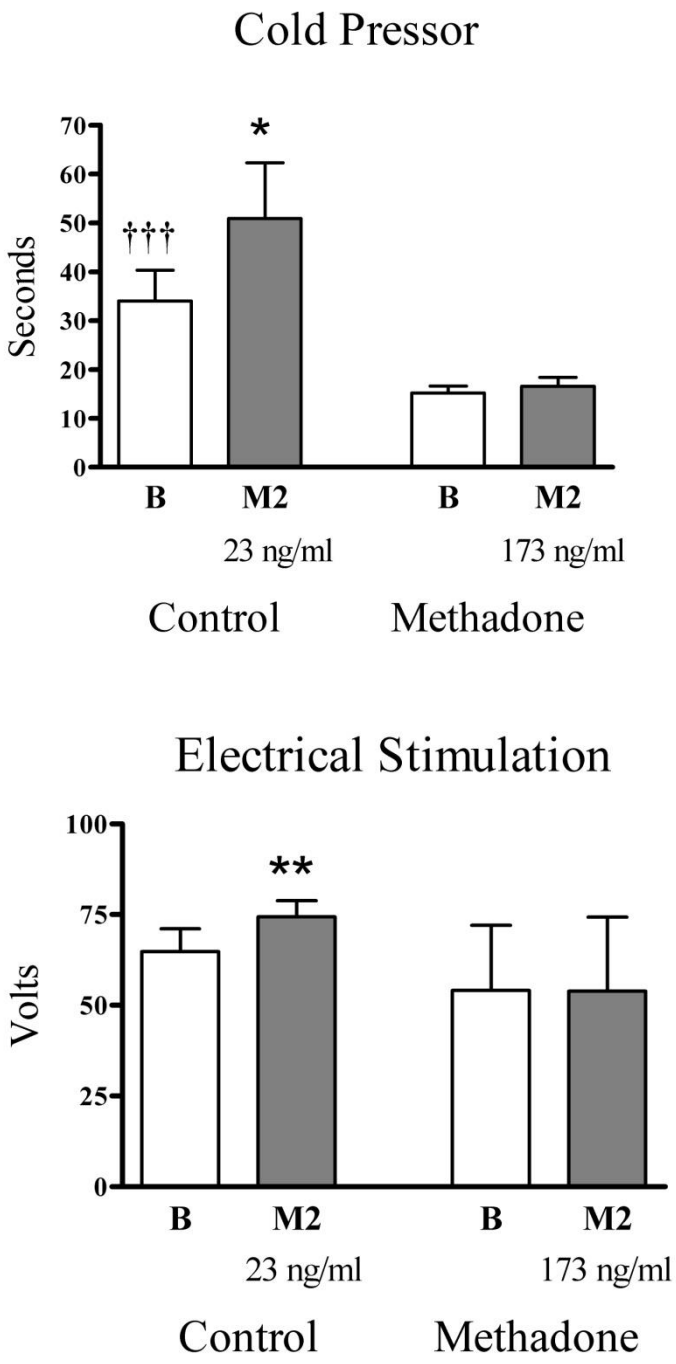

Figure 4 Cold pressor (upper panel) and electrical stimulation (lower panel) pain tolerance responses at baseline (B) and Morphine Infusion 2 (M2) (Mean plasma morphine concentrations). *P<0.05, **P<0.01 compared to baseline (0 ng/ml). †††P<0.001 methadone subjects versus control subjects.

3.3.4. Electrical stimulation

Control and combined methadone subjects' pain tolerance responses in the electrical stimulation test at baseline and Morphine 2 are shown in Figure 4 (lower panel). Electrical stimulation values for combined methadone subjects (54±4 range 20-80 volts) were not significantly different to controls (65±6 range 38-100 volts) (P=0.16) at baseline. Within-group comparisons revealed that pain tolerance values for control participants increased significantly (P=0.007) from baseline to Morphine 2(74±5 range 60-100 volts) (P<0.01; 95% CI 3 to 16), but not baseline to Morphine 1 (68±5 range 48-100 volts) (P>0.05).

There were no significant changes (P=0.9) in pain tolerance values for combined methadone subjects from baseline to Morphine 1 (55±5 range 24-86 volts) or Morphine 2(54±5 range 20-88 volts). Within-group comparisons showed that there were no significant differences in antinociceptive effects in the electrical stimulation pain tolerance test for methadone subjects in the 11-45 (P=0.43), 46-80 (P=0.56) or 81-115 (P=0.37) mg per day group from baseline to Morphine 2. There were also no significant differences (P>0.16) between the groups (11-45, 46-80 or 81-115) of methadone subjects at baseline or Morphine 2 in the electrical stimulation pain tolerance test (Figure 5 middle panel).

Trend analysis shows that there was no significant change in the linear component of trend for electrical stimulation pain tolerance at baseline (P=0.19) or Morphine 2 (P=0.07) between dose groups.

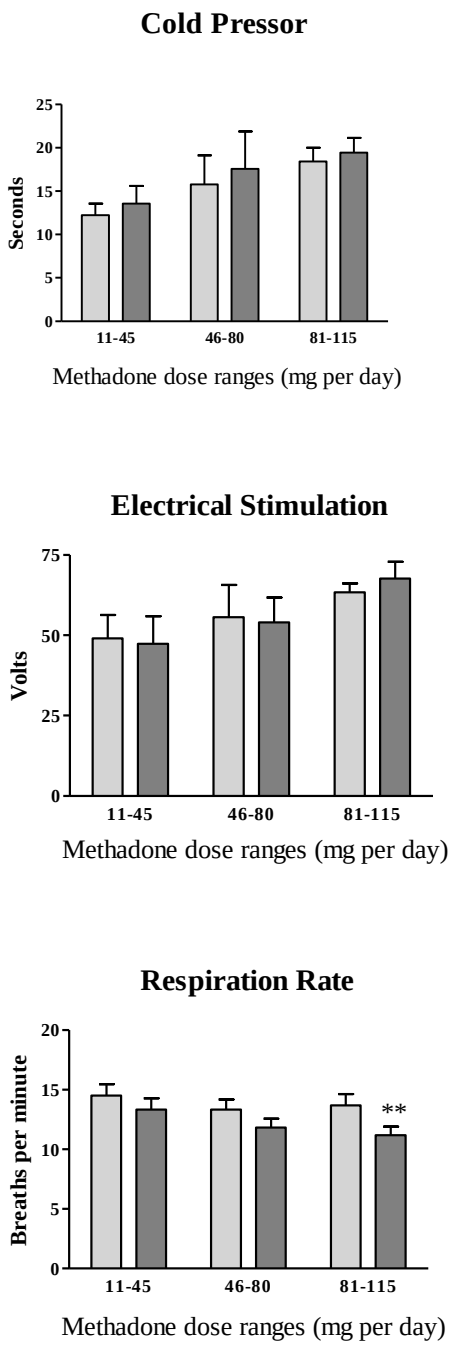

Figure 5 Cold pressor pain tolerance (upper panel), electrical stimulation pain tolerance (middle panel) and respiration rate (lower panel) values at baseline (light grey bars) and morphine concentration 2 (M2) (dark grey bars) for daily methadone dose ranges 11-45, 46 to 80 and 81-115 mg per day. Data are represented as mean ± SEM. **P<0.01 compared to baseline.

3.3.5. Respiration

Respiration rate (breaths per minute) relative to baseline and Morphine 2 is shown in Figure 6. Respiration rate for combined methadone subjects (14±0.5 range 10-18 breaths per minute) was significantly lower than for control participants (17±0.8 range 14-22 breaths per minute) at baseline (P=0.004; 95% CI 1.1 to 4.9). Within-group comparisons revealed that the respiration rate for control participants did not decrease significantly (P=0.09) between baseline and Morphine 1 (16±0.6 range 13-19 breaths per minute) or baseline and Morphine 2 (15±0.9 range 10-19 breaths per minute). Respiration rate for combined methadone subjects decreased significantly (P=0.0008) from baseline to Morphine 2 (12.3±0.5 range 9-16 breaths per minute) (P<0.01; 95% CI 0.8 to 2.6), but not baseline to Morphine 1 (13±0.6 range 9-16) (P>0.05). Within-group comparisons showed that while there was no change in respiration rate for methadone participants in dose groups 11-45 (P=0.16) and 46-80 (P=0.06) mg per day, there was a significant decrease in respiration rate for the 81-115 (P=0.004; CI 1.2 to 3.8) mg group from baseline to Morphine 2 (Figure 5). There were no significant differences (P>0.18) in respiration rate between the groups (11-45, 46-80 or 81-115) of methadone subjects at baseline or Morphine 2.

Trend analysis shows that there was no significant change in the linear component of trend for change in respiration rate at baseline (P=0.5) or Morphine 2 (P=0.08) between dose groups.

One subject in the methadone group had reduced respirations (nine breaths per minute) and one subject in the control group had reduced respirations (ten breaths per minute) following Morphine 2. There were no adverse events as a result of these decreases in respiration rate.

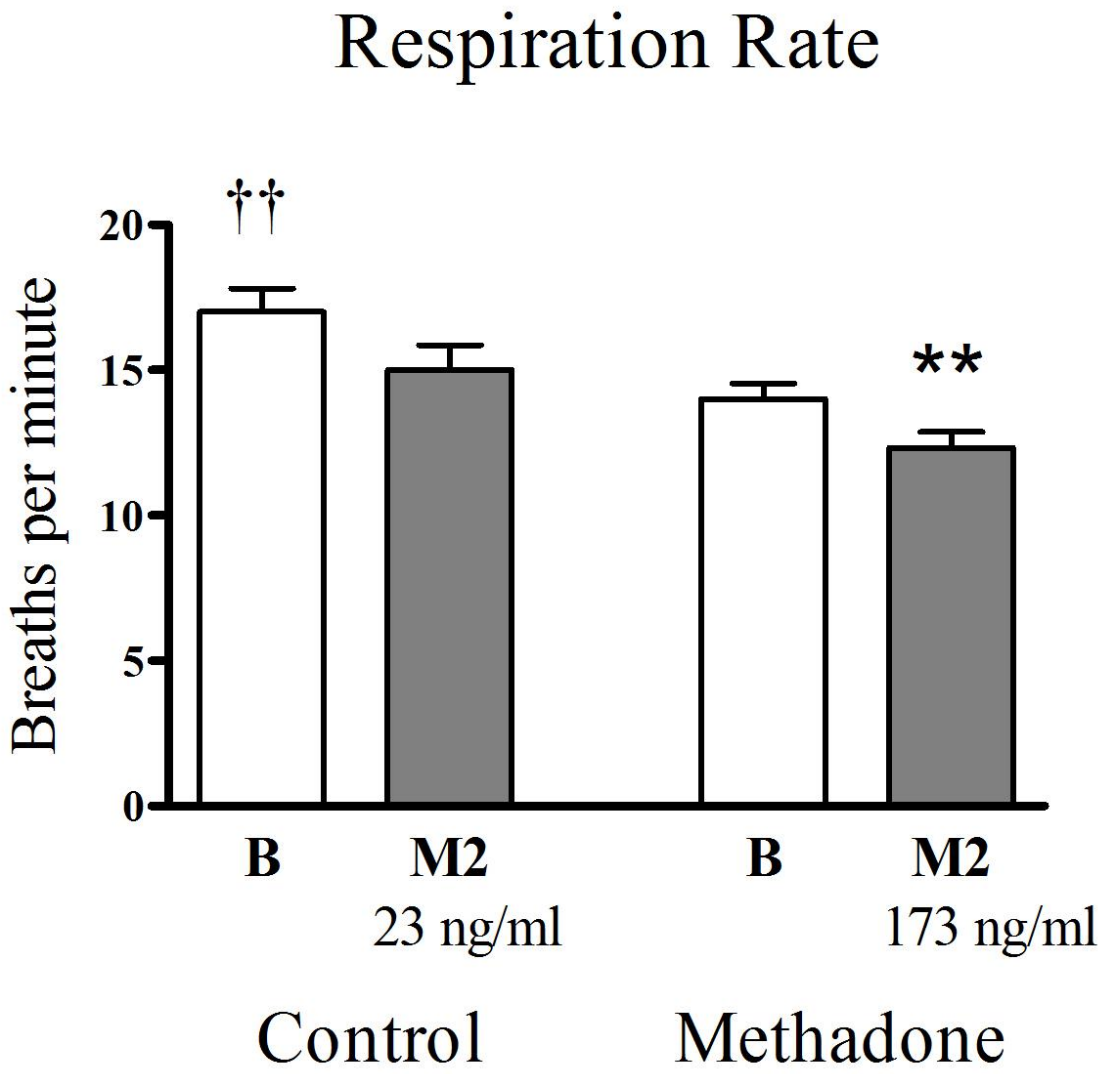

Figure 6 Respiration rate responses at different plasma morphine concentrations, Baseline (B) and Morphine Infusion 2 (M2). Data (as mean ± SEM) are shown for methadone maintained and healthy control participants. **P<0.01 compared to baseline (0 ng/ml). ††P<0.01 methadone subjects versus control subjects.

3.3.6. Post methadone maintenance dosing

3.3.6.1. Cold Pressor

Combined methadone participants' cold pressor pain detection threshold and pain tolerance values at Pre (0 hours) and Post (2 hours) methadone dose administration on the saline administration day are shown in Figure 7. This was measured on the saline administration day.

There was a significant increase for methadone participants at 2 hours post methadone dose administration for pain detection threshold (P=0.002; 95% CI 1.2 to 4) and pain tolerance (P=0.003; 95% CI 1.5 to 6). Within-group comparisons show that while there was no change in cold pressor pain detection threshold values for methadone participants in daily dose groups 11-45 (P=0.05) or 81-115 (P=0.07) mg per day, there was a significant increase from time of dose to 2 hours post methadone administration for daily dose group 46-80 (P=0.04 95% CI 0.5 to 9) mg per day. In the cold pressor pain tolerance test, while there was no change in values for methadone participants in daily dose groups 11-45 (P=0.42) or 81-115 (P=0.07) mg per day, there was a significant increase from time of dose to 2 hours post methadone administration for daily dose group 46-80 (P=0.04 95% CI 0.3 to 11) mg per day.

3.3.6.2. Electrical stimulation

Combined methadone participants' electrical stimulation pain detection threshold and pain tolerance values at Pre (0 hours) and Post (2 hours) methadone dose administration on the saline administration day are shown in Figure 7.

There was a significant increase at 2 hours post methadone dose administration for electrical stimulation pain tolerance (P=0.04; 95% CI 0.2 to 11), but not pain detection threshold (P=0.63). There was no change in electrical stimulation pain detection threshold values for methadone participants in daily dose groups 11-45 (P=0.51), 46-80 (P=0.16) or 81-115 mg/day (P=0.8) between time of dose and two hours post methadone administration. In the electrical stimulation pain tolerance test, within-group comparisons show that there was no change in values for methadone participants between time of dose and 2 hours post methadone administration for daily dose groups 11-45 (P=0.40), 46-80 (P=0.05) or 81-115 (P=0.48).

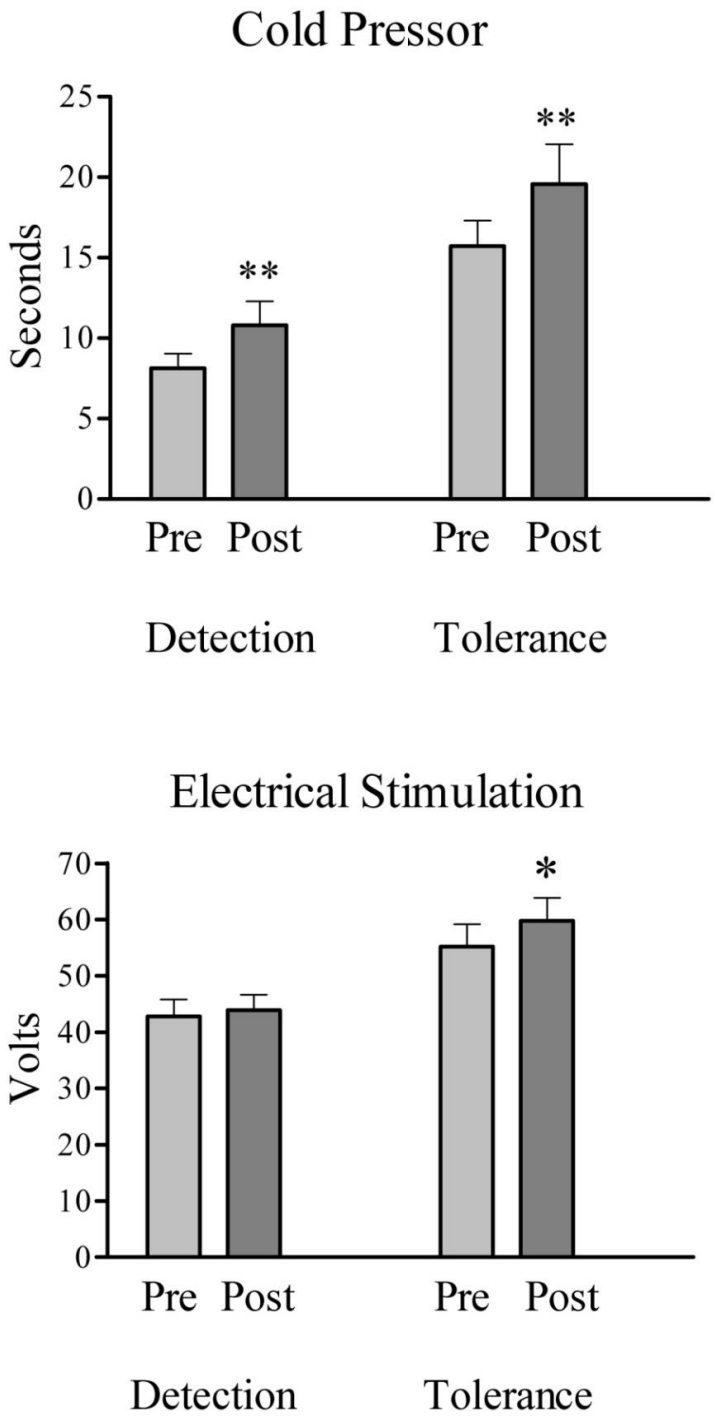

Figure 7 Pain detection threshold and pain tolerance values in cold pressor (upper panel) and electrical stimulation (lower panel) immediately prior to (Pre) and 2 hours after (Post) methadone administration. Data (as mean ± SEM) were collected from methadone subjects on days when only saline was administered. *** P<0.001, * P<0.05 0 vs. 2 hours.

3.3.7. Plasma methadone concentration and cold pressor

Linear regression analysis showed that there was not a statistically significant relationship (r^2=0.20, P=0.06) between plasma R-(-)- methadone concentrations and cold pressor pain tolerance scores at baseline on the saline administration day (Figure 8).

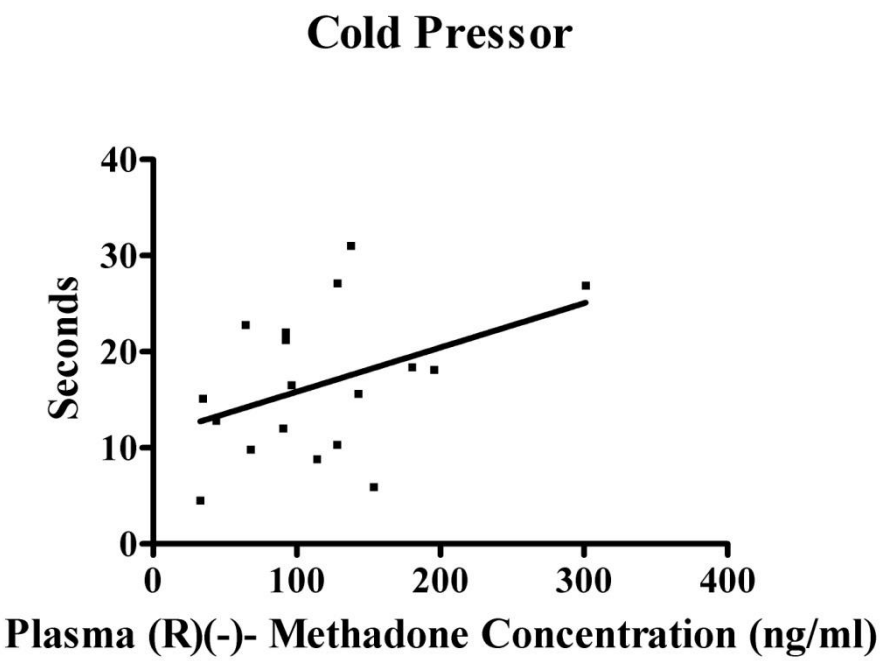

Figure 8 Linear regression analysis of plasma R-(-) methadone concentrations and cold pressor pain tolerance values at baseline on the saline administration day (r^2=0.20, P=0.06) in the 18 methadone maintained subjects. Spearman's correlation was p=0.08, r=0.4, 95% confidence intervals of -0.07 to 0.7.

3.3.8. Adverse events

There were no serious adverse events in the course of the study. Seven control subjects required metoclopramide hydrochloride 10 mg (Pfizer, Perth, Australia) intramuscularly for mild vomiting with good effect. The adverse events occurred at various times up to two hours after the second of the morphine infusions (M2). One control subject was unable to complete the procedure due to difficulty in obtaining venous access.

3.4. Discussion

Very high doses of intravenous morphine (55 mg) failed to provide antinociception for methadone subjects in either the electrical stimulation or cold pressor tests. Power analysis indicated that a statistically significant antinociceptive effect is likely to be obtained with a sample size of eighty methadone participants; however, with a mean increase of 1.4 seconds in the cold pressor test, this would not be clinically significant. This high dose of morphine is nearly five times the 12 mg dose shown to be effective in controls using the same methods. It is also more than five times the morphine dose (10 mg/70 kg body weight) that has been shown to significantly depress ventilation and elevate end tidal carbon dioxide tension in controls (Arunasalam et al. 1983, Daykin et al. 1986). While it had little antinociceptive effect in methadone subjects, 55 mg of morphine significantly decreased respiration rate, but only by an average of 2 breaths per minute.

Whilst methadone participants showed virtually complete tolerance to the antinociceptive effects of high dose morphine, tolerance to the respiratory depressant effects was less complete. This is consistent with other evidence indicating that the respiratory depressant effects of methadone and other opioids may exhibit incomplete tolerance (Crettol et al. 2007, Megarbane et al. 2007). There were no differences in the baseline respiratory and antinociceptive responses of the different methadone groups in their response to morphine. This indicates extensive tolerance exists even in those on doses and plasma concentrations of methadone that are relatively low by usual standards of maintenance treatment. Interestingly, the greatest degree of respiratory depression was found following the administration of morphine to the high dose methadone group. Rather than being more tolerant, the high plasma methadone concentration increased the high morphine respiratory depressant effect.

The mechanisms underlying tolerance have been described at both the intracellular and supracellular level (e.g. at the level of neuronal circuits) (Chen et al. 2010). The adaptations occurring at the intracellular level include mu opioid receptor phosphorylation, beta-arrestin binding, receptor endocytosis and recycling (Dang and Christie 2012). Differences in rate and extent of tolerance development for different opioid effects may be due to regional differences in

adaptations through the brain. Alternatively, supracellular adaptations may operate to modulate tolerance to specific opioid effects (Nestler 1997, Dang and Christie 2012).

It has been suggested, on the basis of case studies and expert opinion, that in comparison with the opioid requirements for pain treatment in healthy controls, methadone maintained patients require either "normal" doses (Kantor et al. 1980), slightly larger doses given more frequently (Kreek and Reisinger 1997) or 3-4 times the "normal" opioid dose (Rapp et al. 1995). The results in this study suggest that, at least in the case of morphine and in the context of experimental pain, five times the morphine dose found to be effective in controls was not able to produce antinociception in methadone subjects. In addition, the observation of respiratory depression, albeit of small magnitude, at these extremely high plasma morphine and R-(-)- methadone concentrations, suggests that pursuing even higher plasma morphine concentrations to achieve antinociception may be unproductive. Alternative strategies such as a different opioid from morphine with different receptor properties (e.g. remifentanil) may be more effective.

There has been an increase in the use of methadone for the treatment of cancer pain (Caraceni et al. 2012) and there is evidence that methadone may provide analgesia in patients refractory to other opioid agonists (Fredheim et al. 2008, Modesto-Lowe et al. 2010). The evidence presented in this study and from previous work in this department (Dyer et al. 1999, Doverty et al. 2001a, Doverty et al. 2001b) suggests that, as the daily methadone dose provides some antinociception 2 hours after dosing, methadone patients may respond to the analgesic effect of additional methadone administered more frequently. Methadone itself may therefore be an appropriate opioid to evaluate for its efficacy in methadone maintenance patients who experience acute pain.

There is evidence from both animal (Celerier et al. 2000, Mao 2002, Lee et al. 2011) and clinical (Compton et al. 2000, Guignard et al. 2000, Doverty et al. 2001a, Chu et al. 2008, Mitra 2008, Lee et al. 2011) studies that sustained or repeated administration of opioids may result in the development of increased pain sensitivity (hyperalgesia). The consequent increase in dose required for analgesia may be interpreted as tolerance (Chu et al. 2008). This study confirmed that methadone participants are hyperalgesic in the cold pressor test and, although not significant in the present study, tolerated less electrical stimulation than controls. A second strategy for pain relief in methadone maintenance patients could therefore be to try to reduce hyperalgesia. Approaches to this could include administration of NMDA antagonists, NOS inhibitors or other compounds aimed at minimizing the adaptations underlying hyperalgesia (Angst et al. 2003, Koppert et al. 2003, Bujalska et al. 2008, Mitra 2008, Chen et al. 2010, Lee et al. 2011). To date, these have not been demonstrated to be effective in humans, but remain an important direction for future research (Mitra and Sinatra 2004, Mitra 2008).

In conclusion, methadone subjects are hyperalgesic relative to healthy controls and cross-tolerant to the antinociceptive effects of morphine at concentrations 3-4 times higher than those reported to be adequate for severe post-surgical pain relief in opioid naïve patients. These high doses of morphine caused small but significant decreases in respiratory rates that were greatest in those maintained on the highest methadone dose.

4. Study 2. Antinociceptive and respiratory effects of high dose morphine in buprenorphine maintained subjects

4.1. Introduction

Buprenorphine is a partial agonist at the mu opioid receptor and its long duration of action and safety profile make it a popular agent for opioid substitution therapy (Johnson et al. 1992, Ling et al. 1998, Johnson et al. 2005, Pinto et al. 2010). While it has proven analgesic effect and good lipophilicity, there have been reservations over its clinical use for analgesia due to misunderstanding over a so-called 'ceiling effect' (bell shaped response curve) for both respiratory depression and analgesia (Kress 2008, Pergolizzi et al. 2010). While there is evidence for a ceiling effect for respiratory depression (see below), the existence of a 'ceiling effect' for analgesia has been challenged in both animal (Christoph et al. 2005) and in human studies (Dahan et al. 2006, Yassen et al. 2006, Pergolizzi et al. 2010). As a consequence, it is likely that buprenorphine will increase in popularity in the future as an analgesic agent for cancer and moderate to severe non-cancer pain treatment (Kress 2008, Pergolizzi et al. 2010). Similarly to methadone, chronic exposure to buprenorphine has been shown to produce both a greater pain sensitivity (hyperalgesia) (Compton et al. 2001) and a cross-tolerance to opioids (Bickel et al. 1988, Rosen et al. 1994, Huxtable et al. 2011). These factors complicate the treatment of acute pain in buprenorphine maintained patients.

The challenge of producing pain relief in opioid maintained patients is well recognised (Mitra and Sinatra 2004, Alford et al. 2006, Huxtable et al. 2011). There is evidence that subjects maintained on methadone for opioid addiction are hyperalgesic to experimental pain and experience little antinociceptive effect at standard clinical doses of morphine (Doverty et al. 2001a, Doverty et al. 2001b). Results described in Study 1 (Antinociceptive and respiratory effects of high dose morphine in methadone maintained subjects (Chapter 3)) and subsequently published suggest that very high doses of morphine provide little antinociception for patients maintained on methadone for the treatment of opioid dependence (Athanasos et al. 2006).

Buprenorphine has a unique pharmacological profile. Full agonists such as morphine, over concentration ranges that cause dose-related increases in analgesia, cause concentration-dependent respiratory depression without any plateau or ceiling in healthy human volunteers (Dahan et al. 2006). In contrast, buprenorphine, while showing full agonism at mu opioid receptors, shows partial agonism at the mu opioid receptors that are involved in respiratory depression (Dahan et al. 2006). It also shows slower receptor dissociation kinetics in comparison with full agonists such as fentanyl (Dahan et al. 2006). As a partial agonist, under appropriate conditions, buprenorphine may act as an agonist or antagonist at the opioid receptors (Strain et al. 1995). Buprenorphine, unlike

full opioid agonists, has an anti-hyperalgesic effect in experimental pain models in healthy subjects (Koppert et al. 2005). What effect this complex pharmacology has on the pain management (with opioids) of buprenorphine maintained patients is not known and the issue has been raised previously (Koltzenburg et al. 2006, Russo 2006) .

Various authors have shown that the magnitude of the daily buprenorphine dose has an effect on the blockade of opioid effects (Bickel et al. 1988, Rosen et al. 1994, Walsh et al. 1995, Comer et al. 2005). Higher doses of buprenorphine have been associated with better retention in treatment (Fareed et al. 2012). A recent study in a population of youths ages 15-21, found that there was a relationship between buprenorphine-naloxone dose and their pain experience. Those with greater pain prior to induction required higher doses of buprenorphine (Chakrabarti et al. 2010). Typical buprenorphine doses range from 2 mg to 20 mg per day. To date, no studies have examined the effect of different daily buprenorphine maintenance doses on the antinociceptive and respiratory responses to morphine.

The aim of the study was to examine whether very high intravenous morphine doses (compared to clinically used analgesic doses with healthy control subjects) produce antinociceptive and respiratory effects in buprenorphine maintained participants and to determine whether the magnitude of the daily buprenorphine dose affects these responses.

4.2. Methods

The subjects, procedures and statistical analyses for the buprenorphine high dose morphine study were as described in detail in chapter 2.

4.2.1. Subjects

In summary, twelve buprenorphine subjects were infused with high dose morphine and their responses during the cold pressor and electrical stimulation tests and respiration rates measured. They were stratified according to buprenorphine dose with four subjects in each of the daily oral dose ranges of 2 to 8mg (n=4), 9 to 15 (n=4) and 16-22 (n=4) mg per day. A control group of ten healthy control subjects, with a similar mean age and weight, was infused with lower doses of morphine and the same responses measured.

4.2.2. Drug administration

As stated previously, morphine sulphate (David Bull Laboratories, Melbourne, Australia) infusions of 1 mg/ml were administered intravenously in two sixty-minute stages to achieve two consecutive target pseudo steady-state plasma concentrations. This procedure has been previously described by Doverty et al (2001a) and utilised a syringe driver infusion pump (3100 Graseby Syringe Pump, Watford, Hertfordshire, UK). Buprenorphine subjects were administered an initial bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 8.3 mg/hr for one hour to

achieve a target pseudo steady-state plasma concentration of 80 ng/ml (Morphine 1). They were then administered an additional bolus of 15.2 mg of morphine sulphate followed by a constant infusion of 16.5 mg/hr for one hour to achieve the second target pseudo steady-state plasma concentration of 180 ng/ml (Morphine 2).

Control subjects were administered an initial bolus of 2.2 mg morphine sulphate followed by a constant infusion of 1.2 mg/hr for one hour to achieve a target pseudo steady-state plasma concentration of 11 ng/ml (Morphine 1). They were then administered 4.95 mg of morphine sulphate followed by a constant infusion of 3.6 mg/hr to achieve the second target pseudo steady-state plasma concentration of 33 ng/ml (Morphine 2).

As stated previously, one control group served as comparison in both the methadone and buprenorphine morphine studies. Subjects were tested on two occasions, at least five days apart; once with morphine, once with saline placebo. The order of administration was randomised.

4.3. Results

Buprenorphine subject and healthy control subject demographics are described in chapter 2.

4.3.1. Plasma morphine concentrations

Pseudo steady-state plasma morphine concentrations in control subjects and the combined three buprenorphine daily dose groups (combined buprenorphine group) in morphine infusion 1 period and morphine infusion 2 period are shown in Figure 9 and Table 4.

One buprenorphine subject had a mean plasma morphine concentration of 22 ng/ml at baseline on the saline administration day. This subject denied having used any illegal substances in the previous 48 hours and showed no signs of intoxication to the clinically experienced staff. The subject was subsequently retested on a saline administration day five days later and had a mean plasma morphine concentration of 0 ng/ml at baseline.

Concentrations				
Buprenorphine Morphine Study				
Plasma Drug Concentration	Group	Baseline	Morphine 1	Morphine 2
Morphine (ng/ml)	Control		7.0±0.4 (Range 5 to 9)	23±1 (19 to 32)
	Combined Buprenorphine		62±4 (42 to 87)	136±10 (92 to 201)
	2-8 mg		70±8 (49 to 91)	175±15 (119 to 201)
	9-15mg		60±4 (48 to 71)	129±9 (48 to 108)
	16-22 mg		57±4 (52 to 71)	109±8 (92 to 129)
Buprenorphine (ng/ml)	Combined Buprenorphine	1.2±0.3 (0.23 to 3.3)	0.95±0.19 (0.16 to 0.23)	1.03±0.23 (0.16 to 3.0)
	2-8 mg	0.71±0.23 (0.42 to 1.17)	0.46±0.12 (0.16 to 0.76)	0.45±0.10 (0.16 to 0.58)
	9-15 mg	1.45±0.45 (0.21 to 2.20)	1.14±0.36 (0.90 to 1.75)	1.40±0.53 (0.26 to 2.7)
	16-22 mg	1.17±0.28 (0.8 to 1.98)	1.23±0.24 (0.79 to 1.79)	1.33±0.22 (0.79 to 1.87)
Norbuprenorphine (ng/ml)	Combined Buprenorphine	1.7±0.3 (0.30 to 3.63)	1.61±0.33 (0.31 to 3.72)	1.85±0.40 (0.34 to 3.53)
			Saline 1	Saline 2
Buprenorphine (ng/ml)	Combined Buprenorphine	1.2±0.3 (0.15 to 4.07)	1.01±0.26 (0.15 to 3.3)	1.18±0.29 (0.15 to 3.03)
	2-8 mg	0.38±0.10 (0.15 to 0.64)	0.30±0.006 (0.15 to 0.41)	0.33±0.08 (0.15 to 0.48)
	9-15 mg	1.59±0.68 (0.23 to 3.30)	1.16±0.46 (0.24 to 2.3)	1.3±0.6 (0.19 to 2.98)
	16-22 mg	1.84±0.76 (0.69 to 4.07)	1.6±0.59 (0.61 to 3.31)	1.81±0.53 (0.63 to 3.03)
Norbuprenorphine (ng/ml)	Combined Buprenorphine	1.78±0.34 (0.28 to 3.9)	1.68±0.3 (0.29 to 3.4)	1.93±0.42 (0.24 to 4.72)

Table 4 Plasma concentrations of morphine, buprenorphine and norbuprenorphine in the buprenorphine morphine study on morphine and saline administration days in buprenorphine maintained subjects. Data are mean±SEM (range).

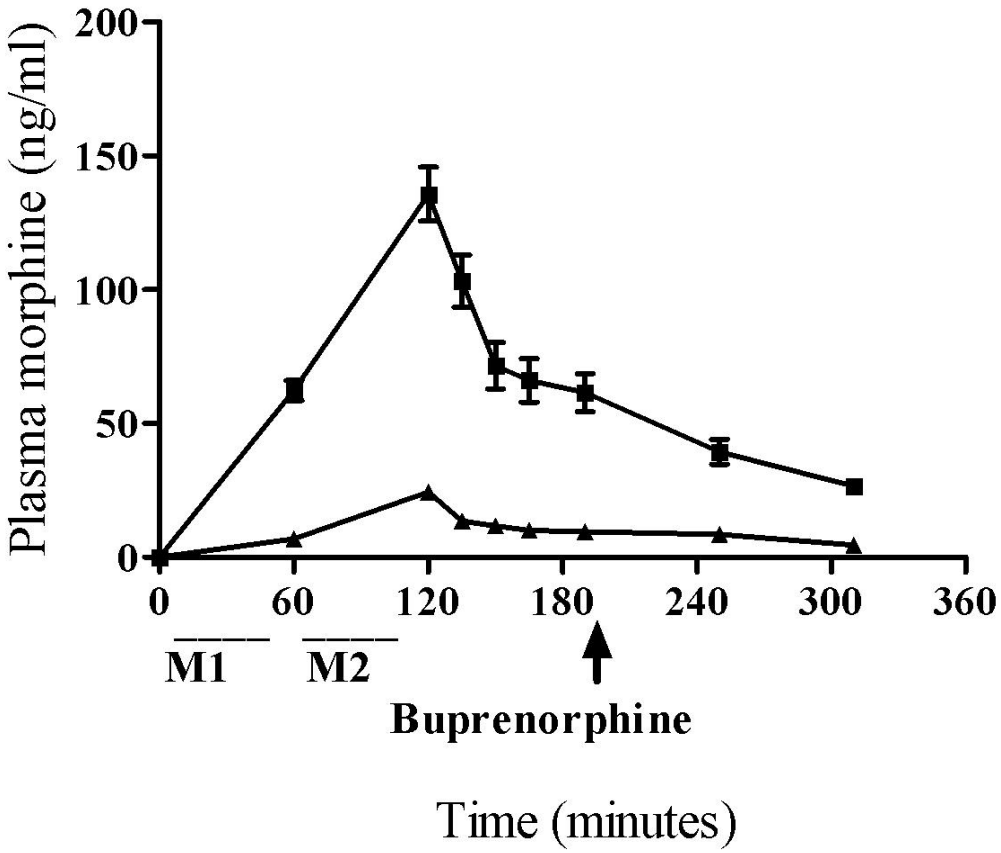

Figure 9 Plasma morphine concentrations in 12 buprenorphine maintained (■) and 10 healthy control subjects (▲). Pseudo steady-state plasma morphine concentration 1 (M1), pseudo-steady-state plasma morphine concentration 2 (M2) and the time of the buprenorphine dose administration are indicated (↑). Data are represented as mean ± SEM.

4.3.2. Plasma buprenorphine and norbuprenorphine concentrations

The combined buprenorphine subjects' mean plasma buprenorphine and norbuprenorphine concentrations on saline and morphine administration days are shown in Figure 10 and Table 4. No significant differences were detected between mean plasma buprenorphine concentrations on morphine and saline placebo days at baseline (P=0.64), morphine/saline infusion 1 (P=0.71) or morphine/saline infusion 2 (P=0.51) or between mean norbuprenorphine concentrations on morphine and saline placebo days at baseline (P=0.71), morphine/saline infusion 1 (P=0.73) or morphine/saline infusion 2 (P=0.83).

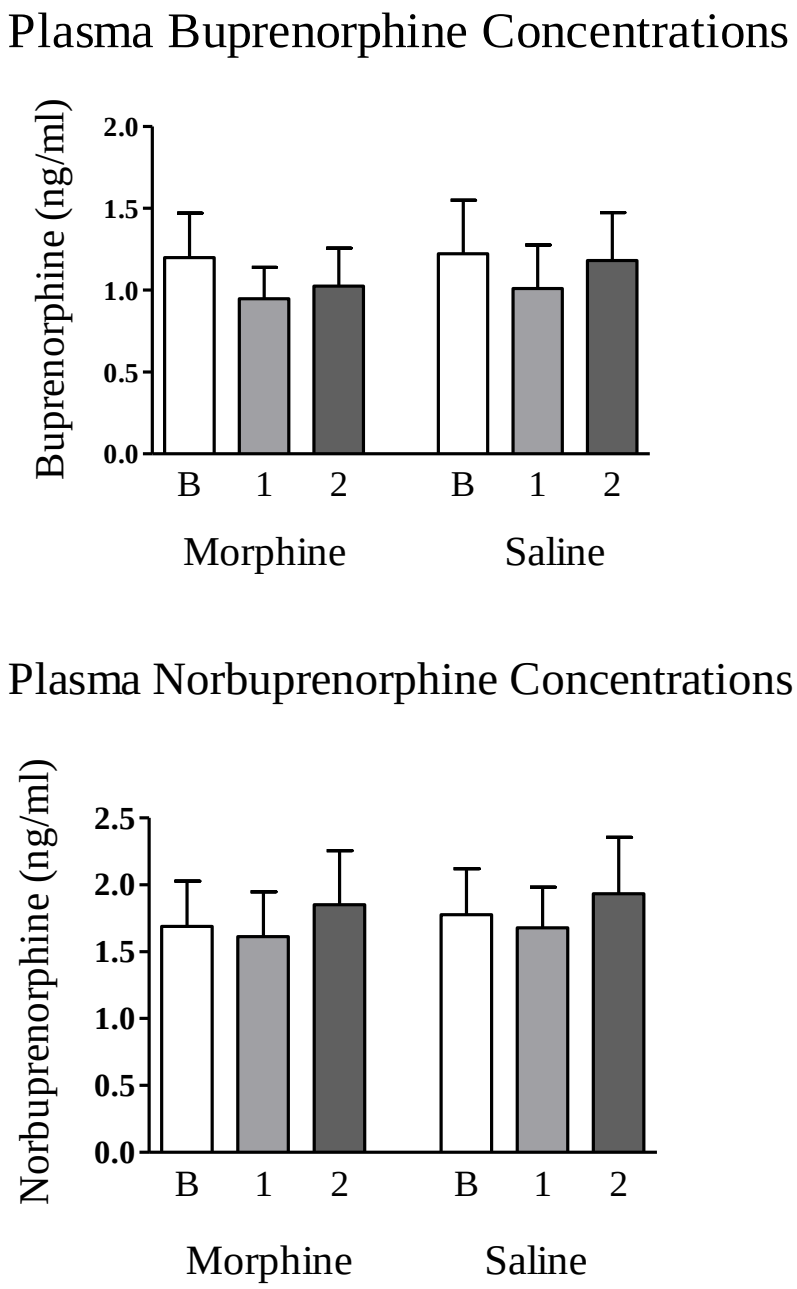

Figure 10 Plasma buprenorphine concentrations (upper panel) at baseline (white), infusion 1 (light grey) and infusion 2 (dark grey) on morphine and saline administration days. Plasma norbuprenorphine concentrations (lower panel) at baseline (white), infusion 1 (light grey) and infusion 2 (dark grey) on morphine and saline administration days. Results are represented as mean ± SEM.

4.3.3. Cold Pressor

Control and combined buprenorphine subjects' pain tolerance responses in the cold pressor test at baseline and morphine infusion 2 are shown in Figure 11 (upper panel) and Table 5. Cold pressor values for combined buprenorphine subjects were significantly different to controls (P=0.009; 95% CI 5 to 30) at 0 hours. Within-group comparisons revealed that pain tolerance values for control participants increased significantly (P=0.04) from baseline to Morphine 2 (52±11 range 6.6-123 seconds) (P<0.05; 95% CI 2 to 34), but not baseline to Morphine 1 (38±7 range 4.6-64 seconds) (P>0.05).

There were no significant changes (P=0.99) in cold pressor pain tolerance values for combined buprenorphine subjects from baseline to morphine infusion 1 or morphine infusion 2. There were also no significant differences in the cold pressor pain tolerance test for buprenorphine subjects in the 2-8 (P=0.45), 9-15 (P=0.66) or 16-22 (P=0.94) mg per day group from baseline to morphine infusion 1 or morphine infusion 2. Similarly, there were no significant differences between the groups (2-8, 9-15 or 16-22 mg per day) of buprenorphine subjects at baseline (P=0.95) or infusion 2 (P=0.93) in the cold pressor pain tolerance test.

Responses				
Buprenorphine Morphine Study				
Response	Group	Baseline	Morphine 1	Morphine 2
Cold Pressor (seconds)	Control	34±6 (4 to 73)	38±7 (5 to 64)	52±11 (7 to 23)*
	Combined Buprenorphine	17±2 (9 to 18) ††	17±2 (4 to 29)	17±2 (4 to 27)
Electrical Stimulation (volts)	Control	65±6 (38 to 100)	68±5 (48 to 100)	74±5 (60 to 100) **
	Combined Buprenorphine	53±5 (24 to 92)	53±4 (24 to 72)	53±5 (34 to 96)
Respiration Rate (breaths per minute)	Control	17.0±0.8 (14 to 22)	16.4±0.6 (13 to 19)	15.0±0.9 (10 to 19)
	Combined Buprenorphine	14.4±0.8 (9 to 20) †	12.8±0.7 (13 to 19)	11.8±0.5 (9 to 15) **
	2-8 mg (P=0.024)	15.5±1.6 (13 to 20)	11.5±0.9 (10 to 13)*	11.5±1.3 (9 to 15)*
	9-15 mg (P=0.004)	15±1.2 (12 to 17)	15±1.1 (12 to 17)	11.5±0.6 (10 to 13)**
	16-22 mg (P=0.016)	14.8±0.5 (14 to 16)	12.3±0.6 (11 to 14)*	12.8±1.3 (10 to 16)*

Table 5 Cold pressor and electrical responses and respiration rates for buprenorphine maintained and control subjects in the buprenorphine morphine study on morphine administration days. Data are mean±SEM (range). † P<0.05, †† P<0.01 between group; * P<0.05, ** P<0.01 between treatments.

4.3.4. Electrical stimulation

Control and combined buprenorphine subjects' pain tolerance responses in the electrical stimulation test at baseline and morphine infusion 2 are shown in Figure 11 (lower panel). Electrical stimulation values for combined buprenorphine subjects were not significantly different to controls (P=0.13) at 0 hours. Within-group comparisons revealed that pain tolerance values for control subjects increased significantly (P=0.007) from baseline to morphine infusion 2 (P<0.01; 95% CI 3 to 16), but not baseline to morphine infusion 1 (P>0.05; 95% CI -2.8 to 10).

There was no significant change (P=0.98) in electrical stimulation pain tolerance values for combined buprenorphine subjects from baseline to morphine infusion 1 or morphine infusion 2. Within-group comparisons showed that there were no antinociceptive effects in the electrical stimulation pain tolerance test for buprenorphine subjects in the 2-8 (P=0.43), 9-15 (P=0.67) or 16-22 (P=0.42) mg per day group from baseline to morphine infusion 2. There were also no significant differences between the groups (2-8, 9-15 or 16-22 mg per day) of buprenorphine subjects at baseline (P=0.95) or morphine infusion 2 (P=0.72) in the electrical stimulation pain tolerance test.

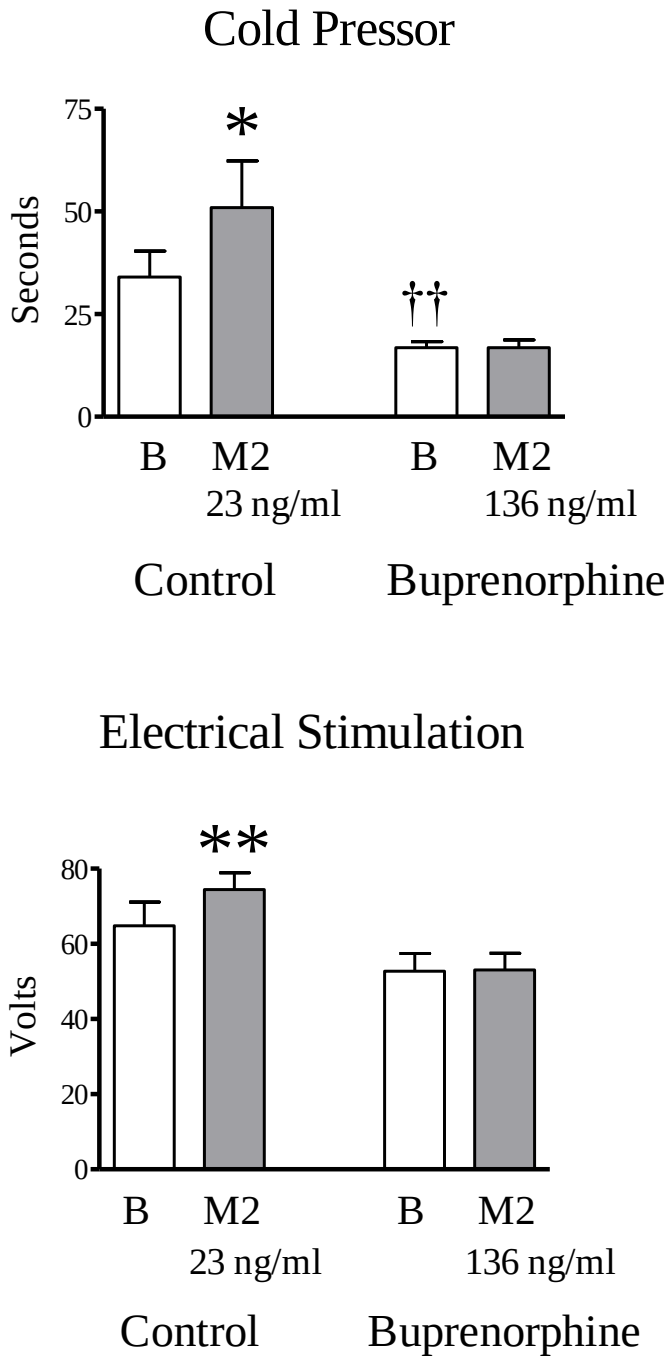

Figure 11 Cold pressor (upper panel) and electrical stimulation (lower panel) mean (± SEM) pain tolerance responses in 10 control and 12 buprenorphine subjects at baseline (B) and morphine infusion 2 (Morphine 2). †† $P<0.01$ between groups; * $P<0.05$; ** $P<0.01$ between treatments. Note different morphine concentrations between buprenorphine and control subjects.

4.3.5. Respiration rate

Respiration rate (breaths per minute) relative to baseline and morphine infusion 2 is shown in Figure 12 and Table 5. Respiration rate for combined buprenorphine subjects was significantly lower than for control subjects at baseline (P=0.03; 95% CI -0.25 to -4.9). Within group comparisons revealed that the respiration rate for control subjects did not decrease significantly (P=0.09) from baseline to morphine infusion 1 or morphine infusion 2. Respiration rate for combined buprenorphine subjects decreased significantly (P=0.006) from baseline to morphine infusion 2 (P<0.01; 95% CI -0.9 to -4.4) but not morphine infusion 1 (P>0.05; 95% CI -2.8 to 10).

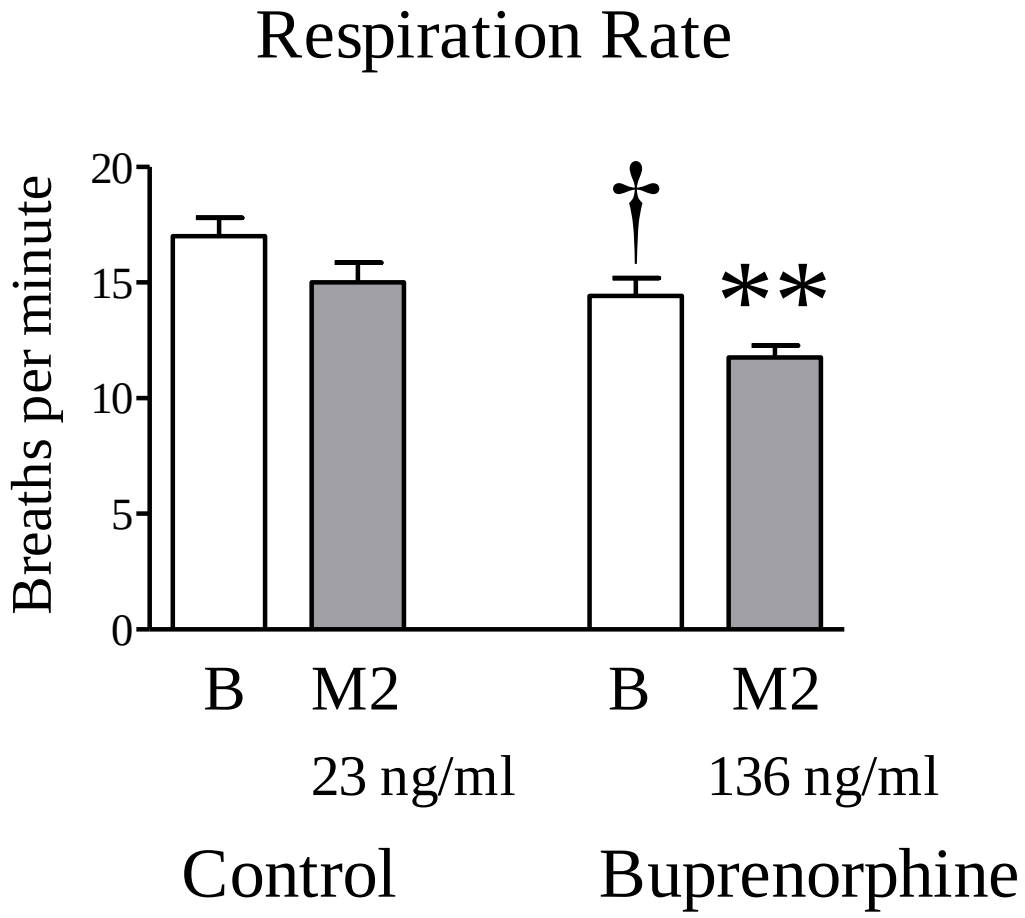

Figure 12 Mean (± SEM) respiration rates (breaths per minute) in 10 control and 12 buprenorphine subjects at baseline and morphine infusion 2 (Morphine 2). † $P<0.05$ between groups, ** $P<0.01$ between treatments. Note different morphine concentrations between the two groups.

Within-group comparisons showed that there were significant changes in respiration rate for dose group 2-8 mg per day (P=0.024) from baseline to morphine infusion 1 (P<0.05; 95% CI -0.56 to -7.4) and baseline to morphine infusion 2 (P<0.05; 95%CI -0.56 to -7.4) (Table 5). There was also significant change in respiration rate for dose group 9-15 mg per day (P=0.004) between baseline and morphine infusion 2 (P<0.01; 95% CI –1.48 to -5.52), but not morphine infusion 1 (P>0.05; 95% CI -2.02 to 2.02). For the dose group 16 to 22 mg per day, there were significant changes (P=0.016) between both baseline and morphine infusion 1 (P<0.05; 95% CI -0.72 to -4.28) and baseline and morphine infusion 2 (P<0.05; 95% CI -0.22 to -3.78). There were no significant differences in respiration rate between the groups (2-8, 9-15 or 16-22 mg per day) of buprenorphine subjects at baseline (P=0.90) or morphine infusion 2 (P=0.67). One buprenorphine participant (nine breaths per minute) and one control participant (ten breaths per minute) achieved the lowest rates of their respective groups.

4.3.6. Concentrations and responses of buprenorphine and methadone subjects

There was a significant difference (p=0.01; 95% CI 9-72) in morphine concentrations between buprenorphine (136±10 range 92-201 ng/ml) and methadone subjects (173±11 range 106-305 ng/ml) at morphine infusion 2. There were no significant differences between buprenorphine and methadone subjects in the cold pressor test at baseline (p=0.2) or at morphine infusion 2 (p=0.3), in the electrical stimulation test at baseline (p=0.8) or at morphine infusion 2 (p=0.9), or for their respective respiration rates at baseline (p=0.6) or at morphine infusion 2 (p=0.5).

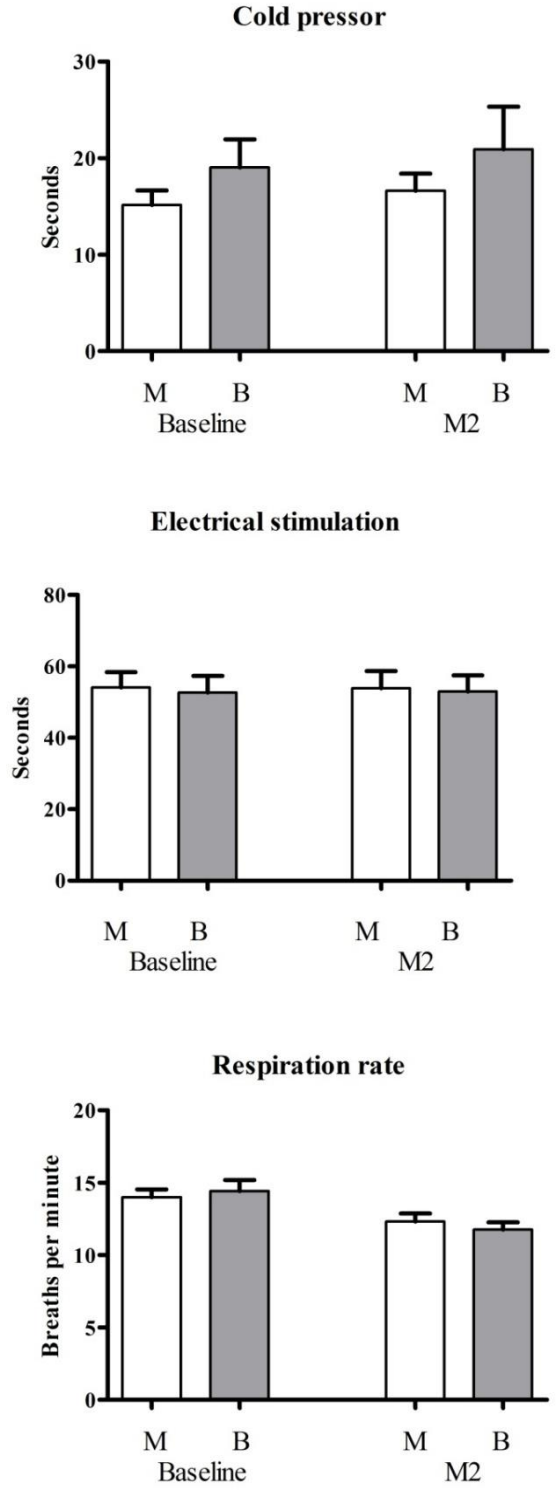

Figure 13 Mean (± SEM) Cold pressor (upper panel), electrical stimulation (middle panel) and respiration rates (lower panel) in 18 methadone (M) and 12 buprenorphine (B) maintained subjects at baseline and second morphine infusion (M2). There were no statistically significant differences between the groups at either baseline or second morphine infusion.

4.3.7. Responses following buprenorphine maintenance dosing

There was no change detected (P=0.21) for buprenorphine subjects in the cold pressor test between time of buprenorphine dose (16.5±2; range 6 to 30 seconds) and 2 hours post dose administration (17±2; range 7 to 32 seconds), or the electrical stimulation test (P=0.68) between time of dose (49±5; range 12 to 80 volts) and 2 hours post administration (50±6; range 22 to 88 volts) (the putative maximum concentration). Likewise there was no change detected in respiration rate (P=0.060) between time of buprenorphine dose (14.4±1; range 9 to 19 breaths per minute) and 2 hours post dose (13.4±0.6 range 10 to 16 breaths per minute).

4.3.8. Adverse events

There were no serious adverse events in the course of the study. No buprenorphine subjects experienced nausea or vomiting. Seven control subjects required metoclopramide hydrochloride 10 mg (Pfizer, Perth, Australia) intramuscularly for mild vomiting with good effect. One control subject was unable to complete the procedure due to difficulty in obtaining venous access.

4.4. Discussion

Very high doses of morphine (55 mg) associated with high plasma concentrations failed to provide antinociception for buprenorphine subjects in either the electrical stimulation or cold pressor tests irrespective of maintenance buprenorphine dose. Morphine doses nearly one-fifth (12 mg) of that administered to buprenorphine subjects, provided antinociception to control subjects in both tests of antinociception. Buprenorphine subjects performed similarly to methadone subjects in the same experimental paradigm (described in chapter three) in at least three respects; firstly, 55 mg of morphine had little antinociceptive effect in buprenorphine and methadone subjects; secondly, this dose statistically significantly decreased respiration rate for both groups (albeit only by an average of 1.5 breaths per minute); and thirdly both buprenorphine and methadone subjects were similarly hyperalgesic in the cold pressor test. As stated, the responses of methadone and buprenorphine maintained subjects in the cold pressor test were very similar (methadone subjects (15±2 range 5 to 25 seconds)) (buprenorphine subjects 17±2 (9 to 18)) (controls 34±6 (4 to 73). Variations in periods of time maintained on methadone ($p=0.96$) and buprenorphine ($p=0.68$) were not significantly correlated to cold pressor responses. There were no apparent pharmacokinetic interactions between morphine and plasma buprenorphine and norbuprenorphine concentrations. There was no change in pain response as a result of changes in plasma buprenorphine at trough and at peak concentrations.

Buprenorphine subjects were tolerant to the antinociceptive effects of very high doses of morphine. They were also hyperalgesic in the cold pressor test. While cross-tolerance to the antinociceptive effects of very high doses of morphine was complete, cross-tolerance did not occur to other effects of morphine. Respiration rates dropped significantly but by a limited amount (approximately 1.5 breaths per minute). Yassen et al (2006) analysed the pharmacokinetic/pharmacodynamic concentration effect relationships for buprenorphine in a clinical study of healthy control subjects. They found a ceiling effect for the respiratory depressant effect of buprenorphine administered acutely. If this was associated with a ceiling effect for analgesia, the utility of buprenorphine would be limited. However, over the concentration range that produced a ceiling effect in respiratory depression, there was no ceiling effect in analgesia (Dahan et al. 2006). This was in contrast to acutely administered morphine. In clinical studies, over a concentration range that produces a systematic increase in analgesia, morphine produces a concentration-dependent respiratory depression without any ceiling effect (Romberg et al. 2004, Dahan et al. 2008). In this study, very high doses of morphine were administered to subjects maintained on buprenorphine with a limited effect on respiratory depression. However, from this study it is unclear whether even higher doses of morphine might produce an unacceptable degree of respiratory depression.

There is increasing evidence that chronic severe pain is more prevalent in people with opioid dependence (Jamison et al. 2000, Rosenblum et al. 2003, Rosenblum et al. 2007, Barry et al. 2011) and this may contribute to the hyperalgesia experienced by the buprenorphine subjects. An observational study found that chronic pain patients with non-cancer chronic pain, taking either methadone or morphine, exhibited similar hyperalgesia in the cold pressor test to methadone and buprenorphine subjects (Hay et al. 2009). Chakrabarti et al (2010) found that people with a greater reported experience of pain prior to induction onto buprenorphine maintenance required greater daily doses. This study found no difference in hyperalgesia experienced at baseline between the three dose ranges (2-8, 9-15, 16-22 mg/day). In addition, methadone and buprenorphine maintained subjects were similarly hyperalgesic compared to controls in the cold pressor test at baseline. There were also no significant differences at baseline in the cold pressor test, electrical stimulation test or for their respective respiration rates.

An important consideration is whether the hyperalgesia experienced by subjects maintained on methadone, buprenorphine or morphine was present prior to induction or whether they were hyperalgesic as a consequence of opioid maintenance. Compton et al (2012) recently examined the pain experiences of opioid dependent individuals entering treatment. The subjects were examined prior to opioid maintenance treatment, once they had been stabilised and when they had been dosing chronically on methadone and buprenorphine. Compton et al (2012) found that prior to induction onto opioid substitution treatment, opioid dependent subjects were hyperalgesic in the cold pressor test. As in the series of studies described in this thesis, control subjects were able to tolerate the ice bath approximately twice as long as opioid dependent subjects. Compton et al (2012) stated that the opioid dependent subjects, prior to induction, had low baseline withdrawal scores (mean of 2.55) and were therefore not experiencing hyperalgesia as a consequence of withdrawal. The withdrawal scale utilised in their study was not described.

Compton et al (2012) also found that opioid addicted subjects were not hyperalgesic in the electrical stimulation test. Recent reviews of opioid induced hyperalgesia have described how pain responses vary with the type of pain stimulus used (Fishbain et al. 2009, Lee et al. 2011). The cold pressor test involves the stimulation of a larger skin surface area than other experimental pain testing modalities (such as electrical stimulation of the ear). Ruscheweyh et al (2010) has suggested that the cold pressor test is therefore a more potent activator of endogenous pain control mechanisms and consequently a more sensitive measure of hyperalgesia.

Compton et al (2012) suggested that subjects prior to induction onto opioid maintenance treatment were hyperalgesic and that transition to treatment with opioids worsens hyperalgesia. Unfortunately, baseline opioid use (time and size of last dose) was based on self-report and the degree to which this influenced pain measure at baseline is not known. Baseline withdrawal scores

were reported as low but withdrawal induced hyperalgesia may have influenced the result. Compton et al (2012) also found that, once stabilised in treatment, there was no difference in pain response between methadone and buprenorphine subjects. This supported the previous work of Compton et al (2001) who also found no significant difference between the responses of the buprenorphine and methadone maintained groups in the cold pressor test. This study found no significant difference in pain response between buprenorphine subjects and the methadone subjects from study 1.

As described in the Introduction, there is a substantial amount of pre-clinical (Chang et al. 2007) and clinical work (Vanderah et al. 2001a, Vanderah et al. 2001b, Ossipov et al. 2003, Chang et al. 2007, Chu et al. 2008) to suggest that while opioid administration provides analgesia, and promotes the development of analgesic tolerance (among other tolerances to different effects), it also sets into motion hyperalgesic processes (See Introduction). A number of authors have suggested that the two phenomena are part of the same process (Chang et al. 2007, Chu et al. 2008). However, neither animal work nor clinical work have identified what the relative contribution of these two phenomena is to the decrease in analgesic effect. This study found that subjects maintained on buprenorphine exhibited both hyperalgesia and opioid tolerance. More recent work, in a population of patients with chronic lower back pain, at daily maintenance doses relatively low compared to methadone and buprenorphine maintained clients, suggests that tolerance can occur in the absence of hyperalgesia (Chu et al. 2012).

The findings from this present study were supported by the overall findings of Compton et al (2012). In spite of the different pharmacology of the two maintenance agents, both methadone and buprenorphine maintained subjects were hyperalgesic in the cold pressor test and there were no significant differences between the two groups in the cold pressor test, electrical stimulation test and their respiration rate at baseline. In addition, it was found that, similarly to methadone subjects, buprenorphine subjects were cross-tolerant to the antinociceptive effects of 55 mg of morphine in the cold pressor and electrical stimulation test, and experienced significantly decreased respiration rates (albeit only by an average of 1.5 breaths per minute) as a consequence of this dose of morphine. As stated earlier, these doses of morphine would produce hypoxia and death in healthy control subjects but produce negligible analgesic effects with buprenorphine maintained subjects. It is unclear whether the decrease in respiration rate would be more pronounced if higher doses of morphine were used in this cohort of subjects.

5. Study 3. Antinociceptive and respiratory effects of high dose morphine and adjuvant analgesics in methadone maintained subjects

5.1. Introduction

Opioids are considered safe, effective and the primary treatment for moderate to severe pain (Collett 2001). Their use has increased in recent years for both cancer and non-cancer pain (Manchikanti et al. 2011, Caraceni et al. 2012). Yet, there are important unwanted side-effects of opioid analgesic therapy. One of these is the development of tolerance. Another is respiratory depression. As drug dosage is increased to overcome tolerance and maintain pain relief, respiratory depression may occur. To counter this, an alternative analgesic to an opioid is sometimes recommended (Mitra and Sinatra 2004, Brill et al. 2006, Macintyre et al. 2010, Huxtable et al. 2011).

Three major classes of alternative analgesics to traditionally used opioids include the non-steroidal anti-inflammatories (NSAIDS) (e.g. ketorolac), NMDA antagonists (e.g. S(+)-ketamine) or a mixed action analgesic (e.g. tramadol). They have different modes of action to opioids acting primarily at the mu opioid receptor (see1.16). Unfortunately, alternative analgesics used alone do not necessarily provide the same degree of pain relief as opioids (Macintyre et al. 2010). In this situation, the combination of an alternative analgesic (adjuvant analgesic) with an opioid may be advantageous (Huxtable et al. 2011). Such a combination approach may have three benefits. It may provide a multimodal coverage of a broad spectrum of pain, enable the individual agents to act in a greater than additive (synergistic) fashion and lower doses of each individual analgesic may result in a lower incidence of individual adverse events.

Non-steroidal anti-inflammatories (NSAIDS) are one of the most commonly used foundations of multimodal analgesia. Maund et al (2011) recently reviewed and contrasted the morphine sparing effect of paracetamol, NSAIDS and cyclo-oxygenase 2 inhibitors. Only the class of NSAIDS resulted in a statistically significant reduction in nausea and post-operative nausea and vomiting (albeit with increased bleeding). Cepeda et al (2005) conducted a large double blind randomised controlled trial of over 1000 patients and compared ketorolac with morphine and their combination for the provision of pain relief following surgery. They found that opioids were more effective than NSAIDS in providing pain relief. However, the addition of NSAIDS to opioid treatment reduced morphine requirements and opioid related side effects in the early postoperative period. In animal work, while ketorolac has been shown to decrease opioid dependence and withdrawal it has been shown to have no effect on tolerance (Trang et al. 2002, Dunbar et al. 2007). In the clinical setting, non-steroidal anti-inflammatories continue to be combined with opioids with

the aim of producing greater analgesia and decreased side-effects for given doses than either drug given separately (White 2008).

There is a substantial amount of evidence to suggest that glutamate via NMDA receptors plays a central role in the development and maintenance of such behavioural manifestations of pain facilitation as hyperalgesia and allodynia. It has been suggested that the NMDA blocking effect of ketamine prevents the development of tolerance and this leads to lower opioid requirements for pain management.(Mao et al. 1995, Mao et al. 1995, Celerier et al. 2000, Kissin et al. 2000, Vanderah et al. 2001a, Rivat et al. 2002, Fischer et al. 2005, Chang et al. 2007). In animal model work, Laulin and colleagues (2002) demonstrated that the combination of an ketamine and fentanyl can reduce or even reverse opioid induced analgesic tolerance. Similarly, Van Elstraete et al (2005) showed that prior administration of ketamine in rats prevented the development of hyperalgesia induced by a single dose of intrathecal morphine.

In two reviews of the addition of ketamine to opioid analgesia, both Subramaniam et al (2004) and Bell et al (2005) concluded that the addition of the NMDA antagonist ketamine to morphine reduces morphine requirements post operatively with minimal adverse effects. More recently, Chazan and co-workers have conducted a number of studies demonstrating how the combination of ketamine and morphine is more effective than morphine alone in decreasing post-operative pain (Chazan et al. 2008, Nesher et al. 2009, Rakhman et al. 2011). Ketamine has also been shown to be particularly effective in providing pain relief to opioid tolerant patients (Bell 1999, Eilers et al. 2001, Sator-Katzenschlager et al. 2001, Haller et al. 2002, Mitra and Sinatra 2004, Bell et al. 2005, Loftus et al. 2010, Laskowski et al. 2011, Weinbroum 2012). It is important to note that a number of these studies and reviews addressed opioid tolerance in chronic pain as well as post-operative pain management.

Tramadol has unique pharmacological actions. It is both an opioid agonist with selectivity for the mu opioid receptor and inhibits noradrenaline and serotonin reuptake (Scott and Perry 2000, Grond and Sablotzki 2004, Enggaard et al. 2006). Its major active metabolite, M1, also has analgesic potency. M1 has higher agonistic effect at the mu-opioid receptor than tramadol and inhibits monoamine reuptake (Goeringer et al. 1997). It has been shown to be more effective in the treatment of neuropathic pain than other weak opioids (Hempenstall et al. 2005). In in vitro studies tramadol reduces NMDA activity (Hara et al. 2005). This is in addition to its activity at the mu opioid receptor and the blocking of the reuptake of the monoamines. As reported above, NMDA antagonists could potentiate the antinociceptive effects of a range of opioid receptor agonists. Clinically, there is evidence that tramadol is effective alone and as an adjuvant to morphine and other opioids in the management of both post-operative pain and cancer pain (Scott and Perry 2000, Webb et al. 2002, Kocabas et al. 2005, Marinangeli et al. 2007).

Other opioid adjuvant analgesics include paracetamol, the gabapentanoids (gabapentin and pregabalin), clonidine and in chronic pain situations, tricyclic antidepressants and anticonvulsants such as sodium valproate (Khan et al. 2011). Paracetamol is not considered as effective in the provision of analgesia and as opioid sparing as other opioid adjuvants (Elia et al. 2005, Remy et al. 2005, Christensen et al. 2011). Gabapentin and pregabalin are used in the management of postoperative pain and some studies have shown them to be opioid sparing. However, recent large scale reviews have failed to find evidence for their effectiveness in acute pain management (Moore et al. 2009, Straube et al. 2010). Clonidine is an alpha 2 receptor agonist and is classed as an imidazoline. It has primarily been used as a treatment for hypertension. It has also been used for its opioid and anesthetic sparing effects (Gregoretti et al. 2009). It is not available in an intravenous form in the United States and this has limited its use in post-operative pain management trials in that country. Such issues as the risk of hypotension, bradycardia and somewhat unpredictable haemodynamic response to different doses have also limited its use in trials (Gregoretti et al. 2009). Tricyclic antidepressants and anticonvulsants, while effective in neuropathic pain, have not been shown to be effective for acute pain situations.

The opioid analgesic adjuvants ketorolac, S-ketamine and tramadol have been shown in experimental and clinical studies to provide analgesia and have different modes of action to opioids acting primarily at the mu opioid receptor. In addition, these drugs are combined with opioids in the clinical setting with the aim of producing greater analgesia and decreased side-effects for given doses than either drug given separately. Either adjuvant analgesics alone or adjuvant analgesics combined with high dose morphine may overcome the limitations of tolerance and side-effects to produce antinociception in the opioid maintained population.

The aim of this study was to examine whether S(+)-ketamine, ketorolac or tramadol alone or in combination with morphine produced antinociception in methadone subjects.

5.2. Methods

The subjects, procedures and statistical analyses for the high dose morphine and opioid adjuvant in methadone maintained subjects study are described in Chapter 2.

5.2.1. Subjects

To summarise, six methadone subjects (3 men and 3 women), ranging between 24 and 39 years with a mean age of 31 years were recruited. Their weight range was between 56 and 86 kg with a mean weight of 70 kg. Their daily methadone dose ranged between 40 and 78 mg per day with a mean dose of 55 mg. The period that they had been maintained on methadone with no dose change ranged between 1.5 and 12 months with a mean of 4 months. The group of subjects had been in the methadone maintenance program in total for a period of between 3 and 28 months with a mean of 7 months.

5.2.2. Drug administration

Infusions of S-ketamine, ketorolac or tramadol, all at 1 mg/ml were administered intravenously in two sixty-minute stages to achieve two consecutive target pseudo steady-state plasma concentrations. Subjects were administered an initial bolus of the adjuvant analgesic (or saline placebo for methadone subjects) followed by a constant infusion for one hour to achieve a target pseudo steady-state plasma concentration (Adjuvant 1). They were then administered a second bolus of the adjuvant analgesic (or saline placebo) followed by a second infusion for one hour to achieve a higher target pseudo steady-state plasma concentration (Adjuvant 2). At the end of the hour of the second infusion, the infusion was paused briefly while a loading dose of morphine was administered. An infusion of morphine was then commenced, the second infusion recommenced and the two infusions were maintained concurrently for one hour (Adjuvant 2/Morphine).

Loading and maintenance doses calculated to achieve target pseudo steady-state plasma concentrations of 15 and 60 ng/ml for S-ketamine, 0.4 and 4.0 mg/L of ketorolac and 288 and 1000 ng/ml of tramadol are shown in Table 3 page 53. While methadone and control subjects received identical loading and maintenance doses of the adjuvant analgesics, they received different loading (control subjects 5 mg, methadone subjects 34 mg) and maintenance doses of morphine (control subjects 3.8 mg, methadone subjects 16.5 mg) to achieve different target pseudo steady-state plasma morphine concentrations (control subjects 33 ng/ml, methadone subjects 180 ng/ml). Table 3 page 53 shows loading and maintenance doses intended to achieve these target pseudo steady-state plasma concentrations. Figure 2 page 54 shows the experimental schema.

Subjects' responses during the cold pressor and electrical stimulation tests and their respiration rates were measured and blood samples taken one hour prior to infusion, at 0 hours and hourly thereafter. Blood samples were also taken at 0.25, 0.5 and 0.75 hours after the end of the last infusion.

5.3. Results

There were no significant differences in the cold pressor or electrical stimulation data, or respiration rates for methadone or control subjects between S-ketamine, tramadol or ketorolac (or saline for methadone subjects) baselines and their respective values at adjuvant 1($p>0.19$) (see Figure 2 for experimental schema).

References to the adjuvant analgesic (or saline) infusion in subsequent figures describe the second of the adjuvant analgesic (or saline) infusions.

5.3.1. Plasma S-ketamine, ketorolac and tramadol concentrations

The plasma concentrations of S-ketamine, tramadol and ketorolac during the adjuvant analgesic (or saline for methadone subjects) (Adjuvant infusion) and the adjuvant analgesic (or saline) combined with morphine infusion (Adjuvant/morphine infusion) are shown in Figure 14 and Table 6. There were no significant differences between control and methadone subjects for S-ketamine infusion ($p=0.15$), S-ketamine/morphine infusion ($p=0.65$), ketorolac adjuvant infusion ($p=0.28$) ketorolac/morphine infusion ($p=0.50$) tramadol infusion ($p=0.36$) or tramadol/morphine infusion ($p=0.60$) concentrations.

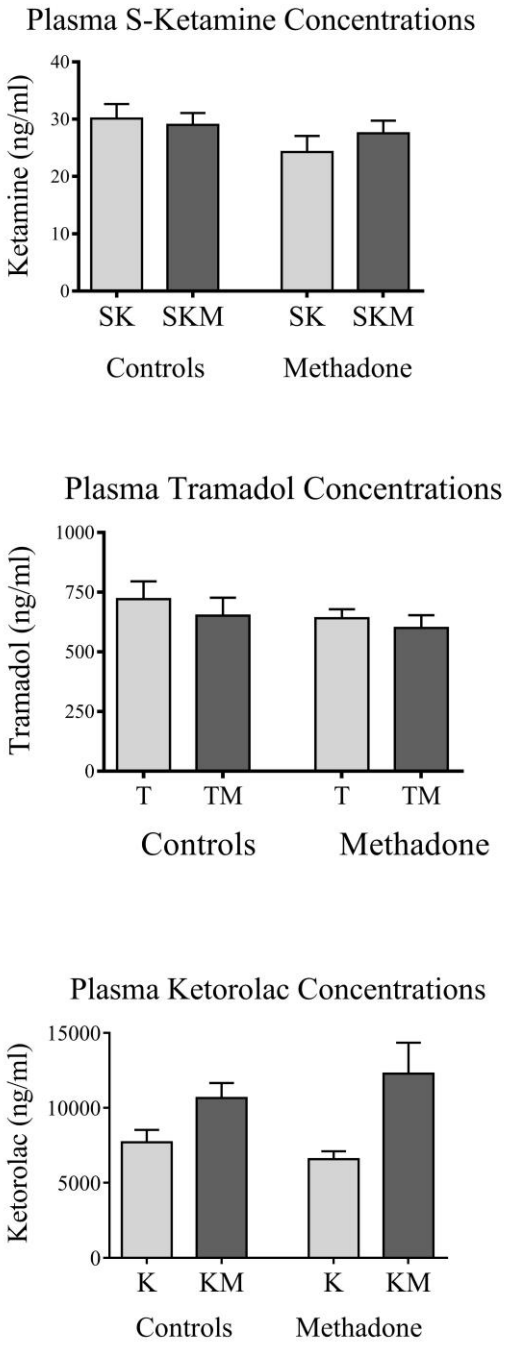

Figure 14 Plasma S-ketamine, tramadol and ketorolac concentrations in 6 control and 6 methadone subjects at adjuvant infusion and adjuvant infusion plus morphine are shown. S-ketamine infusion (SK) and S-ketamine/morphine infusion (SKM) (upper panel), Tramadol infusion (T) and tramadol/morphine infusion (TM) (middle panel), and Ketorolac infusion (K) and ketorolac/morphine infusion (KM) (lower panel) are indicated. Results are represented as mean ± SEM.

5.3.2. Plasma morphine concentrations

Plasma morphine concentrations during the adjuvant/morphine infusion in control and methadone subjects on S-ketamine, ketorolac and tramadol administration days, and methadone subjects on the saline placebo administration day are shown in Figure 15 and Table 6. There was no significant difference in plasma morphine concentrations between the S-ketamine, tramadol and ketorolac days for control subjects (p=0.50) and between the S-ketamine, tramadol, ketorolac and saline days for methadone subjects (p=0.67) at this time point.

5.3.3. Plasma R-methadone concentrations

Methadone subjects' mean plasma R-methadone concentrations at baseline, adjuvant infusion, adjuvant/morphine infusion and 3 hours post dose are shown in Figure 16, Figure 17 and Table 6. There was no significant difference in plasma R-methadone concentrations between the S-ketamine, tramadol, ketorolac and saline days at baseline (p=0.39), adjuvant infusion (p=0.57), adjuvant/morphine infusion (p=0.46) and 3 hours post dose (p=0.09).

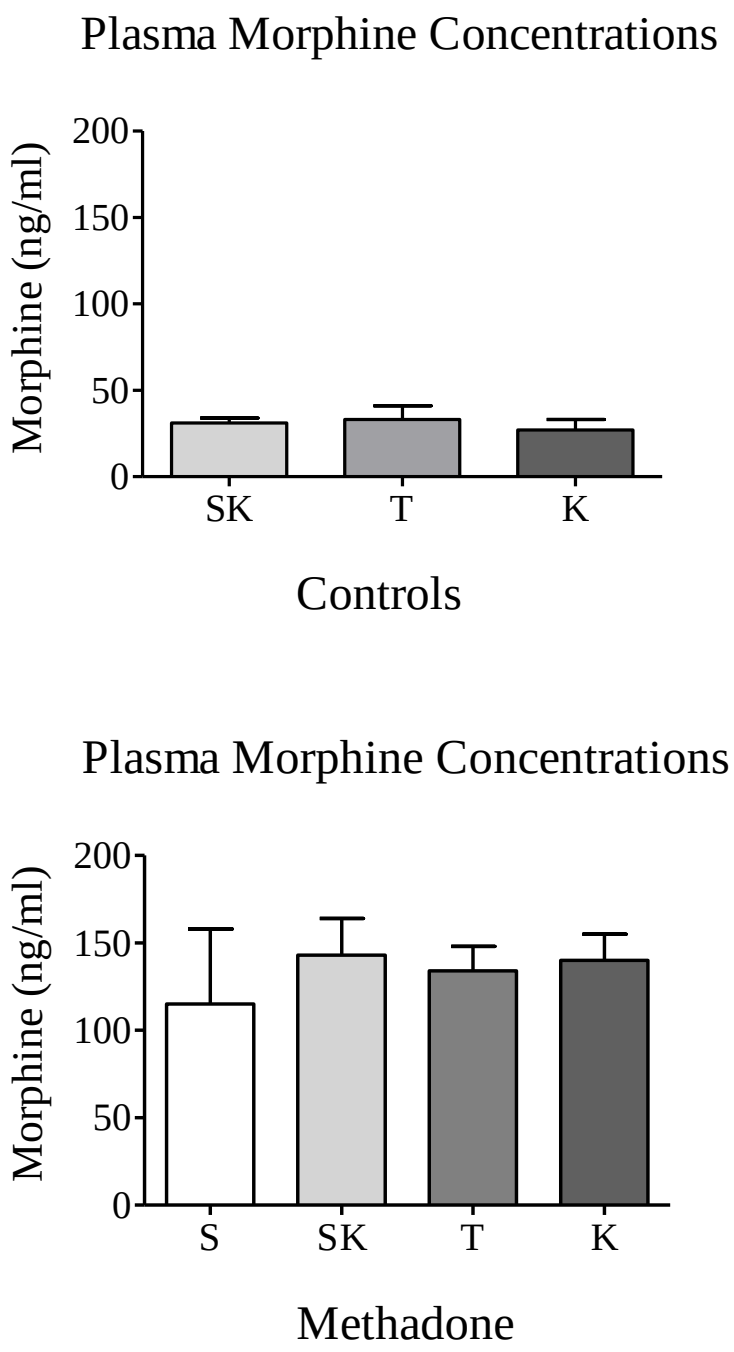

Figure 15 Plasma morphine concentrations during S-ketamine/morphine infusion (SK), tramadol/morphine infusion (T), ketorolac/morphine infusion (K) and saline placebo/morphine infusion (S) (methadone subjects) in 6 control (upper panel) and 6 methadone subjects (lower panel) are shown. Results are represented as mean ± SEM.

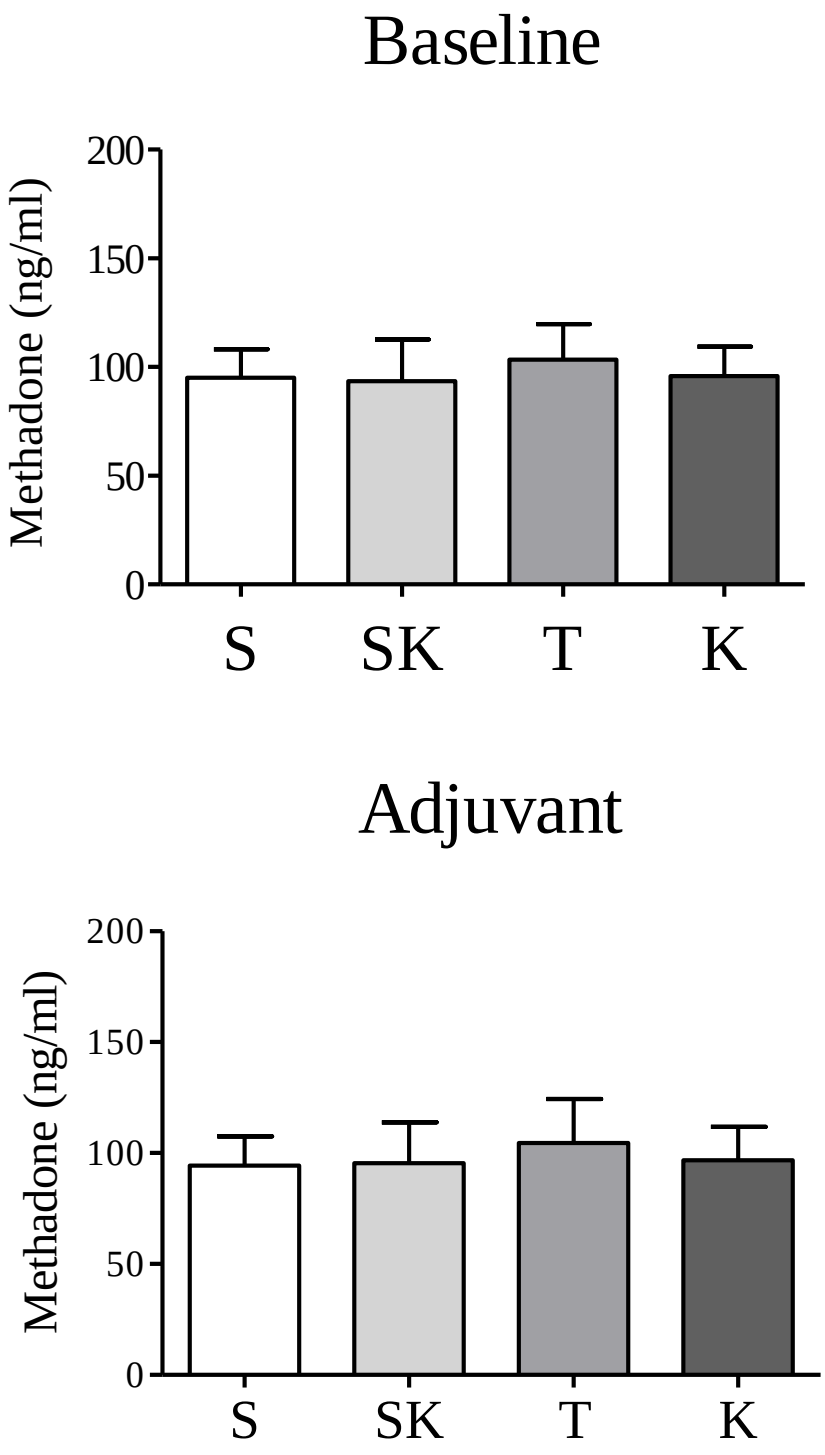

Figure 16 Plasma methadone concentrations in 6 methadone subjects at baseline and at adjuvant/saline placebo infusion (Adjuvant) on saline placebo (S), S-ketamine (SK), tramadol (T) and ketorolac (K) administration days are shown. Results are represented as mean ± SEM.

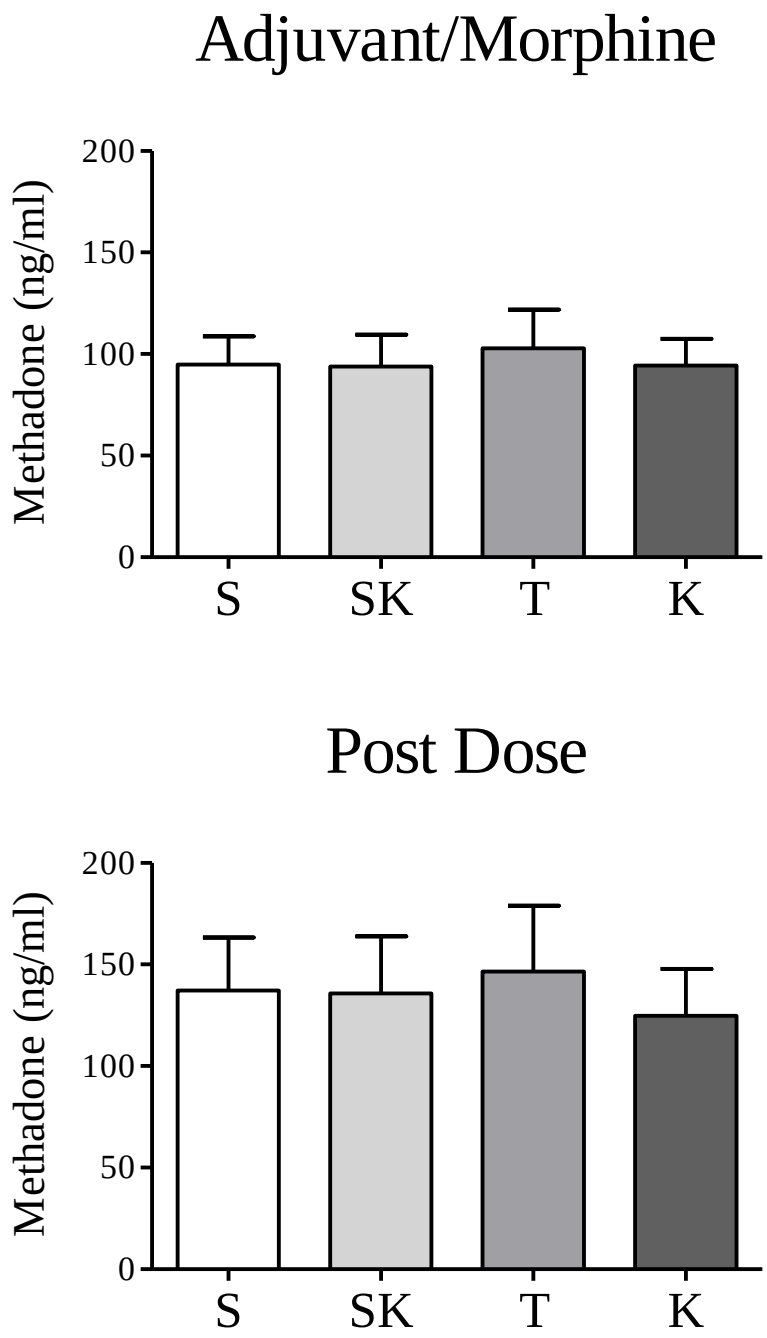

Figure 17 Plasma methadone concentrations in 6 methadone subjects at adjuvant/saline placebo and morphine infusion (Adjuvant/Morphine) and three hours post methadone administration (Post Dose) on saline placebo (S), S-ketamine (SK), tramadol (T) and ketorolac (K) administration days are shown. Results are represented as mean ± SEM.

Plasma Concentrations				
Plasma Drug Concentration	Group	Baseline	Analgesic	Analgesic And Morphine
S-ketamine (ng/ml)	Control		30±3 (21 to 37)	29±2 (22 to 34)
	Methadone		24±3 (range 13 to 32)	28±2 (21 to 33)
Tramadol (ng/ml)	Control		721±74 (439 to 1000)	651±75 (418 to 976)
	Methadone		642±37 (533 to 760)	600±55 (478 to 765)
Ketorolac (ug/ml)	Control		7704±826 (5106 to 10508)	10661±990 (8230 to 14325)
	Methadone		6575±525 (5095 to 8646)	12281±2067) (7730 to 22108)
Morphine (Co-S-ketamine Infusion) (ng/ml)	Control			31±3 (21 to 41)
	Methadone			143±28 (46 to 219)
Morphine (Co-Tramadol Infusion) (ng/ml)	Control			33±8 (16 to 71)
	Methadone			134±14 (84 to 178)
Morphine (Co-Ketorolac Infusion) (ng/ml)	Control			27±6 (12 to 45)
	Methadone			140±15 (84 to 196)
Morphine (Co-Saline Infusion) (ng/ml)	Methadone			115±4.3 (101 to 130)
Methadone (S-ketamine Day) (ng/ml)	Methadone	94±19 (34 to 156)	95±19 (33 to 146)	94±16 (37 to 139)
Methadone (Tramadol Day) (ng/ml)	Methadone	103±16 (55 to 155)	105±20 (45 to 168)	103±19 (48 to 156)
Methadone (Ketorolac Day) (ng/ml)	Methadone	96±14 (56 to 131)	97±15 (42 to 158)	94±13 (41 to 127)
Methadone (Saline Day) (ng/ml)	Methadone	95±13 (50 to 130)	94±13 (47 to 124)	95±14 (47 to 132)

Table 6 Plasma drug concentrations on S-ketamine, tramadol, ketorolac and saline administration days for control and methadone maintained subjects. Morphine administrated during S-ketamine, Tramadol, Ketorolac and Saline infusions described as Morphine (Co-S-Ketamine Infusion), Morphine (Co-Tramadol Infusion), Morphine (Co-Ketorolac Infusion) and Morphine (Co-Saline Infusion). Concentrations are mean±SEM (range).

5.3.4. Cold pressor

5.3.4.1. S-ketamine administration day

On the S-ketamine administration day, cold pressor pain tolerance responses for control and methadone subjects at baseline, S-ketamine infusion and S-ketamine/morphine infusion are shown in Figure 18 (upper panel) and Table 7. Pain tolerance responses for control subjects were significantly higher than for methadone subjects at baseline (P=0.02; CI 4.5 to 40). Within group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.041) from baseline to the S-ketamine/morphine infusion (P<0.05; 95% CI 2 to 34), but not baseline to S-ketamine infusion (P>0.05; 95% CI -12 to 21). Pain tolerance responses for methadone subjects increased significantly (P=0.034) from baseline to S-ketamine/morphine infusion (P<0.05; 95% CI 0.17 to 4.4), but not baseline to S-ketamine infusion (P>0.05; 95% CI -1 to 3.2). While control subjects increased pain tolerance responses between baseline and S-ketamine/morphine infusion by a mean of 46% (17.8 seconds), methadone subjects increased their pain tolerance responses by a mean of 14% (2.3 seconds).

5.3.4.2. Tramadol administration day

Cold pressor pain tolerance responses for control and methadone subjects on the tramadol administration day at baseline, tramadol infusion and tramadol/morphine infusion are shown in Figure 18 (middle panel) and Table 7. Pain tolerance responses for control subjects were not significantly higher than for methadone subjects at baseline (P=0.1341). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.015) from baseline to tramadol/morphine infusion (P<0.05; 95% CI 11 to 77), but not baseline to tramadol infusion (P>0.05; 95% CI -12 to 54). There were no significant changes (P=0.21) in pain tolerance responses for methadone subjects from baseline to tramadol infusion or tramadol/morphine infusion.

5.3.4.3. Ketorolac administration day

On the ketorolac administration day, cold pressor pain tolerance responses for control and methadone subjects at baseline, ketorolac infusion and ketorolac/morphine infusion are shown in Figure 18 (lower panel) and Table 7. Pain tolerance responses for control subjects were significantly higher than for methadone subjects at baseline (P=0.016; CI 5 to 39). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.021) from baseline to the ketorolac/morphine infusion (P<0.05; 95% CI 3 to 30), but not baseline to ketorolac infusion (P>0.05; CI -10 to 17). Pain tolerance responses for methadone subjects did not increase significantly (P=0.32) from baseline to ketorolac or ketorolac/morphine

infusion. There were also no significant differences (P=0.21) in cold pressor responses between infusions for methadone subjects on the saline administration day.

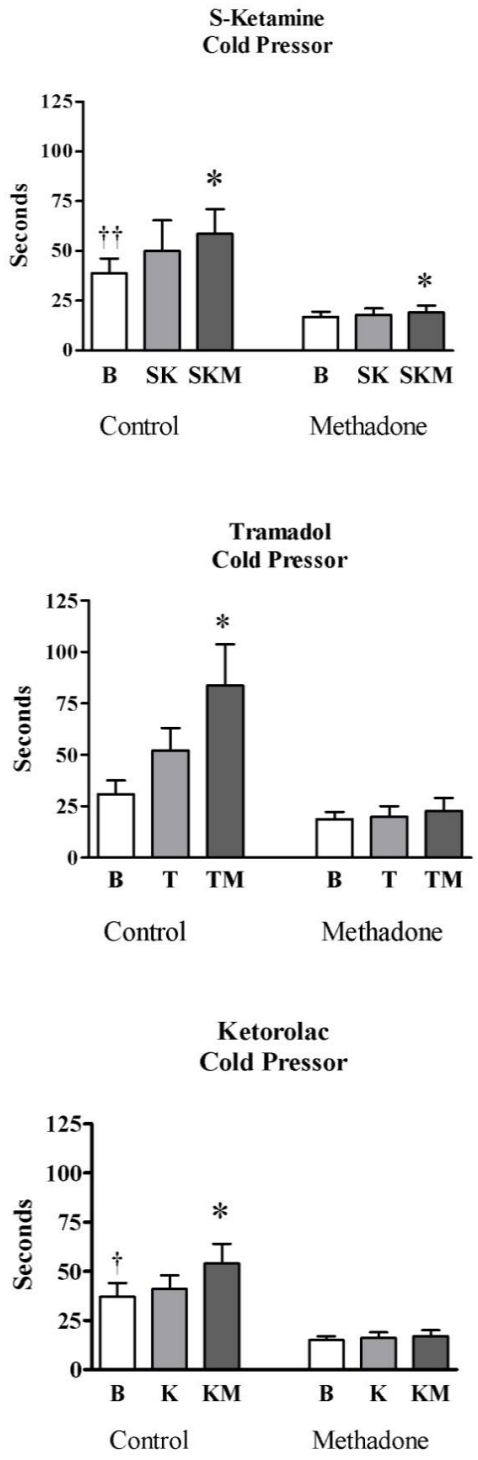

Figure 18 Cold pressor pain tolerance responses on S-ketamine (upper panel), tramadol (middle panel) and ketorolac (lower panel) administration days in 6 control and 6 methadone subjects at baseline (B), adjuvant (S-Ketamine (SK), Tramadol (T), Ketorolac (K)) and adjuvant/morphine (S-Ketamine and morphine (SKM), Ketorolac and morphine (KM), Tramadol and morphine (TM)) infusions. Results are represented as mean ± SEM. ††P<0.01, † P<0.05 between groups, * P<0.05 between treatments.

5.3.5. Electrical stimulation

5.3.5.1. S-Ketamine day

Electrical stimulation pain tolerance responses for control and methadone subjects on the S-ketamine day at baseline, S-ketamine infusion and S-ketamine/morphine infusion are shown in Figure 19 (upper panel) and Table 7. Pain tolerance responses for control subjects were not significantly higher than for methadone subjects at baseline (P=0.39). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.034) from baseline to S-ketamine/morphine infusion (P<0.05 95% CI 0.5 to 19), but not from baseline to S-ketamine infusion (P>0.05; 95% CI -0.2 to 18). There were no significant changes (P=0.77) in electrical stimulation pain tolerance responses for methadone subjects from baseline to S-ketamine infusion or S-ketamine infusion/morphine.

5.3.5.2. Tramadol day

Pain tolerance responses in the electrical stimulation test on the tramadol day are shown in Figure 19 (middle panel) and Table 7. Pain tolerance responses for control subjects were not significantly higher than for methadone subjects at baseline (P=0.73). Within group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.007) from baseline to tramadol infusion (P<0.05; 95% CI 2.9 to 22) and from baseline to tramadol/morphine infusion (P<0.05; 95% CI 3.3 to 23). There were no significant changes (0.072) in pain tolerance responses for methadone subjects from baseline to tramadol infusion or tramadol/morphine infusion (P=0.2).

5.3.5.3. Ketorolac day

Control and methadone subjects' pain tolerance responses in the electrical stimulation test on the ketorolac administration day are shown in Figure 19 (lower panel) and Table 7. Pain tolerance responses for control subjects were not significantly higher than for methadone subjects at baseline (P=0.28). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.026) from baseline to the ketorolac/morphine infusion (P<0.05; 95% CI 4 to 28), but not from baseline to S-ketamine infusion (P>0.05; 95% CI -6 to 18). There were no significant changes (P=0.82) in pain tolerance responses for methadone subjects from baseline to ketorolac infusion or ketorolac/morphine infusion. There were also no significant differences (P=0.094) in cold pressor responses between infusions for methadone subjects on the saline administration day.

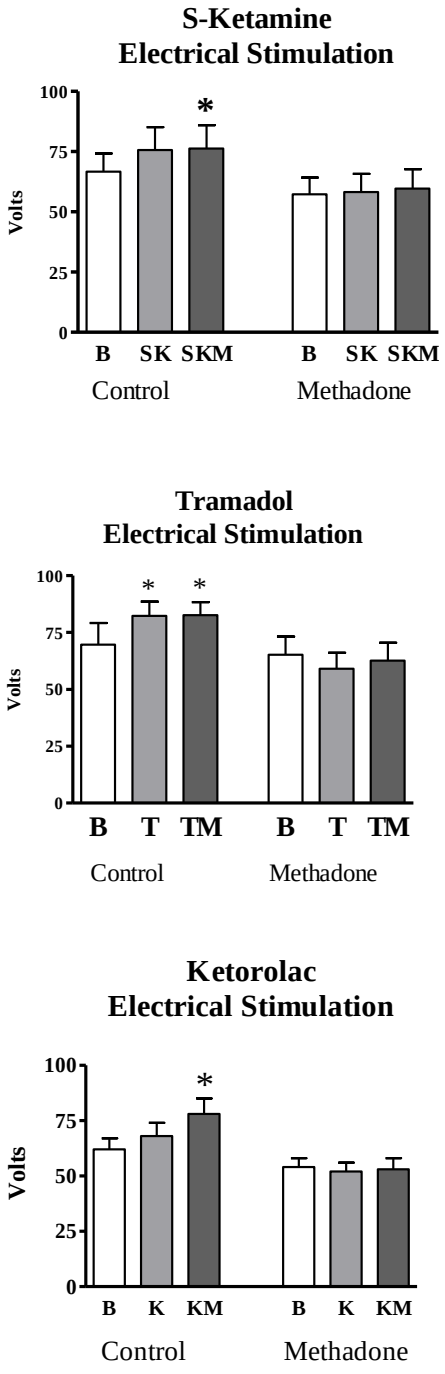

Figure 19 Electrical stimulation pain tolerance responses on S-ketamine (upper panel), tramadol (middle panel) and ketorolac (lower panel) administration days in 6 control and 6 methadone subjects at baseline (B), adjuvant (S-Ketamine (SK), Tramadol (T), Ketorolac (K)) and adjuvant/morphine (S-Ketamine and morphine (SKM), Ketorolac and morphine (KM), Tramadol and morphine (TM)) infusions. Results are represented as mean ± SEM. * $P<0.05$ between treatments.

5.3.6. Respiration rate

5.3.6.1. S-ketamine day

Respiration rate (breaths per minute) for control and methadone subjects on the S-ketamine day at baseline, S-ketamine infusion and S-ketamine/morphine infusion are shown in Figure 20 (upper panel) and Table 7. Respiration rate for control subjects was significantly higher than methadone subjects at baseline (P=0.027; 95% CI 0.67 to 8.67). There were no significant changes for control (P=0.46) and methadone subjects (P=0.98) from baseline to S-ketamine infusion or S-ketamine/morphine infusion.

5.3.6.2. Tramadol day

Control and methadone subjects' respiration rate at baseline, tramadol infusion and tramadol/morphine infusion on the tramadol administration day is shown in Figure 20 (middle panel) and Table 7. Respiration rate for control subjects was significantly higher than methadone subjects at baseline (P=0.026; 95% CI 0.63 to 7.71). Within group comparisons revealed that the respiration rate for control subjects decreased significantly (P=0.0003) between baseline and tramadol infusion (P<0.01; 95% CI -2.3 to -8.4) and baseline and tramadol/morphine infusion (P<0.01; 95% CI -2.6 to -9.1). Respiration rate for methadone subjects did not decrease significantly (0.12) from baseline to tramadol infusion or tramadol/morphine infusion.

5.3.6.3. Ketorolac day

Respiration rate at baseline, ketorolac infusion and ketorolac/morphine infusion on the ketorolac administration day is shown in Figure 20 (lower panel) and Table 7. Respiration rate for control subjects was significantly higher than methadone subjects at baseline (P=0.023; 95% CI 0.9 to 9.1). There were no significant changes for control (P=0.18) and methadone subjects (P=0.37) from baseline to ketorolac infusion or ketorolac/morphine infusion. There were also no significant differences (P=0.14) in respiration rates between infusions for methadone subjects on the saline administration day.

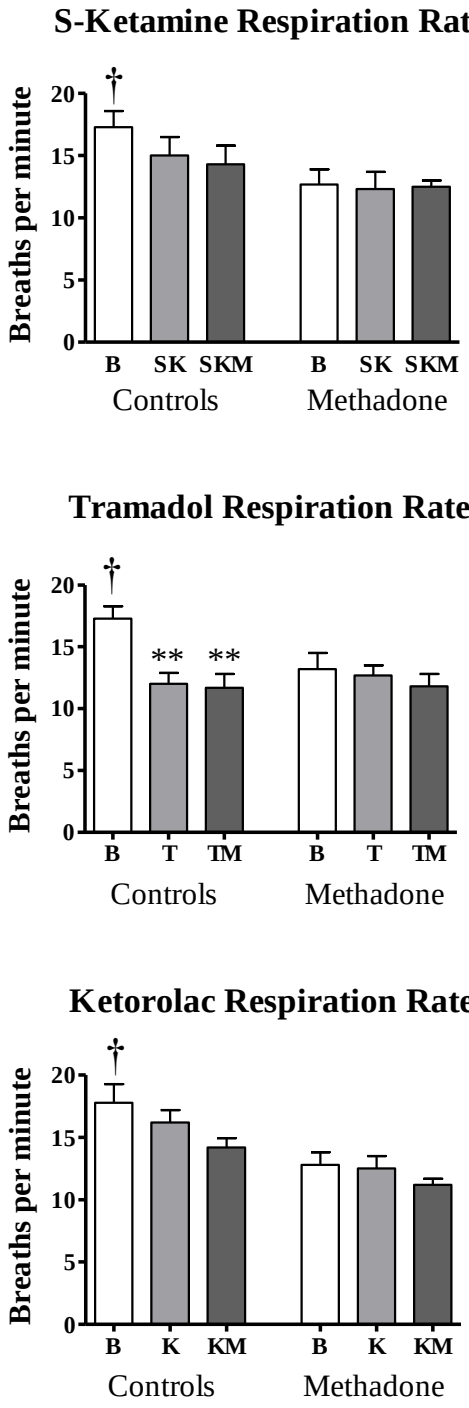

Figure 20 Respiration rates on S-ketamine (upper panel), tramadol (middle panel) and ketorolac (lower panel) administration days in 6 control and 6 methadone subjects at baseline (B), adjuvant (S-Ketamine (SK), Tramadol (T), Ketorolac (K)) and adjuvant/morphine (S-Ketamine and morphine (SKM), Ketorolac and morphine (KM), Tramadol and morphine (TM)) infusions. Results are represented as mean ± SEM. † $P<0.05$ between groups, ** $P<0.01$ between treatments.

5.3.7. Adverse events

There were no serious adverse events in the study. One healthy control subject withdrew from the study because of decreased SPO_2 (below 93% SPO_2 for more than one minute), which resolved within an hour and required 0.2 mg of naloxone hydrochloride (Mayne Pharma, Mulgrave, Australia) intramuscularly. One methadone maintained subject and three healthy control subjects experienced mild vomiting during the tramadol/morphine administration. One healthy control subject required metoclopramide hydrochloride 10 mg (Pfizer, Perth, Australia) intramuscularly for vomiting. Five methadone maintenance and 3 healthy control subjects reported moderate feelings of disassociation during the S-ketamine infusions. These episodes lasted from 30 seconds to seven minutes. Four healthy control subjects reported mild and indistinct hallucinatory experiences during the tramadol infusions. These episodes lasted from one minute to five minutes.

Responses				
Response	Group	Baseline	Analgesic	Analgesic/ Morphine
Cold Pressor S-ketamine (seconds)	Control	39±7 (12 to 61)††	43±11 (17 to 77)	57±14 (16 to 106)*
	Methadone	17±2.7 (7.1 to 26)	18±3.3 (8.4 to 32)	19±3.5 (8.2 to 32)*
Cold Pressor Tramadol Day (seconds)	Control	31±7 (14 to 56)	52±11 (20 to 91)	75±22 (32 to 180)*
	Methadone	19±3.5 (9.5 to 34)	20±5 (8.6 to 44)	23±6.3 (8.4 to 52)
Cold Pressor Ketorolac Day (seconds)	Control	37±7 (18 to 65)†	41±7 (19 to 72)	54±10 (25 to 101)*
	Methadone	15±2.2 (5.9 to 22)	16±2.7 (6.4 to 26)	17±2.7 (7.3 to 27)
Cold Pressor Saline Day (seconds)	Methadone	19±5.9 (7.6 to 48)	19±7.3 (6.4 to 55)	23±9.2 (7.5 to 68)
Electrical Stimulation S-ketamine Day (seconds)	Control	67±7.6 (40 to 90)	76±9.5 (42 to 100)	76±9.7 (44 to 100)*
	Methadone	57±7 (36 to 80)	58±7.5 (40 to 90)	60±8 (36 to 92)
Electrical Stimulation Tramadol Day (seconds)	Control	70±9.5 (48 to 100)	82±6.3 (64 to 100)*	83±5.7 (68 to 100)*
	Methadone	65±8 (44 to 100)	59±7.1 42 to 88)	63±7.8 (40 to 94)
Electrical Stimulation Ketorolac Day (seconds)	Control	62±5.3 (46 to 80)	68±5.6 (48 to 82)	78±7.4 (54 to 100)*
	Methadone	54±4.2 (40 to 70)	52±3.9 (40 to 64)	53±4.8 (42 to 68)
Electrical Stimulation Saline Day (seconds)	Methadone	58±8.2 (34 to 90)	54±8.8 (30 to 90)	53±7.7 (34 to 86)
Respiration Rate S-ketamine Day (breaths per minute)	Control	17.3±1.3 (12 to 22)†	15.0±1.5 (10 to 20)	14.3±1.5 (8 to 18)
	Methadone	12.7±1.2 (9 to 16)	12.3±1.4 (8 to 18)	12.5±0.5 (11 to 14)
Respiration Rate Tramadol Day (breaths per minute)	Control	17.3±1 (14 to 20)†	12.0±1 (10 to 16)**	11.7±1 (10 to 17)**
	Methadone	13.1±1.3 (10 to 18)	12.7±0.8 (10 to 16)	11.8±1.0 (9 to 16)
Respiration Rate Ketorolac Day (breaths per minute)	Control	17.8±1.5 (12 to 22)†	16.2±0.9 (12 to 18)	14.2±0.8 (12 to 16)
	Methadone	12.8±1.1 (10 to 16	12.5±0.6 (11 to 14)	11.2±0.5 (9 to 13)
Respiration Rate Saline Day (breaths per minute)	Methadone	12.1±0.6 (10 to 14)	12.5±1.1 (8 to 15)	10.5 (9 to 13)

Table 7 Cold pressor and electrical stimulation responses, and respiration rates on S-ketamine, tramadol and ketorolac administration days for control and methadone maintained subjects. Data are mean±SEM (range). † P<0.05, †† P<0.01 between groups, * P<0.05, ** P<0.01 between treatments.

5.4. Discussion

The study examined the antinociceptive effects of adjuvant analgesics (S-ketamine, tramadol and ketorolac) combined with morphine in methadone maintained and healthy control subjects. The study confirmed that methadone maintained subjects were hyperalgesic compared to healthy control subjects in the cold pressor test. Each of the adjuvants, combined with morphine, provided statistically significant antinociception to the group of healthy control subjects. Tramadol and ketorolac, combined with high dose morphine failed to provide statistically significant antinociception to methadone maintained subjects. S-ketamine and high dose morphine provided statistically significant improvement in methadone subjects, but the improvement was of the order of a mean of two seconds or 12% from baseline and therefore unlikely to have clinical significance.

A central aim of opioid adjuvant therapy is the maximisation of analgesic effect with the minimum of adverse events through activation of different antinociceptive mechanisms (Kalso 2005). The study examined three adjuvants alone and combined with morphine. These were ketorolac, tramadol and ketamine. Each of these drugs are used as analgesics in the clinical settings and there is a large body of evidence supporting their effectiveness in the clinical setting for the management of pain (See section 1.16). Yet in this study, no adjuvant alone was able to produce clinically significant antinociception in either the cold pressor or the electrical stimulation test for either healthy control or methadone maintained subjects. It is important to examine the factors that may have affected the results in this study. These same factors may have impacted on the lack of results in the other studies in this series that encompass this thesis. Factors such as the nature of experimental pain testing, differences in antinociceptive test, size of dose or method of administration could also have contributed to the lack of effect in the studies in this thesis compared to other studies.

Ing Lorenzini et al (2011) (20 mg intravenous ketorolac, cold pressor test), Compton (2003) (10 mg oral ketorolac, cold pressor test) and Romundstad (2006) (30 mg intravenous ketorolac, mechanical pressure pain)failed to demonstrate the antinociceptive effect of ketorolac in experimental pain models. Ing Lorenzini (2011) and Romundstad (2006) found a significant antinociceptive effect of ketorolac when in combination with another agent (paracetamol). In a recent review of the efficacy of non-opioid analgesics in experimental human pain models, Staahl et al (2009) found that non-steroidal anti-inflammatories were effective in attenuating nociception produced by electrical stimulation in healthy volunteers. However, ketorolac was not included as one of the non-steroidal anti-inflammatories tested. In this study, ketorolac was not found to produce antinociceptive effect in electrical stimulation. It has been suggested that the cold pressor test is mediated via nociceptors of cutaneous veins and the activation of alpha delta and C fibres (Arendt-Nielsen et al. 2007). The drug ketorolac has a mechanism of action on prostaglandin

synthesis. Ing Lorenzini et al (2011) have suggested that this may be the reason for the lack of effect of ketorolac in the cold pressor test.

There have been inconsistent findings concerning the ability of ketamine to induce antinociception in experimental settings. Gottrup et al (2000) (subcutaneous ketamine 5mg in 2 ml, capsaicin-induced pain and hyperalgesia), Gottrup et al (2004) (subcutaneous ketamine 5mg in 2 ml, capsaicin-induced pain and hyperalgesia) and Wallace et al (2002) (intravenous ketamine 16 mg, capsaicin-induced pain and hyperalgesia) failed to demonstrate ketamine induced antinociception in experimental pain models. However, S-ketamine was shown to significantly decrease pain in studies by Koppert et al (2001) (intravenous 0.4 mg/kg, transcutaneous electrical stimulation) and Gottrup (2006) (30 minute intravenous infusion of ketamine 0.24 mg/kg, patients with nerve injury pain and induced brush and pinprick pain).

Filitz et al (2008) (intravenous 75mg tramadol, transcutaneous electrical stimulation) demonstrated a slight but significant pain reduction from tramadol of 12%. In their discussion of the results, Filitz et al (2008) noted that the marginal analgesic effect of tramadol that they found in their study was contradictory to the clinical experience with tramadol. They suggested that while there are many components to the mechanisms of action of tramadol (including the metabolite O-desmethyl-tramadol (M1), the main mechanism is the activation of inhibitory descending pathways in the spinal cord (see section 1.16). They suggest that their model of transcutaneous electrical stimulation might be more sensitive to mu receptor activity (which is limited in the case of tramadol and its metabolite M1) rather than the antinociceptive activity produced by descending inhibitory systems as these are activated after longer lasting periods of nociceptive stimulation. Such an explanation may have relevance for the lack of effect of tramadol found in this study. The cold pressor test and the electrical stimulation test may be more sensitive to mu receptor activity than the antinociceptive effect of descending inhibitory systems.

There are several different tests used to measure antinociception. The modalities include mechanical (von Frey hairs (Hay et al. 2008), pressure (Luginbuhl et al. 2003)) electrical (intradermal electrical stimulation (Troster et al. 2006)), thermal (heat lamp (Andrews 1943)) mechanical/chemical (pinprick hyperalgesia (Troster et al. 2006)), vascular (tourniquet pain (Beecher 1966)) as well as the cold pressor and electrical stimulation described in this series of studies. It has been noted that phasic stimuli (pain occurring in phases) (such as electrical stimulation) differ in a number of ways from that of tonic pain (deep prolonged sensations of pain) (such as the cold pressor test). Phasic pain has been described as flickering, pulsing, shooting, pricking and sharp. Tonic pain has been described as pressing, wrenching, smarting and aching. It has been suggested that there are differences in quality, neurology and functional aspects of the different pain sensations elicited by different modalities (Beecher 1966, Chen et al. 1989). As

suggested by Filitz et al (2008) with regards to their own work, differences in the modalities of the experimental pain models used to examine the antinociceptive effectiveness of the adjuvants in other studies may explain the lack of effect of adjuvants alone in their (and this) study.

There are also several different antinociception measurement tools employed in experimental antinociception research. This series of studies used pain detection (when pain is first detected) and pain tolerance (the maximum amount of pain tolerated). Visual analogue scales and the Mc Gill Pain Questionnaire are among a range of many other measures employed (Chen et al. 1989). Different measurements may lead to differing results. In addition, there is also little consistency in the instructions given between different groups in different studies performing the same antinociceptive measure. Different instructions will involve different emphasis on certain aspects of measurement and may also lead to differing results. Different antinociception measurement and different instructions may also have contributed to the lack of effect observed.

Another contributing factor to lack of antinociception may have been drug doses utilised. The rationales for drug doses chosen are described in section 2.8.2. Unfortunately the plasma morphine concentrations reached were between a mean of 30% (ketorolac administration day) and 42% (saline administration day) below targets for methadone maintained subjects and this may have contributed to the lack of effects.

As shown in Figure 14, plasma concentrations achieved differed from the target concentrations for each of the adjuvants and morphine. Plasma S-ketamine concentrations were approximately 50% below maximum targets, tramadol was approximately 30% below targets and ketorolac was approximately 2 to 3 times above target concentrations. Plasma morphine concentrations in this study were approximately on target for control subjects. For methadone subjects, plasma morphine concentrations were between 30% and 40% below targets. The inability to reach target plasma concentrations for S-ketamine and tramadol may have contributed to the lack of effects found in this study.

Antinociceptive effect may not have been achieved due to the length of dosing. Subjects were maintained at the maximum dose of adjuvants for approximately 2 hours and the maximum dose of morphine for approximately 1 hour. Longer dosing of subjects may have produced antinociceptive effects in this study but possibly at the expense of increased respiratory depression.

To summarise, three major factors may have contributed to the lack of antinociceptive effect for the adjuvants alone in this study. Firstly, the cold pressor and electrical stimulation tests were used in this series of studies to measure antinociception. The aim of using more than one pain modality was to increase the range of antinociceptive effects that might be measured. However, there are many other pain modalities utilised to produce and measure antinociception. These

different tests may have been able to measure improvements in antinociception where the tests utilised in this study were not able to. Secondly, concentration effect relationship extrapolations are approximate estimates. There was variation in what was targeted in terms of plasma drug concentrations and what was achieved. This may have affected the provision of antinociception. Thirdly, subjects were maintained at maximum doses for adjuvants for two hours and morphine for one hour. Longer dosing schedules may have provided an antinociceptive effect. These limitations may apply to other studies in this series of studies and a summary of limitations is described in 7.2.2 page 153.

In conclusion, the combination of S-ketamine, tramadol or ketorolac and morphine provided clinically significant antinociception to healthy controls but not methadone maintained subjects. The study confirmed that methadone maintained subjects are hyperalgesic relative to healthy control subjects. The most effective combination in the healthy control population was tramadol and morphine. A number of factors may have contributed to the lack of antinociceptive effect produced by opioid adjuvants alone and these will be discussed further in the final chapter of this thesis.

6. Study 4. Antinociceptive and respiratory effects of high dose morphine and adjuvant analgesics in buprenorphine maintained subjects

6.1. Introduction

As described in section 1.11.2, Buprenorphine has a complex pharmacology. Predominately, buprenorphine displays partial agonism at the mu-opioid receptor. Its activity at the mu opioid receptor is responsible for such effects as spinal and supra-spinal analgesia, respiratory depression and miosis (Gutstein and Akil 2006). Studies suggest that it has a 'ceiling effect' for respiratory depression but no similar 'ceiling' for analgesia(Dahan et al. 2006, Megarbane et al. 2006, Yassen et al. 2006, Yassen et al. 2007, Pergolizzi et al. 2010). The effect of this complex pharmacology on the management of pain in buprenorphine maintained patients is not known.

The central aim of opioid adjuvant therapy in pain management is the maximisation of analgesic effect through activation of different antinociceptive mechanisms (Kalso 2005). A number of studies have shown that ketamine provides superior pain relief in opioid tolerant patients (Bell 1999, Eilers et al. 2001, Sator-Katzenschlager et al. 2001, Haller et al. 2002, Loftus et al. 2010). There is less evidence for the effectiveness of such commonly used opioid adjuvants as tramadol or ketorolac in the provision of analgesia for opioid tolerant patients but the focus on opioid adjuvant therapy for pain management in this population is increasing (See section 1.16) (Bourne 2010, Macintyre et al. 2010, Huxtable et al. 2011).

The aim of this study was to examine whether S-ketamine, tramadol or ketorolac alone, or in combination with high dose morphine, produced antinociception and respiratory effects in buprenorphine maintained subjects. Controls were administered the same doses of opioid adjuvants but lower doses of morphine.

6.2. Methods

The subjects, procedures and statistical analyses for the buprenorphine adjuvant study were as described in chapter 2.

6.2.1. Subjects

In summary, six buprenorphine subjects (3 men and 3 women), aged between 25 and 37 years with a mean age of 32 years were enrolled. Their weights ranged from 55 to 85 kg with a mean of 70 kg. They had been receiving a daily buprenorphine dose between 2 and 16 mg with a mean of 10 mg with no dose change for 2 to 9 months with a mean of 5 months. The group had been in buprenorphine maintenance treatment in total ranging between 2 to 18 months with a mean of 8 months.

Six healthy control subjects (3 men and 3 women) were also recruited. They were aged between 24 and 39 years with a mean age of 31 years. The weight range of the group ranged from 56 to 86 kg with a mean of 68 kg. The healthy control group served as controls for both the methadone and buprenorphine maintained subject groups in the adjuvant analgesic studies.

6.2.2. Drug administration

Infusions of S-ketamine, ketorolac or tramadol 1 mg/ml were administered intravenously in two sixty-minute stages to achieve two consecutive target pseudo steady-state plasma concentrations. Subjects were administered an initial bolus of the adjuvant analgesic (or saline placebo for buprenorphine subjects) followed by a constant infusion for one hour to achieve a target pseudo steady-state plasma concentration (Adjuvant 1). They were then administered a second bolus of the adjuvant analgesic (or saline placebo) followed by a second infusion for one hour to achieve a higher target pseudo steady-state plasma concentration (Adjuvant 2). At the end of the hour of the second infusion, the infusion was paused briefly while a loading dose of morphine was administered. An infusion of morphine was then commenced, the second infusion recommenced and the two infusions were maintained concurrently for one hour (Adjuvant 2/Morphine).

Loading and maintenance doses calculated to achieve target pseudo steady-state plasma concentrations of 15 and 60 ng/ml for S-ketamine, 0.4 and 4.0 mg/L of ketorolac and 288 and 1000 ng/ml of tramadol are shown in Table 3 page 53. While buprenorphine and control subjects received identical loading and maintenance doses of the adjuvant analgesics, they received different loading (control subjects 5 mg, buprenorphine subjects 34 mg) and maintenance doses of morphine (control subjects 3.8 mg, buprenorphine subjects 16.5 mg) to achieve different target pseudo steady-state plasma morphine concentrations (control subjects 33 ng/ml, buprenorphine subjects 180 ng/ml). Table 3 page 53 shows loading and maintenance doses intended to achieve these target pseudo steady-state plasma concentrations. Figure 2 page 54 shows the experimental paradigm.

Subjects' responses during the cold pressor and electrical stimulation tests and their respiration rates were measured and blood samples taken one hour prior to infusion, at 0 hours and hourly thereafter. Blood samples were also taken at 0.25, 0.5 and 0.75 hours after the end of the last infusion.

6.3. Results

There were no significant differences in the cold pressor or electrical stimulation tests, or respiration rate for either buprenorphine or control subjects between S-ketamine, tramadol, ketorolac (or saline for buprenorphine subjects) baselines and their respective values at infusion 1 ($p>0.32$).

References to the adjuvant analgesic (or saline) infusion describe the second of the adjuvant analgesic (or saline) infusions.

6.3.1.1. Plasma S-ketamine, ketorolac and tramadol concentrations

Plasma S-ketamine and tramadol concentrations for control and buprenorphine subjects during the respective infusions are shown in Figure 21 and Table 8. There were no significant differences between the adjuvant analgesic concentrations for buprenorphine and control subjects for the S-ketamine (P=0.55), S-ketamine/morphine (P=0.88), tramadol (P=0.85), tramadol/morphine (P=0.90), ketorolac (p=0.63) or ketorolac/morphine infusions (p=0.67).

Plasma Drug Concentrations (ng/ml)				
Adjuvant Analgesic Alone and With Morphine Study				
Plasma Drug Concentration	Group	Baseline	Analgesic	Analgesic/morphine
S-ketamine	Control		30±3 (21 to 37)	29±2 (22 to 34)
	Buprenorphine		28±3 (19 to 33)	29±3 (20 to 36)
Tramadol	Control		721±74 (439 to 1000)	651±75 (418 to 976)
	Buprenorphine		701±71 (470 to 926)	664±67 (447 to 880)
Ketorolac	Control		7704±826 (5106 to 10508)	10661±990 (8230 to 14325)
	Buprenorphine		7214±553 (5491 to 9284)	11274±1001 (8580 to 15757)
Morphine (Co-S-ketamine Infusion)	Control			31±3 (21 to 41)
	Buprenorphine			114±12 (64 to 154)
Morphine (Co-Tramadol Infusion)	Control			33±8 (16 to 71)
	Buprenorphine			149±22 (85 to 226)
Morphine (Co-Ketorolac Infusion)	Control			27±6 (12 to 45)
	Buprenorphine			128±6 (118 to 147)
Morphine (Co-Saline Infusion)	Buprenorphine			132±9 (109 to 166)
Buprenorphine (S-ketamine Day)	Buprenorphine	0.86±0.26 (0.27 to 2.09)	0.92±0.30 (0.25 to 2.27)	0.85±0.25 (0.19 to1.96)
Buprenorphine (Tramadol Day)	Buprenorphine	0.96±0.3 (0.28 to 2.26)	0.70±0.17 (0.23 to 1.34)	0.80±0.24 (0.22 to 1.77)
Buprenorphine (Ketorolac Day)	Buprenorphine	1.42±0.56 (0.38 to 3.78)	1.11±0.36 (0.30 to 2.36)	1.0±0.34 (0.30 to 2.3)
Buprenorphine (Saline Day)	Buprenorphine	0.83±0.23 (0.2 to 1.55)	0.87±0.21 (0.17 to 1.49)	0.77±0.21 (0.16 to 1.4)
Norbuprenorphine (S-ketamine Day)	Buprenorphine	2.03±0.69 (0.4 to 4.68)	2.20±0.80 (0.37 to 5.44)	2.09±0.70 (0.47 to 4.33)
Norbuprenorphine (Tramadol Day)	Buprenorphine	1.87±0.68 (0.22 to 4.31)	2.16 to 0.89 (0.27 to 6.16)	2.31±0.94 (0.26 to 6.25)
Norbuprenorphine (Ketorolac Day)	Buprenorphine	2.15±0.88 (0.66 to 4.85)	2.17±0.88 (0.15 to 5.79)	2.3±0.88 (0.15 to 5.92)
Norbuprenorphine (Saline Day)	Buprenorphine	1.6±0.66 (0.25 to 4.11)	1.79±0.73 (0.21 to 4.62)	1.82±0.72 (0.15 to 4.79)

Table 8 Plasma drug concentrations on S-ketamine, tramadol, ketorolac and saline administration days for buprenorphine maintained and control subjects. Data are mean±SEM (range).

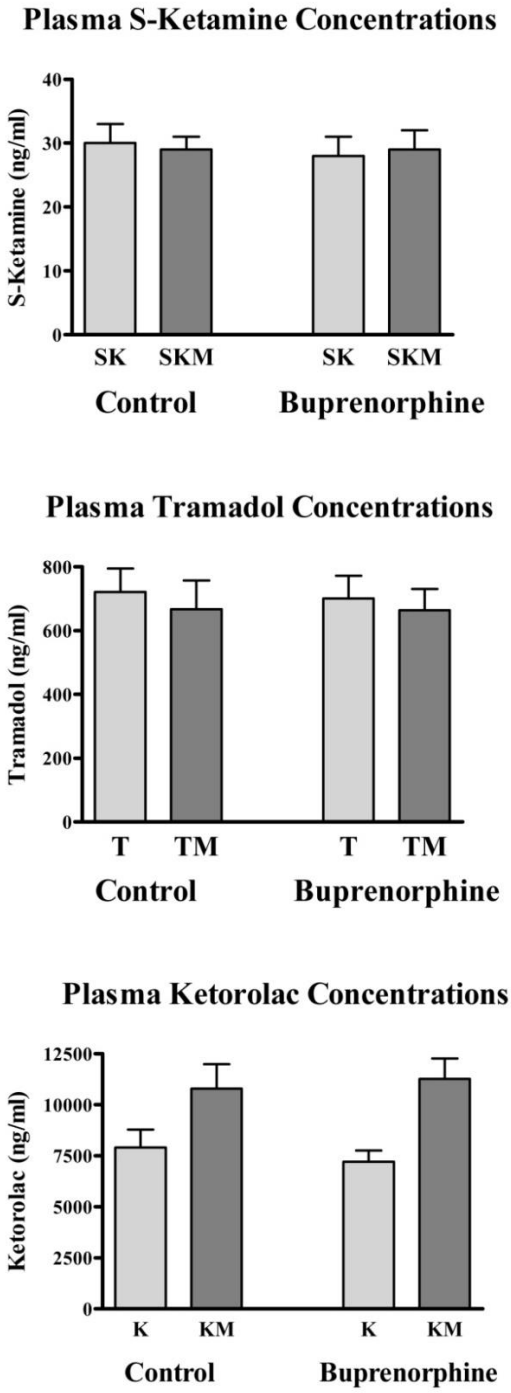

Figure 21 Plasma S-ketamine, tramadol and ketorolac concentrations in 6 control and 6 buprenorphine subjects are shown. S-ketamine infusion (SK), S-ketamine/morphine infusion (SKM) (upper panel), tramadol infusion (T), tramadol/morphine infusion (TM) (middle panel) and ketorolac infusion (K), ketorolac/morphine infusion (KM) are indicated. Results are represented as mean ± SEM.

6.3.1.2. Plasma morphine concentrations

Plasma morphine concentrations during the S-ketamine (SK), ketorolac (K), tramadol (T) and saline (S) infusions combined with morphine are shown in Figure 22 and Table 8. There was no significant difference (P=0.50) in plasma morphine concentrations between the S-ketamine, tramadol (T) and ketorolac (K) days for control subjects and no significant difference in morphine concentrations between the infusions of saline, S-ketamine, tramadol and ketorolac combined with morphine infusions for buprenorphine subjects (P=0.43).

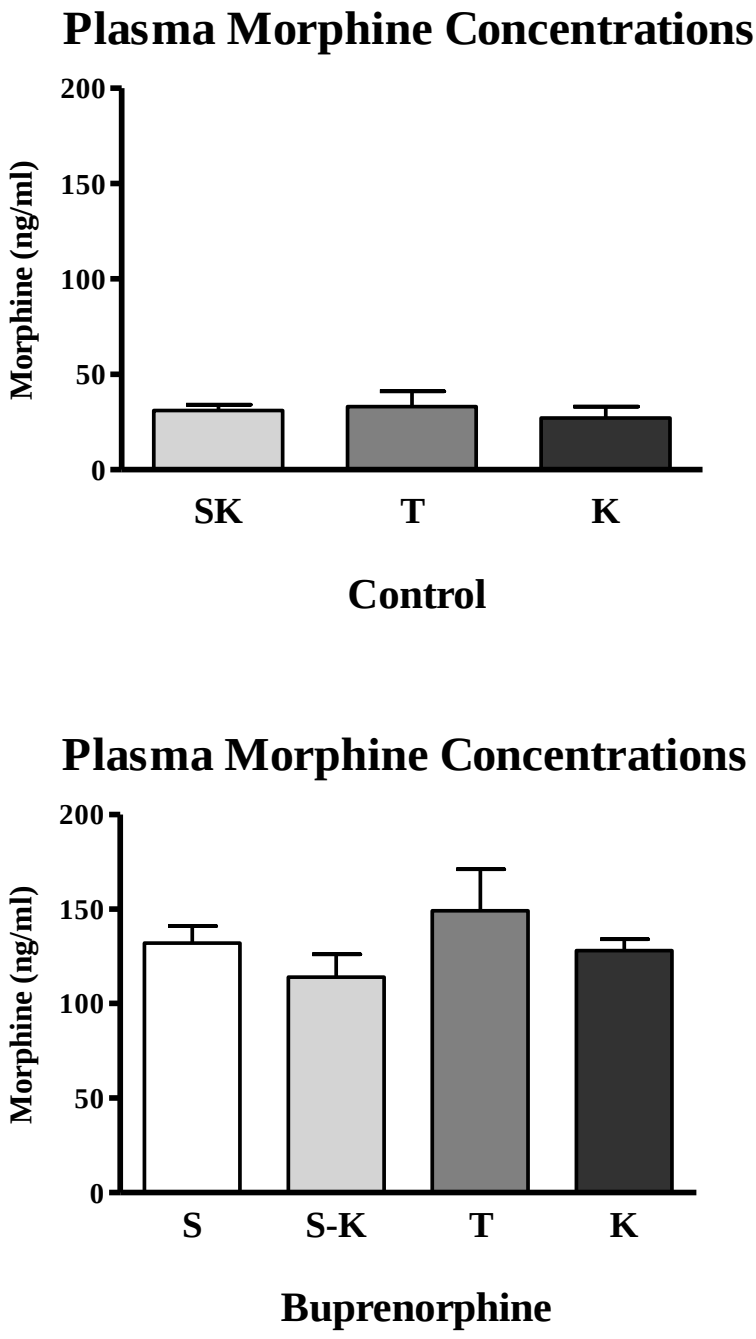

Figure 22 Plasma morphine concentrations during S-ketamine/morphine infusion (SK), tramadol/morphine infusion (T), ketorolac/morphine infusion (K) and saline placebo/morphine infusion (S) (buprenorphine subjects) in 6 control (upper panel) and 6 buprenorphine subjects (lower panel) are shown. Results are represented as mean ± SEM.

6.3.1.3. Plasma buprenorphine, norbuprenorphine concentrations

Plasma buprenorphine and norbuprenorphine concentrations on S-ketamine, tramadol, ketorolac and saline days for buprenorphine subjects are shown in Figure 23 and Table 8. There were no significant differences in buprenorphine concentrations between the saline, S-ketamine, tramadol or ketorolac days at baseline (P=0.10), or the adjuvant analgesic or saline (Adjuvant) (P=0.16) or adjuvant analgesic or saline/morphine (Adjuvant/Morphine) (P=0.46) infusions. There were also no significant differences in norbuprenorphine concentrations between the treatment days at baseline (P=0.17), or the adjuvant analgesic or saline (P=0.70) or adjuvant analgesic or saline/morphine (P=0.36) infusions.

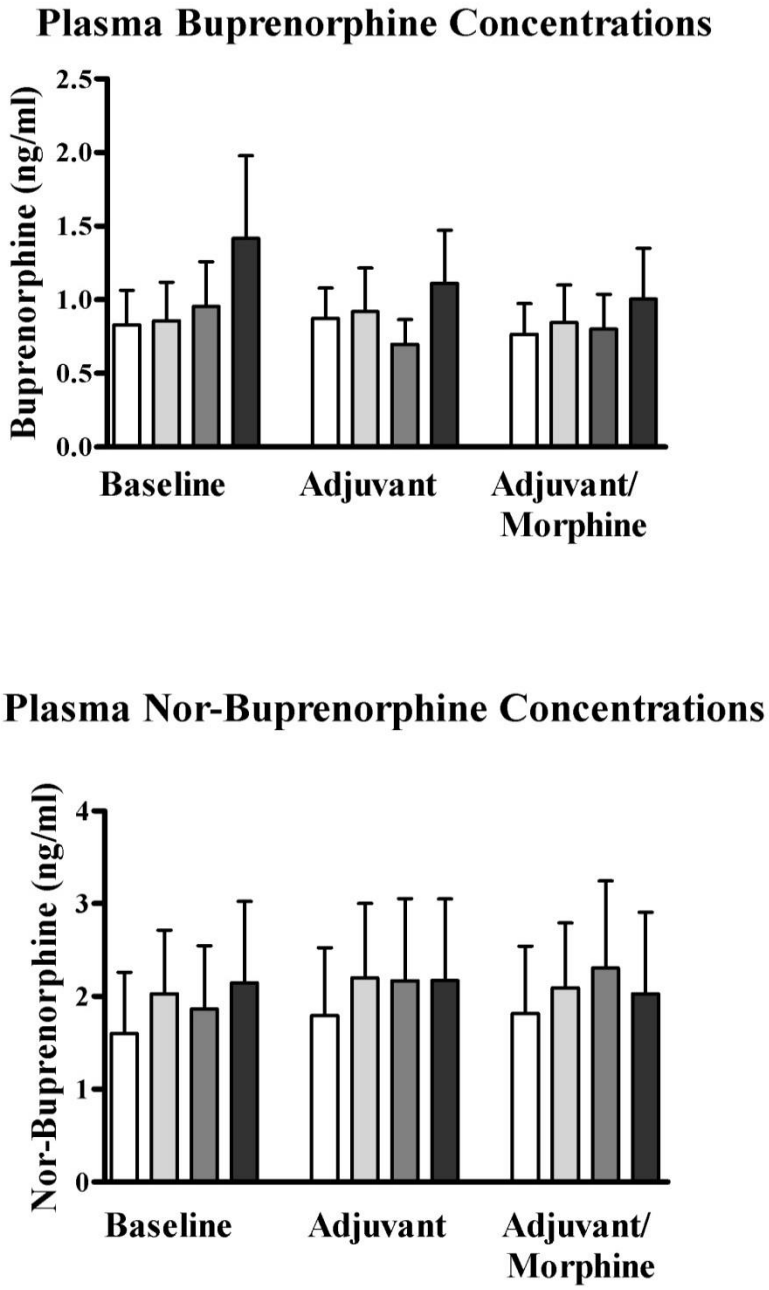

Figure 23 Plasma buprenorphine (upper panel) and norbuprenorphine (lower panel) concentrations at baseline, adjuvant analgesic or saline infusion (Adjuvant), and adjuvant analgesic or saline/morphine infusion (Adjuvant/Morphine) on saline (white bar), S-ketamine (light grey bar), tramadol (darker grey bar) and ketorolac (darkest grey bar) administration days. Results are represented as mean ± SEM.

6.3.2. Responses

6.3.2.1. Cold pressor

Cold pressor pain tolerance responses for control and buprenorphine subjects on the S-ketamine administration day at baseline, and the S-ketamine and S-ketamine/morphine infusions are shown in Figure 24 (upper panel) and Table 9. Pain tolerance responses for buprenorphine subjects were significantly lower than for control subjects at baseline (P=0.012; 95% CI -6.2 to -40). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.041) from baseline to the S-ketamine/morphine infusion (P<0.05; 95% CI 2 to 34), but not from baseline to the S-ketamine infusion (P>0.05; 95% CI -12 to 21). Pain tolerance responses for buprenorphine subjects also increased significantly (P=0.019) from baseline to the S-ketamine/morphine infusion (P<0.05; 95% CI 0.75 to 6.9) but not from baseline to the S-ketamine infusion (P>0.05; 95% CI -1.7 to 4.4).

The cold pressor pain tolerance responses for control and buprenorphine subjects on the tramadol administration day at baseline, and the tramadol and tramadol/morphine infusions are shown in Figure 24 (middle panel) and Table 9. Pain tolerance responses for buprenorphine subjects were significantly lower than for control subjects at baseline (P=0.035; 95% CI -1.5 to -32). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly from baseline to the tramadol/morphine infusion (P<0.05; 95% CI 11 to 77), but not from baseline to the tramadol infusion (P>0.05; 95% CI -12 to 54). Pain tolerance responses for buprenorphine subjects also increased significantly from baseline to the tramadol/morphine infusion (P<0.01; 95% CI 1.7 to 9.5) but not baseline to the tramadol infusion (P>0.05; 95% CI -1.8 to 6.0).

Control and buprenorphine subjects' cold pressor pain tolerance responses on the ketorolac administration day are shown in Figure 24 (lower panel) and Table 9. Buprenorphine subjects had significantly lower (P=0.007; 95% CI -8 to -41) cold pressor pain tolerance responses than for control subjects at baseline on the ketorolac administration day. Pain tolerance responses for control subjects increased significantly from baseline to the ketorolac/morphine infusion (P<0.05; 95% CI 3 to 30), but not from baseline to the ketorolac infusion (P>0.05; 95% CI -10 to 17). Cold pressor tolerance responses for buprenorphine subjects did not increase significantly (P=0.064) between baseline and the ketorolac infusion, or baseline and the ketorolac/morphine infusion on the ketorolac administration day. There was also no significant difference (P=0.11) in cold pressor responses between infusions for buprenorphine subjects on the saline administration day.

Responses				
Adjuvant Analgesic Alone and With Morphine Study				
Response	Group	Baseline	Analgesic	Analgesic/Morphine
Cold Pressor S-ketamine Day (seconds)	Control	39±7 (12 to 61)	43±11 (17 to 77)	57±14 (16 to 106)*
	Buprenorphine	15.9±1.4 (12 to 20) †	17.3±2.1 (12 to 26)	19.7±2.2 (13 to 26)*
Cold Pressor Tramadol Day (seconds)	Control	31±7 (14 to 56)	52±11 (20 to 91)	75±22 (32 to 180)*
	Buprenorphine	14.2±1.8 (8.6 to 19) †	16.3±2.2 (8 to 23)	19.8±3.1 (11 to 29)*
Cold Pressor Ketorolac Day (seconds)	Control	37±7 (18 to 65)	41±7 (19 to 72)	54±10 (25 to 101)*
	Buprenorphine	12.8±1.1 (9.5 to 17) †	14.3±1.5 (10 to 20)	16.3±1.9 (10 to 23)
Cold Pressor Saline Day (seconds)	Buprenorphine	13.9±1.6 (9 to 20)	14.1±1.9 (8 to 23)	15.9±1.8 (9.2 to 22)
Respiration Rate S-ketamine Day (breaths per minute)	Control	17±1 (12 to 22)	15±2 (10 to 20)	14±2 (8 to 80)
	Buprenorphine	13.7±1.2 (9 to 17)	13.7±0.8 (10 to 15)	12.2±0.9 (9 to 14)
Respiration Rate Tramadol Day (breaths per minute)	Control	17.3±1 (14 to 20)	12.0±1 (10 to 16)**	11.7±1 (10 to 17)**
	Buprenorphine	13.3±1 (11 to 18)†	12.8±1.1 (8 to 15)	11.3±0.9 (8 to 14)
Respiration Rate Ketorolac Day (breaths per minute)	Control	17.8±1.5 (12 to 22)	16.2±0.9 (12 to 18)	14.2±0.8 (12 to 16)
	Buprenorphine	12.8±0.9 (11 to 16) †	13.2±1.3 (8 to 17)	12±1.4 (9 to 18)
Respiration Rate Saline Day (breaths per minute)	Buprenorphine	13.7±1.4 (9 to 18)	13.2±0.8 (12 to 17)	11.5±0.9 (8 to 14)

Table 9 Cold pressor and electrical stimulation responses, and respiration rates on S-ketamine, tramadol and ketorolac administration days for buprenorphine maintained and control subjects. Data are mean±SEM (range). † $P<0.05$ between groups, *$P<0.05$, ** $P<0.01$ between treatments.

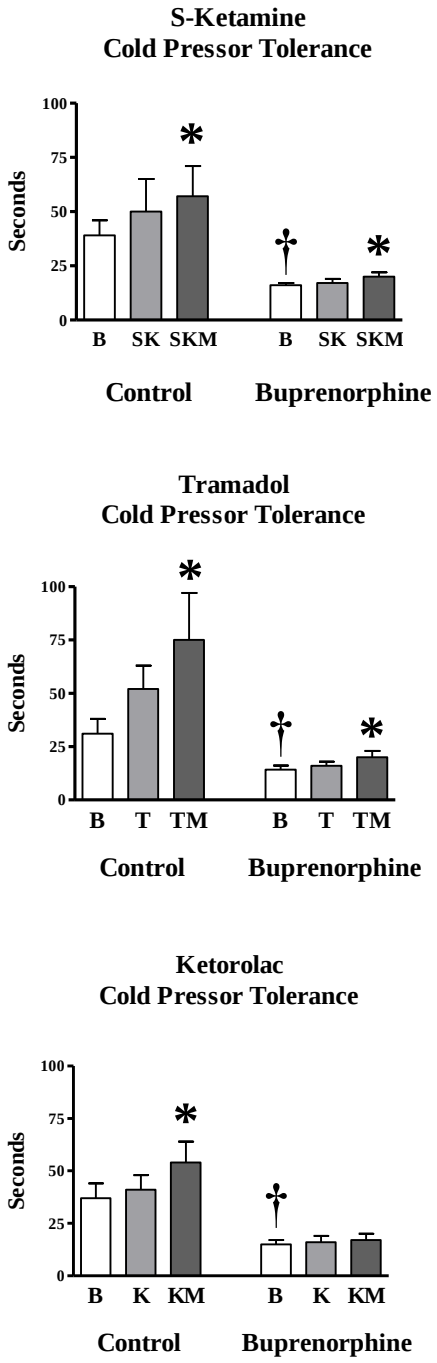

Figure 24 Cold pressor mean (±SEM) pain tolerance responses for 6 buprenorphine and 6 control subjects at respective baselines and S-ketamine infusion (SK) and S-ketamine/morphine infusion (SKM) (upper panel), tramadol infusion (T) and tramadol/morphine infusion (TM) (middle panel), and ketorolac infusion (K) and ketorolac/morphine infusion (KM) are shown (lower panel).
† $P<0.05$ between groups, * $P<0.05$ between treatments.

The percentage changes in the cold pressor pain tolerance test for buprenorphine subjects from baseline to the S-ketamine/morphine and tramadol/morphine infusions, and for control subjects from baseline to the S-ketamine/morphine, and from baseline to the tramadol/morphine infusions are shown in Figure 25. Buprenorphine subjects improved 23±6% from baseline during the S-ketamine/morphine infusion and 41±14% from baseline during the tramadol/morphine infusion. For reference, the control subjects' improvement of 45±25% from baseline during the S-ketamine/morphine infusion is also shown. In the tramadol/morphine infusion, the control subjects' improved their cold pressor pain tolerance score by 156±50% from baseline.

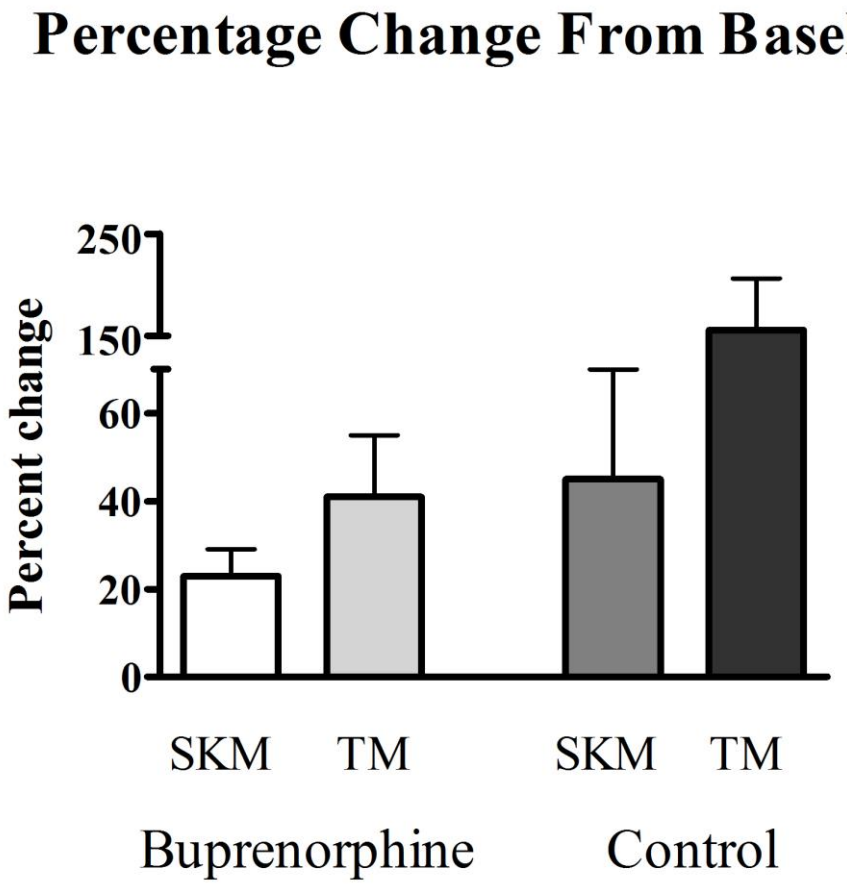

Figure 25 The percentage changes from baseline for 6 buprenorphine subjects during S-ketamine/morphine infusion (SKM), tramadol/morphine infusion (TM), and 6 control subjects during S-ketamine/morphine infusion (SKM) and tramadol/morphine infusion (TM) are shown. Results are represented as mean ± SEM. The Y axis is in two segments to describe the extent of percentage change for control subjects during the tramadol/morphine infusion.

6.3.2.2. Electrical stimulation

Electrical stimulation pain tolerance responses for control and buprenorphine subjects on the S-ketamine administration day at baseline, and the S-ketamine and S-ketamine/morphine infusions are shown in Figure 26 (upper panel) and Table 9. Pain tolerance responses for buprenorphine subjects were not significantly different (P=0.25) than for control subjects at baseline. Within group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.032) from baseline to the S-ketamine/morphine infusion (P<0.05; 95% CI 0.5 to 19), but not from baseline to the S-ketamine infusion (P>0.05; 95% CI -0.2 to 18). Pain tolerance responses for buprenorphine subjects did not increase significantly (P=0.16) between baseline and the S-ketamine infusion or the S-ketamine/morphine infusion.

The tramadol administration day electrical stimulation pain tolerance responses for control and buprenorphine subjects at baseline, and the tramadol and tramadol/morphine infusions are shown in Figure 26 (middle panel) and Table 9. Pain tolerance responses for buprenorphine subjects were not significantly different than for control subjects at baseline (P=0.14). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.007) from baseline to the tramadol infusion (P<0.05; 95% CI 2.9 to 22) and from baseline to the tramadol/morphine infusion (P<0.05; 95% CI 3.3 to 23). Pain tolerance responses for buprenorphine subjects did not increase significantly (P=0.48) between baseline and the S-ketamine infusion or the S-ketamine/morphine infusion.

Control and buprenorphine subjects' electrical stimulation pain tolerance responses on the ketorolac administration day are shown in Figure 26 (lower panel) and Table 9. Pain tolerance responses for buprenorphine subjects were not significantly different than for control subjects at baseline (P=0.26). Within-group comparisons revealed that pain tolerance responses for control subjects increased significantly (P=0.026) from baseline to the ketorolac/morphine infusion (P<0.05; 95% CI 4.0 to 28), but not the ketorolac infusion (P<0.05; 95% CI -6.0 to 18). Pain tolerance responses for buprenorphine subjects did not increase significantly (P=0.15) between baseline and the ketorolac infusion or the ketorolac/morphine infusion. There was also no significant difference (P=0.32) in electrical stimulation responses between infusions for buprenorphine subjects on the saline administration day.

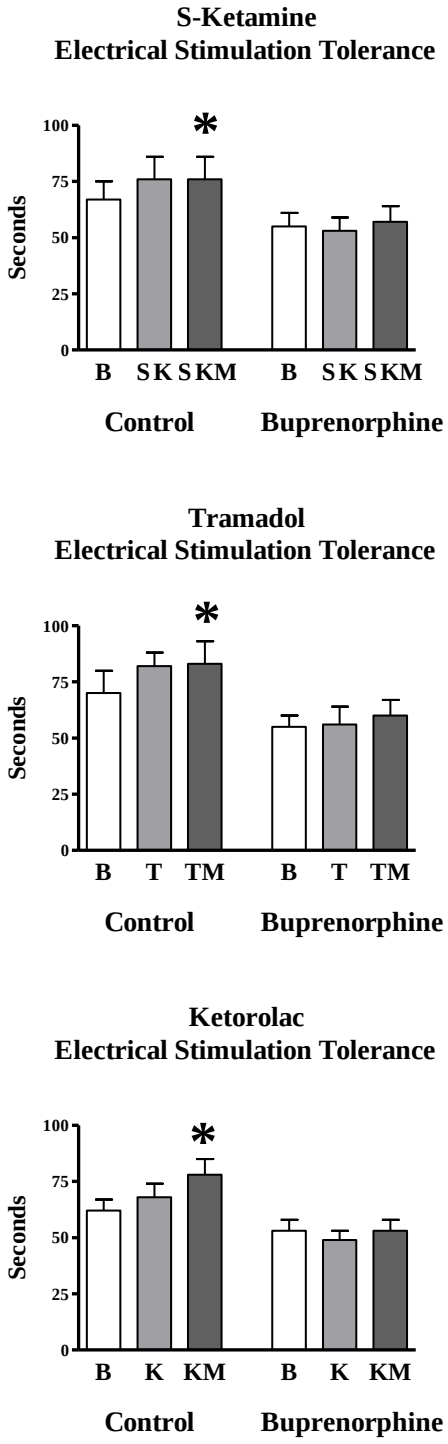

Figure 26 Electrical stimulation pain tolerance responses on S-ketamine infusion (SK) and S-ketamine/morphine infusion (SKM) (upper panel), tramadol infusion (T) and tramadol/morphine infusion (TM) (middle panel), and ketorolac infusion (K) and ketorolac/morphine infusion (KM) are shown (lower panel). Results are represented as mean ± SEM. * $P<0.05$ between treatments.

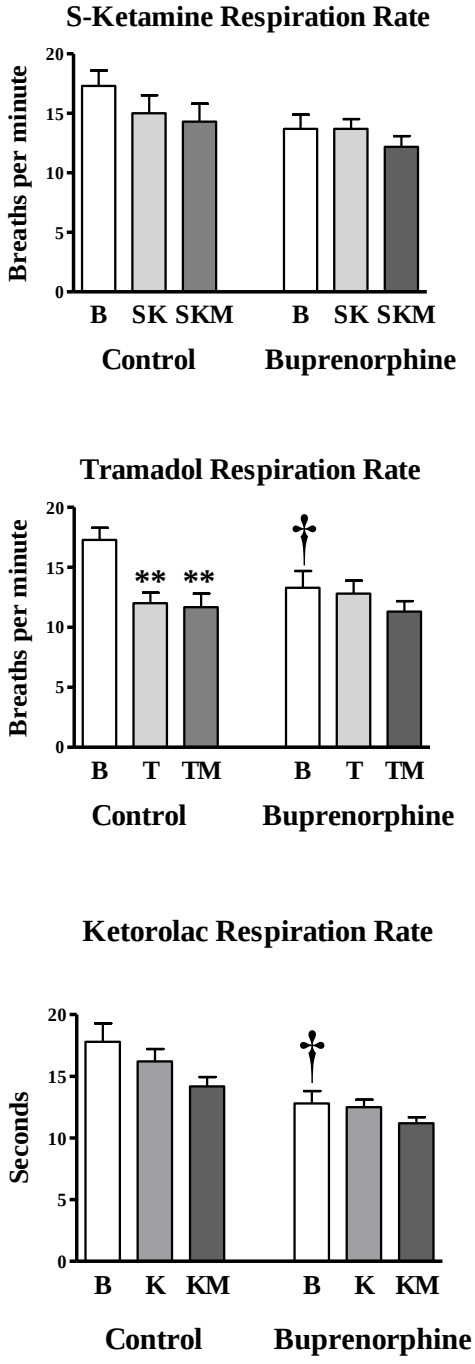

Figure 27 Respiration rates on S-ketamine infusion (SK) and S-ketamine/morphine infusion (SKM) (upper panel), tramadol infusion (T) and tramadol/morphine infusion (TM) (middle panel), and ketorolac infusion (K) and ketorolac/morphine infusion (KM) are shown (lower panel). Results are represented as mean ± SEM. † $P<0.05$ between groups, ** $P<0.01$ between treatments.

6.3.2.3. Respiration rate

The respiration rate (breaths per minute) for control subjects, relative to baseline, and when on the S-ketamine and S-ketamine/morphine infusions are shown in Figure 27 (upper panel) and Table 9. Respiration rate for buprenorphine subjects was not significantly lower (P=0.064) than for control subjects at baseline. There were no significant changes for control subjects (P=0.46) from baseline to the S-ketamine or the S-ketamine/morphine infusions. There were also no significant changes for buprenorphine subjects (P=0.25) from baseline to the S-ketamine or S-ketamine/morphine infusions.

The respiration rate relative to baseline, and the tramadol and tramadol/morphine infusions are shown in Figure 27 (middle panel) and Table 9. Respiration rate for buprenorphine subjects was significantly lower than for control subjects at baseline (P=0.017; 95% CI 0.88 to 7.1). Within-group comparisons revealed that the respiration rate for control subjects decreased significantly (P=0.0003) between baseline and the tramadol infusion (P<0.01; 95%CI -2.3 to -8.4) and the tramadol/morphine infusion (P<0.01; 95% CI -2.6 to -9). Respiration rates for buprenorphine subjects did not decrease significantly (P=0.14) between baseline and the tramadol and the tramadol/morphine infusion.

Control and buprenorphine subjects' respiration rates on the ketorolac administration day are shown in Figure (lower panel) and Table 9. The respiration rate for buprenorphine subjects was significantly lower than for control subjects at baseline (P=0.018; 95% CI -1.1 to -8.9). Within-group comparisons revealed that the respiration rate for control subjects did not decrease significantly (P=0.17) between baseline and the ketorolac and ketorolac/morphine infusions. The respiration rate for buprenorphine subjects did not decrease significantly (P=0.08) between baseline and the ketorolac and the ketorolac/morphine infusions. There was also no significant difference (P=0.22) in cold pressor responses between infusions for buprenorphine subjects on the saline administration day.

6.3.3. Adverse events

There were no serious adverse events in the study. Two buprenorphine subjects experienced mild and indistinct hallucinatory experiences during the tramadol infusions. Four buprenorphine subjects reported moderate feelings of disassociation during the S-ketamine infusions.

As described in Study 3, one healthy control subject withdrew from the study because of decreased SPO_2 (below 93% SPO_2 for more than one minute) which resolved within an hour and required 0.2

mg of naloxone hydrochloride (Mayne Pharma, Mulgrave, Australia) IM. Three control subjects reported moderate feelings of disassociation during the S-ketamine infusions. Four control subjects reported mild and indistinct hallucinatory experiences during the tramadol infusions.

6.3.4. Methadone, buprenorphine maintained subject comparisons

As stated earlier, there were no significant differences in plasma adjuvant concentrations between buprenorphine and methadone subjects in the S-ketamine/morphine (p=0.8), tramadol/morphine (p=0.8) and ketorolac/morphine (p=0.8) infusions.

6.4. Discussion

The study examined the antinociceptive effects of adjuvant analgesics (S-ketamine, tramadol and ketorolac) alone and combined with morphine in buprenorphine maintained and healthy control subjects. The study confirmed that buprenorphine subjects were hyperalgesic compared to healthy control subjects in the cold pressor test. Each of the adjuvants, combined with morphine, provided statistically significant antinociception to the group of healthy control subjects. The adjuvants S-ketamine and tramadol, combined with morphine, provided statistically significant antinociceptive effects in the cold pressor test for buprenorphine maintained subjects. An important question is whether these improvements for buprenorphine subjects were clinically relevant.

As described in section 1.11.2, buprenorphine has a very different pharmacological profile from methadone. Koppert et al (2005) found that in healthy volunteers, buprenorphine administered both sublingually and intravenously, had little analgesic action but pronounced antihyperalgesic action. Interestingly, it has also been suggested that methadone may have antihyperalgesic effect. Meyer et al (2005), in related work with an electrically induced hyperalgesia model, found that the antihyperalgesic properties of methadone were present but less pronounced than those of ketamine and buprenorphine. Koppert (2006) and Russo (2006) proposed an antihyperalgesic/analgesia ratio with ketamine having the most pronounced antihyperalgesic effect with buprenophine and then methadone in decreasing order of effect. Some authors have suggested that the antihyperalgesic action of buprenorphine may be a result of antagonistic effect at the kappa opioid receptors (Pergolizzi et al. 2010, Andresen et al. 2011). In a communication, Koppert (2006) suggested caution with the extrapolation of their experimental results to the clinical situation and suggested the need for further investigation. The findings of Koppert et al (2005) with regards to the antihyperalgesic nature of buprenorphine have not been replicated conclusively to date. A recent study by Andresen et al (2011) failed to show an antihyperalgesic effect for buprenorphine.

If buprenorphine did possess stronger antihyperalgesic effect compared to methadone, differences might be found in the degree of hyperalgesia between those maintained on the two agents. However, in this series of studies, there was statistically no difference between the hyperalgesia experienced in the cold pressor test between subjects maintained on methadone and

subjects maintained on buprenorphine. There was also no significant difference between methadone and buprenorphine subjects' respiratory rate. Both methadone and buprenorphine subjects were found to be cross-tolerant to the antinociceptive effects of high doses of intravenous morphine. Compton et al (2012) also found no difference between the hyperalgesia experienced by those maintained on methadone and those subjects maintained on buprenorphine in the cold pressor test. They also found that opioid dependent subjects were not hyperalgesic in the electrical stimulation test. They suggested that this pain sensitivity in the cold pressor text was present prior to stabilisation on the long acting opioids.

While studies 1 and 2 did not find that methadone and buprenorphine subjects differed in their degree of hyperalgesia and their antinociceptive or respiratory response to morphine, results from this study suggest that buprenorphine subjects may differ from methadone subjects in terms of their response to adjuvants and high dose morphine. In Study 3, while the adjuvant analgesics ketorolac and tramadol alone or combined with high dose morphine, failed to produce statistically significant pain relief with methadone subjects, S-ketamine in combination with morphine produced a statistically significant improvement. However, the change was a mean of approximately 2 seconds or 12% improvement from baseline, and therefore could not be considered clinically significant. This study found that S-ketamine and tramadol combined with high-dose morphine produced statistically significant improvement in antinociception in the cold pressor test for buprenorphine subjects of a mean of 3.8 (S-ketamine) and 5.6 (tramadol) seconds. A central question is whether this improvement was clinically relevant.

One of the most important things to consider when ensuring adequate pain control is the method for evaluation of pain (Carr et al. 1992, Carr and Goudas 1999). Carr (1992) and Carr and Goudas (1999) contend that effectiveness of pain treatment cannot be interpreted without considering the percentage of pain reduction or changes in the Numerical Rating Scale (1-10). Cepeda et al (2003) found that in patients with post-surgical acute pain, both the meaning of changes in the Numerical Rating Scale and the meaning of percent pain reduction depend upon baseline pain intensity. Cepeda et al (2003) examined 700 post-surgical patients during opioid titration to ascertain what reduction in pain was considered to be clinically significant. The researchers found that a 20% reduction in pain corresponded to "minimal improvement", a 35% decrease in pain corresponded to "much improvement" and a 45% decrease in pain corresponded to "very much improvement". While Cepeda et al (2003) were referring to post-surgical acute pain and not experimental pain, this guide may be considered a broad indication to a patient's perception of improvement in pain.

When this guide is applied to the results from these sets of studies, it suggests that S-ketamine plus high dose morphine produced an improvement in experimental pain for methadone

subjects of 12% from baseline. This is well below "minimal improvement" in the Cepeda et al (2003) guide and therefore not clinically significant.

S-ketamine plus high dose morphine produced an improvement in pain for buprenorphine subjects of 23% from baseline. This can be considered more than "minimal improvement". The most promising of the combinations for buprenorphine patients was tramadol and high dose morphine which produced a 39% improvement from baseline. According to the Cepeda et al (2003) suggested guide, this was in the "much improved" category. In comparison, for the control subjects, S-ketamine and morphine produced a 45% improvement and was in the "very much improved category". Interestingly, tramadol and morphine produced a 151% improvement in the control subjects group which exceeded the scope of the Cepeda et al (2003) guidelines.

However, it is important to consider what these changes mean in absolute as well as percentage terms for buprenorphine subjects. These changes amount to improvements in the cold pressor test from approximately 14 or 16 seconds at baseline to a maximum of 20 seconds. That is, these are improvements in the order of 4 to 6 seconds. This is in comparison with matched healthy control subjects that had a baseline latency of between 30 and 35 seconds. Buprenorphine subjects improved their pain tolerance, but at best fell far short of the healthy control subjects' baseline. Whether this represents meaningful improvement for buprenorphine subjects is unclear as they remained hyperalgesic.

The anti-hyperalgesic effect of buprenorphine may be tissue and pain modality specific. Andresen et al (2011) compared transdermal buprenorphine and fentanyl in healthy volunteers using a variety of pain tests. Buprenorphine, but not fentanyl, had an antinociceptive effect on bone associated pain and mechanical pain stimulation in the ultraviolet B (or medium wave) light-induced primary hyperalgesic area. Both drugs produced antinociception in the thermal stimulation test. Neither drug had an antinociceptive effect in the nerve growth factor induced muscle soreness nor capsaicin induced hyperalgesia (Andresen et al. 2011). These observed differences in analgesic or antihyperalgesic effect reinforce the observation that antinociceptive response is dependent on the drug, the modality of the experimental pain test and the tissues affected. A strength of this series of studies is that different drugs (morphine, ketorolac, S-ketamine and tramadol) different modalities (cold pressor and electrical stimulation) and different tissues (hand and forearm, ear) were utilised to observe possible effects.

In conclusion, the study confirmed that buprenorphine subjects, similarly to methadone subjects, were hyperalgesic in the cold pressor test compared with healthy control subjects. The combination of S-ketamine, tramadol or ketorolac and morphine provided clinically significant antinociception to healthy control subjects. While the combinations of S-ketamine or tramadol and

high dose morphine provided statistically significant antinociception to buprenorphine subjects, it is not clear whether this change represents a clinically significant improvement.

7. Summary of major findings and conclusion

As described in chapter 1, the aims of this series of studies were to investigate whether subjects maintained on methadone or buprenorphine required a higher therapeutic plasma concentration range (high plasma concentrations) of morphine with or without the addition of opioid adjuvants (ketorolac or S-ketamine or tramadol) to produce acute experimental antinociception in comparison with a group of non-opioid tolerant healthy controls.

The main findings in this series of studies are as follows:

High plasma concentrations of morphine alone did not produce antinociception in methadone and buprenorphine subjects in comparison with a group of non-opioid tolerant health controls.

In methadone subjects, tramadol and ketorolac, when combined with high dose morphine, failed to provide antinociception in methadone subjects. The combination of S-ketamine and high dose morphine provided statistically, but not clinically significant improvement in antinociception.

In buprenorphine subjects, ketorolac and high dose morphine did not provide antinociception. While the combinations of S-ketamine or tramadol and high dose morphine provided statistically significant antinociception in the cold pressor test, it was not clear whether this change represented a clinically significant improvement.

Other findings were that there was no difference between the hyperalgesia experienced in the cold pressor test between subjects maintained on methadone and subjects maintained on buprenorphine at baseline. In terms of respiration rate at baseline, there was no significant difference between the groups. Both methadone and buprenorphine groups were cross tolerant to the antinociceptive and respiratory effects of high doses of morphine. The magnitude of the daily maintenance dose made no difference in the antinociceptive responses of the different methadone (11-45, 46-80, 81-115 mg per day) and buprenorphine (2 to 8, 9-15, 16-22 mg) groups. The respiration rates of subjects on methadone (14±0.5 range 10-18 to 12.3±0.5 range 9-16 breaths per minute) and buprenorphine (14.4±0.8 range 9-20 to 11.8±0.5 range 9-15 breaths per minute) decreased significantly from baseline and both groups were equally at risk of respiratory depression at the high doses of morphine utilised.

As stated above, there were some statistical differences between the antinociceptive responses of methadone subjects, buprenorphine subjects and healthy controls in the morphine and opioid adjuvant studies, but whether these differences were clinically significant is unclear. In study 3, high doses of morphine and adjuvants, while providing antinociception to healthy controls, did not

provide antinociception in methadone subjects. In study 4, S-ketamine and tramadol combined with high-dose morphine produced statistically significantly enhanced antinociception in the cold pressor test, with a mean of 3.8 seconds (23% improvement from baseline) (S-ketamine) (16±1.4 range 12-20 to 20±2.2 range 13-26) and tramadol plus morphine with a mean of 5.6 seconds (39% improvement from baseline) (14±1.8 range 9-19 to 20±3.1 range 11 to 29). However, even at best (20±3.1 range 11 to 29 (tramadol and morphine)) the subjects were still hyperalgesic compared to healthy control subjects at baseline (34±6 range 4-73 seconds) and therefore the clinical significance was likely to be limited. The clinical implications will be discussed in further detail below.

7.1. Clinical implications of research findings

The findings from this series of studies add to the body of evidence concerning the management of acute pain in the methadone and buprenorphine maintained population.

7.1.1. Study 1. Antinociceptive and respiratory effects of high dose morphine in methadone maintained subjects

This study examined the antinociceptive and respiratory effects of high dose morphine in methadone maintained subjects. Methadone maintained subjects were hyperalgesic in the cold pressor test (15±2 range 5-25 seconds) compared to healthy controls (34±6 range 4-73 seconds). Large scale case studies have suggested that the opioid tolerant population require three to four times the amount of opioid to manage post-operative pain than the opioid naïve population (Rapp et al. 1995, de Leon-Casasola 1996). There have been no other large scale studies examining the management of post-operative pain among opioid tolerant patients since these studies. Other more recent research with smaller numbers has confirmed that opioid tolerant patients require higher doses of opioids to provide pain management than the opioid naïve population (McCarthur et al. 2007, McCarthur et al. 2008, Patanwala et al. 2008). Study 1 in this thesis found that high dose morphine failed to provide statistically significant antinociception for methadone subjects. Power analysis indicated that a statistically significant effect was likely to be found with a sample size of eighty methadone subjects. However, the improvement would be in the order of 1.4 seconds in the cold pressor test and therefore of little clinical utility. The study found that high plasma concentrations of morphine had a statistically significant negative effect on the respiration rate but only by an average of 2 breaths per minute (14±0.5 range 10-18 breaths per minute decreased to 12±0.5 range 9-16 breaths per minute).

The magnitude of the daily maintenance dose made no difference in the baseline respiratory and antinociceptive responses of the different methadone groups. That is, the magnitude of the daily maintenance dose made no difference to the hyperalgesia experienced by the subjects. This suggests that hyperalgesia (antinociceptive response at baseline) exists even if the

subject is maintained on low doses of methadone and is likely to be a function of exposure to opioids rather than being related to dose.

A question for further investigation is whether hyperalgesia is a consequence of opioid maintenance treatment or whether it is a feature of the opioid addiction prior to entry into the opioid maintenance programme. One recent study has investigated this question. Compton et al (2012) examined patients (opioid addicted but not chronic pain) prior to entry into opioid maintenance treatment and found that opioid dependent patients were hyperalgesic in the cold pressor test and remained so after stabilisation on methadone or buprenorphine. As opioid use at time of baseline testing was verbally reported but not objectively confirmed (e.g. by plasma examination), it is not clear whether opioid maintenance treatment exacerbated the hyperalgesia experienced in the cold pressor test (Compton et al. 2012).

In Study 1 of this thesis, tolerance to the respiratory depressant effects of high dose morphine was less complete than tolerance to the antinociceptive effects of high dose morphine. Other studies (human and animal) have found that the respiratory depressant effects of methadone and other opioids also exhibit incomplete tolerance (Paronis and Woods 1997, Dyer et al. 1999). Interestingly, in my study, the higher daily methadone dose group had the greatest degree of respiratory depression. Intuitively, if respiratory opioid tolerance was a function of plasma opioid concentration, the higher the maintained daily methadone dose, the greater the respiratory tolerance. However, in this population, the high plasma methadone concentrations increased the respiratory depressant effect resulting from the highest morphine concentrations. The decrease was from 14 breaths per minute to 11 breaths per minute. This respiratory depression, at these very high plasma morphine and R-(-) methadone concentrations, even of this small magnitude, suggests that pursuing even higher plasma morphine concentrations to achieve antinociception may be unproductive as unacceptable respiratory depression may occur before an antinociceptive effect. For this reason, other agents should be considered for this population.

7.1.2. Study 2. Antinociceptive and respiratory effects of high dose morphine in buprenorphine maintained subjects

Study 2 examined the antinociceptive and respiratory effects of high dose morphine in subjects maintained on buprenorphine for the treatment of opioid dependence. A number of authors have suggested that the magnitude of the daily buprenorphine dose has an effect on the blockade of opioid effects (Bickel et al. 1988, Rosen et al. 1994, Walsh et al. 1995, Comer et al. 2005). Patients are commonly maintained on daily doses of buprenorphine from 2 mg to 20 mg per day. This study also investigated whether different daily doses may have produced different effects with regards to opioid pain management.

High doses of morphine failed to provide antinociception for buprenorphine subjects in the experimental pain tests compared to controls. In spite of the pharmacological differences between the full agonist methadone and the partial agonist buprenorphine, buprenorphine maintained subjects performed similarly to the methadone maintained subjects in at least three respects.

Firstly, high doses of morphine had little antinociceptive effect in both buprenorphine and methadone subjects. Secondly, this dose significantly decreased the respiration rate for both groups (methadone 14±0.5 range 10-18 decreased to 12±0.5 range 9-16 breaths per minute: buprenorphine 14±0.8 range 9 to 20 decreased to 12±0.5 range 9 to 15 breaths per minute). Dahan et al (2006) suggested that there may be a ceiling effect for the respiratory depressant effects of buprenorphine but no ceiling effect for the analgesic effects. The results from this study neither supported nor rejected this suggestion. However, from this study, the drop in respiration rate with high dose morphine has implications if higher doses of morphine were to be utilised in this population. Thirdly, both buprenorphine and methadone subjects were similarly hyperalgesic in the cold pressor test at baseline. The reason(s) for the similarities between these two pharmacologically distinct entities is/are unclear.

Buprenorphine is a partial mu agonist with high receptor affinity and slow disassociation kinetics. Some authors have suggested that it acts as an antagonist at the kappa receptor (Lewis 1985, Walsh and Eissenberg 2003) and that this produces an antihyperalgesic effect (Pergolizzi et al. 2010, Andresen et al. 2011). In contrast, methadone is primarily a mu agonist. There is little clinical evidence that patients maintained on buprenorphine exhibit greater dependence or tolerance than patients maintained on methadone. Indeed, several studies suggest that buprenorphine maintained patients exhibit less physical dependence than methadone patients, and that this contributes to the favouring of one opioid over the other. The withdrawal syndrome associated with buprenorphine is reported to be shorter and less distressing than the withdrawal syndrome associated with methadone (Fudala et al. 1990, San et al. 1992, Walsh and Eissenberg 2003, Reed et al. 2007). However, both methadone and buprenorphine subjects exhibited similar antinociceptive and respiratory profiles in the first two studies of this thesis. In the Compton et al study (2012), following stabilisation on either methadone and buprenorphine, there was no difference in pain response between patient groups. There are three possible hypotheses for the genesis of hyperalgesia in this population. Firstly, these subjects may have predisposing hyperalgesia prior to opioid use (this hypothesis would be very difficult to test). Secondly, opioid use (misuse) prior to opioid maintenance produces hyperalgesia (as suggested by Compton et al (2012)). And thirdly, opioid maintenance produces hyperalgesia. This hyperalgesia may provide the basis for the antinociceptive cross tolerance. Recent work by Treister (2012) and Chu (2012) have provided important findings with regards to the nature of opioid induced hyperalgesia. Treister et al (2012) found that abstinence for 5 months or more following periods on opioid

maintenance may reverse opioid induced hyperalgesia. Chu et al (2012) found that with certain populations, and with mean doses of 78 mg per day of sustained release morphine, pain tolerance can occur in the absence of hyperalgesia (See Introduction for further discussion of these studies).

In summary, higher doses of morphine than administered in these studies are unlikely to be effective in this population. As a result of the decrease in respiration rate, albeit limited at the doses studied here, this may not be a safe option. Other agents need to be considered. This was explored in Studies 3 and 4.

7.1.3. Study 3. Antinociceptive and respiratory effects of high dose morphine and adjuvant analgesics in methadone maintained subjects

This study examined the antinociceptive and respiratory effects of high dose morphine and the opioid adjuvant analgesics ketorolac, S-ketamine and tramadol in methadone maintained subjects. Three major classes of alternative analgesics to traditionally used opioids include the non-steroidal anti-inflammatory drugs (NSAIDS) (e.g. ketorolac), NMDA antagonists (e.g. S-ketamine) or a mixed action analgesics (e.g. tramadol). These three classes of alternative analgesics have different modes of action to opioids that act primarily at the mu opioid receptor. There is experimental and clinical evidence to support their use as analgesic agents alone and in combination with opioids (Rohdewald et al. 1988, Gwirtz et al. 1995, Park et al. 1995, Hummel et al. 1997, Leung et al. 2001, Blais et al. 2002, Hernandez-Delgadillo et al. 2002, Webb et al. 2002, Weinbroum 2003, Subramaniam et al. 2004, Cepeda et al. 2005, Kocabas et al. 2005, Elvir-Lazo and White 2010). In combination with opioids, they may provide superior pain management than opioids alone and may decrease opioid-related side effects

In the clinical area, such as in post-surgical recovery wards, high doses of morphine, alone and in combination with adjuvant analgesics provide analgesia in the opioid dependent population (McCarthur et al. 2007, McCarthur et al. 2008, Macintyre et al. 2010, Huxtable et al. 2011). The doses to achieve this analgesia were high and at the discretion of the clinician. Yet, in this study, high doses of morphine and adjuvants, while providing antinociception to healthy controls did not provide antinociception in methadone subjects (S-ketamine and high dose morphine provided statistically significant antinociceptive improvement in methadone subjects, but the improvement was of the order of a mean of two seconds or 12% from baseline and therefore unlikely to have clinical significance). In Studies 3 and 4, morphine was not studied alone. Therefore, the relative contribution of morphine/adjuvants to antinociception in healthy controls cannot be ascertained.

For the healthy control subjects, the most effective combination was tramadol and morphine. In the cold pressor test for this population, there was a significant mean change of more than 150% from baseline (compared to S-ketamine/morphine which produced a mean of 45%

significant improvement from baseline). Of note is that in spite of research that suggests that tramadol has a weak effect at the mu opioid receptor and would therefore produce limited respiratory depression (Grond and Sablotzki 2004, Filitz et al. 2008), this study found that the tramadol alone in healthy controls produced mean significant respiratory decrease from a baseline of 17±1 (range 14 to 20) to 12±1 (range 10-16) breaths per minute. The addition of morphine to the tramadol infusion produced no further significant change in respiratory depression with 12±1 (range10-17) breaths per minute. This suggests that either tramadol has a stronger opioid effect than previously reported or that some unknown mechanism was depressing the respiration rate. Interestingly, in the cold pressor test on the tramadol administration day for control subjects, at baseline subjects were able to keep their arms in the water for 31±7 (range 14 to 56) seconds, at tramadol alone infusion 52±11 (range 20 to 91) seconds and at tramadol plus morphine 75±22 (range 32 to 180) seconds. The reason for the increase in antinociception without further decrease in respiratory rate with the addition of morphine is unclear.

Opioid adjuvant therapy has important therapeutic potential among the opioid non-tolerant (healthy control) population. These results contrast with some of the published literature and further research with larger numbers of subjects could be explored to unequivocally confirm these findings.

7.1.4. Study 4. Antinociceptive and respiratory effects of high dose morphine and adjuvant analgesics in buprenorphine maintained subjects

This study examined the antinociceptive and respiratory effects of high dose morphine in subjects maintained on buprenorphine for the treatment of opioid dependence. As described previously, buprenorphine is a substantially different pharmacological entity from methadone. In the previous study, Study 3, the adjuvant analgesics ketorolac and tramadol alone or combined with high dose morphine, failed to produce statistically significant pain relief in methadone subjects. In contrast, in Study 3, S-ketamine in combination with morphine produced a statistically improvement. However, the change was a mean of approximately 2 seconds or 12% improvement from baseline, and could not be considered clinically significant. This study found that S-ketamine and tramadol combined with high-dose morphine produced statistically significant improvement in antinociception in the cold pressor test, with a mean of 3.8 seconds (23% improvement) (S-ketamine) (16±1.4 range 12-20 to 20±2.2 range 13-26) and tramadol plus morphine with a mean of 5.6 seconds (39% improvement) (14±1.8 range 9-19 to 20±3.1 range 11 to 29). It is more difficult to judge whether this difference would be clinically significant (Cepeda et al. 2003). It can be argued that even at best (20 seconds) the subjects were still hyperalgesic compared to healthy control subjects at baseline (34±6 range 4-73 seconds) and therefore the significance was limited.

7.1.5. Comparison of methadone subjects to buprenorphine subjects

Compton et al (2012) found that opioid dependent subjects were hyperalgesic prior to induction onto opioids and that there was no difference in the hyperalgesia experienced in the cold pressor test by those maintained on methadone and buprenorphine subsequent to stabilisation of 12 to 18 weeks. While these findings need to be replicated, they may have relevance to the findings of this study as was discussed in Study 4.

7.2. Strengths and limitations

7.2.1. Strengths

Methadone and buprenorphine subjects are a difficult group of subjects to enrol and retain in a series of experimental pain studies.

The subjects volunteered to be involved in very demanding (involving repeated experience of pain) and long (in excess of ten hours) days. However, this study was able to enrol and retain these subjects in spite of these extremely unpleasant conditions. As mentioned in the limitations, longer infusions times would have been difficult and the Research Ethics Committee may have objected to submitting these individuals to further inconvenience and possibly increased risk.

These subjects in this study had a history of illicit drug dependency. The factors of illicit drug use, crime to support illicit drug dependency and difficulties with personal relationships that the opioid dependent population in general experience, may have contributed negatively to the subjects' abilities to comply with the study regime. In spite of this, the study was able to enrol and retain adequate numbers to make rigorous analyses.

One of the strengths of the study is that the plasma concentrations of the relevant analgesics were measured, rather than relying on doses only. Knowledge of the plasma concentrations enabled much better interpretation of the results than would have been possible with doses alone. To my knowledge, few other studies have measured and related doses, plasma concentrations and responses.

Even at these concentrations that were below targets, respiratory depression occurred. At higher doses the risk of respiratory depression may have been unacceptable.

The purpose of this series of studies was to accurately measure antinociceptive responses. Such responses are dependent on the subject characteristics, the drug, the modality of the experimental pain test and the tissues affected. A strength of the design of this series was that different drugs from four different pharmacological groups (morphine, ketorolac, S-ketamine and tramadol), different drug doses, different subject's daily maintenance dose ranges and different modalities (cold pressor and electrical stimulation) were utilised to measure possible effects.

7.2.2. Limitations

The original design of the experiment (in studies 1 and 2) included six methadone subjects in three daily dose ranges of methadone and six buprenorphine subjects in three daily dose ranges of buprenorphine tested with high doses of morphine. In study 2, following the testing of four buprenorphine subjects in each of the three daily dose ranges and no differences found in the cold pressor tests, electrical stimulation tests, or in terms of respiration rate, it was decided that it was unethical to subject more subjects to more pain testing.

Methadone and buprenorphine subjects were stratified into commonly used daily dose groups to examine the possible effects of small, medium or large daily dosing. Subjects were subsequently combined and analysed as larger groups. Overall numbers for the combined methadone (n=18) and combined buprenorphine groups (n=12) were small, but as mentioned in the strengths section, the subjects were recruited from a difficult population.

The series of studies in this thesis were not investigations of subjects with clinical pain. Rather these studies were double blind randomised controlled trials in strictly controlled experimental environments. The reason for such a design was to minimise many confounding variables (e.g. different types of clinical pain, different intensities, different parts of the body affected) and isolate the effect of an opioid. However, great caution needs to be taken in translating results from the experimental situation to the clinical arena. The study used two models of experimental pain and the subjects were not experiencing clinical pain.

Concentration-effect relationship extrapolations are approximate estimates. The intended target concentrations were derived from concentration/effect relationships in non-opioid dependent individuals (Berkowitz et al. 1975, Dahlstrom et al. 1982, Gourlay et al. 1986, Lehmann et al. 1990, Arendt-Nielsen et al. 1996, Mandema and Stanski 1996, Tucker et al. 1999). There was great variability between subjects in the concentrations that were achieved at the same dose and, generally, the achieved concentrations were either less than or greater than predicted. There may be a number of reasons for this. Subjects in these studies were not opioid tolerant and may have had different characteristics (e.g. different ages, weights, diseases, co-medications) resulting in clearance values which differed (increased or decreased) from those references. For healthy controls, methadone and buprenorphine maintained clients, plasma ketorolac concentrations were approximately 2 to 3 times above target concentrations, tramadol was approximately 30% below targets and S-ketamine concentrations were approximately 50% below targets. Plasma morphine concentrations were approximately on target for control subjects. For methadone and buprenorphine subjects, plasma morphine concentrations were between 30% and 40% below targets. It is important to note that respiratory depression occurred at morphine concentrations that were below target for opioid tolerant subjects. If higher concentrations had been achieved, there would have been a greater risk of respiratory depression.

The morphine dose (12 mg) was uniform for healthy controls in all studies and not determined according to weight (mg/kg). This dose of morphine had previously been shown to

produce antinociception in opioid naïve subjects without serious adverse effects (Doverty et al. 2001b). The dosing regime was chosen based on evidence that a plasma morphine concentration of approximately 15 ng/ml is adequate for minimum effective post-surgical analgesia (Dahlstrom et al. 1982, Gourlay et al. 1986) and approximately 50 ng/ml provided analgesia for moderate to severe post-surgical pain (Berkowitz et al. 1975) . The decision to have a uniform dose for all healthy controls rather than calculating according to weight could have been considered a limitation. However, at that dose there was negligible difference in pharmacokinetics between the 102 kg healthy control (plasma morphine concentration 19 ng/ml) and the 59 kg healthy control (plasma morphine concentration 21 ng/ml) (range of healthy controls was 19 to 31 ng/ml).

7.3. Directions for future research

There are a number of possible strategies that may provide effective pain relief in patients maintained on opioids for the treatment of opioid dependence. Two such strategies are the use of high potency short acting opioids such as remifentanil and the use of ultra-low dose naltrexone combined with opioids.

Hay et al (2006) examined the effect of an intravenous infusion of remifentanil in methadone subjects. They found a five-fold increase in pain tolerance values while decreasing respiration rates from a mean of 14 breaths per minute to a mean of 8 breaths per minute. As they stated, while there is potential for remifentanil to be used as an analgesic for the treatment of acute pain in methadone patients, the doses required may be many-fold greater than those used in healthy control subjects, respiratory depression may be a concern and the analgesic effects dissipate rapidly after the cessation of the remifentanil infusion. Such an approach may not be safely achieved in contexts where there are not significant supports, for example, outside of a hospital high dependency unit.

Hay et al (2011) have also examined the antinociceptive effect of buprenorphine combined with ultra-low dose naltrexone. They found that the ratio of 166:1 of buprenorphine to naltrexone increased cold pressor tolerance by 30.9% compared to buprenorphine alone with minimal respiratory depression and without an increase in adverse effects. The antinociceptive effects of ultra-low dose naltrexone have been studied in combination with buprenorphine, methadone and morphine clinically (Cruciani et al. 2003, Chindalore et al. 2005, Bijur et al. 2006, Hamann and Sloan 2007, La Vincente et al. 2008, Farahmand et al. 2012), and in animal models (Crain and Shen 1996, Crain and Shen 2000, Crain and Shen 2001). Studies have shown that ultra-low dose naltrexone also attenuates the development of tolerance in animal models (McNaull et al. 2007, Mattioli et al. 2010). The ultra-low doses of antagonist (picomolar to nanomolar range) are thought to selectively block the anti-analgesic, excitatory Gs-coupled mu opioid receptors without affecting the binding to the inhibitory Gi/Go-coupled receptor (Crain and Shen 2000, La Vincente et al. 2008). The effect of ultra-low dose naltrexone and opioids on the experience of hyperalgesia and antinociceptive tolerance of opioid maintained patients is unknown. Such an approach may yet

prove to be efficacious in the provision of pain management in the opioid tolerant population and warrants further investigation.

7.4. Conclusion

Opioids still remain the major drug family for the treatment of acute severe pain. Opioids such as methadone and buprenorphine remain important agents for the treatment of opioid dependency. Yet, opioid use produces a range of unwanted side effects such as hyperalgesia, tolerance and respiratory depression. The development of hyperalgesia is the predominant reason why opioid tolerant individuals do not respond to clinically used doses of opioids. The results of these studies, albeit in experimental pain, further reinforce this point. The important clinical problem of safe and efficacious analgesia for acute pain in this population remains unresolved with the pharmacological agents currently available.

Appendix

Appendix 1: Athanasos P, Smith C, White J, Somogyi A, Bochner F and Ling W. (2006) Methadone maintenance patients are cross-tolerant to the antinociceptive effects of high morphine concentrations. Pain. Jan; 120: 267-275

Athanasos, P., Smith, C.S., White, J.M., Somogyi, A.A., Bochner, F. & Ling, W. (2006) Methadone maintenance patients are cross-tolerant to the antinociceptive effects of very high plasma morphine concentrations.
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Bibliography

Abadinsky, H. (2008). Drug Use and Abuse. A Comprehensive Introduction. Sixth Edition., Cengage Learning**:** 35.
Alford, D. P., Compton, P. and Samet, J. H. (2006). "Acute pain management for patients receiving maintenance methadone or buprenorphine therapy." Ann Intern Med **144**(2): 127-134.
Amass, L., Bickel, W. K., Higgins, S. T. and Badger, G. J. (1994). "Alternate-day dosing during buprenorphine treatment of opioid dependence." Life Sci **54**(17): 1215-1228.
Andersen, G., Christrup, L. and Sjogren, P. (2003). "Relationships among morphine metabolism, pain and side effects during long-term treatment: an update." J Pain Symptom Manage **25**(1): 74-91.
Andresen, T., Staahl, C., Oksche, A., Mansikka, H., Arendt-Nielsen, L. and Drewes, A. M. (2011). "Effect of transdermal opioids in experimentally induced superficial, deep and hyperalgesic pain." Br J Pharmacol **164**(3): 934-945.
Andrews, H. L. (1943). "The Effect of Opiates on the Pain Threshold in Post-Addicts." J Clin Invest **22**(4): 511-516.
Anggard, E., Gunne, L. M., Homstrand, J., McMahon, R. E., Sandberg, C. G. and Sullivan, H. R. (1975). "Disposition of methadone in methadone maintenance." Clin Pharmacol Ther **17**(3): 258-266.
Angst, M. S., Koppert, W., Pahl, I., Clark, D. J. and Schmelz, M. (2003). "Short-term infusion of the mu-opioid agonist remifentanil in humans causes hyperalgesia during withdrawal." Pain **106**(1-2): 49-57.
Ardakani, Y. H. and Rouini, M. R. (2007). "Pharmacokinetics of tramadol and its three main metabolites in healthy male and female volunteers." Biopharm Drug Dispos **28**(9): 527-534.
Arendt-Nielsen, L., Curatolo, M. and Drewes, A. (2007). "Human experimental pain models in drug development: translational pain research." Curr Opin Investig Drugs **8**(1): 41-53.
Arendt-Nielsen, L., Nielsen, J., Petersen-Felix, S., Schinder, T. W. and Zbinden, A. M. (1996). "Effect of racemic mixture and the (S+)-isomer of ketamine on temporal and spatial summation of pain." Anaesthesia **77**: 625-631.
Arias, A., Feinn, R. and Kranzler, H. R. (2006). "Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis." Drug Alcohol Depend **83**(3): 262-268.
Arunasalam, K., Davenport, H. T., Painter, S. and Jones, J. G. (1983). "Ventilatory response to morphine in young and old subjects." Anaesthesia **38**(6): 529-533.
Athanasos, P., Farquarson, A., Compton, P., Psaltis, P. and Hay, J. L. (2008). "ECG characteristics of methadone and buprenorphine maintained subjects." Journal of addictive diseases **27**(3).
Athanasos, P., Smith, C. S., White, J. M., Somogyi, A. A., Bochner, F. and Ling, W. (2006). "Methadone maintenance patients are cross-tolerant to the antinociceptive effects of very high plasma morphine concentrations." Pain **120**: 267-275.
Ausubel, D. P. (1966). "The Dole-Nyswander treatment of heroin addiction." JAMA **195**(11): 949-950.
Ball, J. and Ross, A. (1991). The Effectiveness of Methadone Maintenance Treatment. New York, Springer Verlag.
Ballantyne, J. C. and LaForge, K. S. (2007). "Opioid dependence and addiction during opioid treatment of chronic pain." Pain **129**(3): 235-255.
Ballantyne, J. C., Loach, A. B. and Carr, D. B. (1988). "Itching after epidural and spinal opiates." Pain **33**(2): 149-160.

Barry, D. T., Beitel, M., Cutter, C. J., Garnet, B., Joshi, D., Rosenblum, A. and Schottenfeld, R. S. (2011). "Exploring relations among traumatic, posttraumatic, and physical pain experiences in methadone-maintained patients." J Pain **12**(1): 22-28.
Bartlett, S. E., Dodd, P. R. and Smith, M. T. (1994). "Pharmacology of morphine and morphine-3-glucuronide at opioid, excitatory amino acid, GABA and glycine binding sites." Pharmacol Toxicol **75**(2): 73-81.
Basbaum, A., Bushnell, C. and Devor, M., Eds. (2008). Pain: Basic Mechanisms. Pain 2008. An updated review. Refresher course syllabus. Seattle, IASP Press.
Beecher, H. K. (1966). "Pain: One Mystery Solved." Science **151**(3712): 840-841.
Beilin, B., Bessler, H., Mayburd, E., Smirnov, G., Dekel, A., Yardeni, I. and Shavit, Y. (2003). "Effects of preemptive analgesia on pain and cytokine production in the postoperative period." Anesthesiology **98**(1): 151-155.
Bekkering, G. E., Soares-Weiser, K., Reid, K., Kessels, A. G., Dahan, A., Treede, R. D. and Kleijnen, J. (2011). "Can morphine still be considered to be the standard for treating chronic pain? A systematic review including pair-wise and network meta-analyses." Curr Med Res Opin **27**(7): 1477-1491.
Belgrade, M. and Hall, S. (2010). "Dexmedetomidine infusion for the management of opioid-induced hyperalgesia." Pain Med **11**(12): 1819-1826.
Bell, R. F. (1999). "Low-dose subcutaneous ketamine infusion and morphine tolerance." Pain **83**(1): 101-103.
Bell, R. F., Dahl, J. B., Moore, R. A. and Kalso, E. (2005). "Peri-operative ketamine for acute post-operative pain: a quantitative and qualitative systematic review (Cochrane review)." Acta Anaesthesiol Scand **49**(10): 1405-1428.
Berkowitz, B. A., Ngai, S. H., Yang, J. C., Hempstead, J. and Spector, S. (1975). "The disposition of morphine in surgical patients." Clinical Pharmacology and Therapeutics **17**(6): 629-635.
Bickel, W. K., Amass, L., Crean, J. P. and Badger, G. J. (1999). "Buprenorphine dosing every 1, 2, or 3 days in opioid-dependent patients." Psychopharmacology (Berl) **146**(2): 111-118.
Bickel, W. K., Stitzer, M. L., Bigelow, G. E., Liebson, I. A., Jasinski, D. R. and Johnson, R. E. (1988). "Buprenorphine: dose-related blockade of opioid challenge effects in opioid dependent humans." J Pharmacol Exp Ther **247**(1): 47-53.
Bijur, P. E., Schechter, C., Esses, D., Chang, A. K. and Gallagher, E. J. (2006). "Intravenous bolus of ultra-low-dose naloxone added to morphine does not enhance analgesia in emergency department patients." J Pain **7**(2): 75-81.
Blais, V., Zhang, J. and Rivest, S. (2002). "In altering the release of glucocorticoids, ketorolac exacerbates the effects of systemic immune stimuli on expression of proinflammatory genes in the brain." Endocrinology **143**(12): 4820-4827.
Bourne, N. (2010). "Acute pain management in the opioid-tolerant patient." Nurs Stand **25**(12): 35-39.
Bowden, M. (2002). Pharmaceutical Acheivers. Philadelphia, Chemical Heritage Foundation.
Bresler, F. (1980). The Chinese Mafia. New York, Stein and Day.
Brewster, D., Humphrey, M. J. and McLeavy, M. A. (1981). "Biliary excretion, metabolism and enterohepatic circulation of buprenorphine." Xenobiotica **11**(3): 189-196.
Brill, S., Ginosar, Y. and Davidson, E. M. (2006). "Perioperative management of chronic pain patients with opioid dependency." Curr Opin Anaesthesiol **19**(3): 325-331.
Bujalska, M., Tatarkiewicz, J., de Corde, A. and Gumulka, S. W. (2008). "Effect of cyclooxygenase and nitric oxide synthase inhibitors on streptozotocin-induced hyperalgesia in rats." Pharmacology **81**(2): 151-157.
Bullingham, R. E., McQuay, H. J., Dwyer, D., Allen, M. C. and Moore, R. A. (1981). "Sublingual buprenorphine used postoperatively: clinical observations and preliminary pharmacokinetic analysis." Br J Clin Pharmacol **12**(2): 117-122.
Bullingham, R. E., McQuay, H. J., Moore, A. and Bennett, M. R. (1980). "Buprenorphine kinetics." Clin Pharmacol Ther **28**(5): 667-672.
Bullingham, R. E., McQuay, H. J., Porter, E. J., Allen, M. C. and Moore, R. A. (1982). "Sublingual buprenorphine used postoperatively: ten hour plasma drug concentration analysis." Br J Clin Pharmacol **13**(5): 665-673.

Burke, A., Smyth, E. and Fitzgerald, G. (2006). Analgesic-Antipyretic Agents; Pharmacotherapy of Gout. Goodman and Gilman's The Pharmacological Basis of Therapeutics. Eleventh Edition. L. Brunton, J. Lazo and K. Parker. New York, McGraw-Hill.
Burkholz, H. (1987). Pain: Solving the Mystery. New York Times. New York.
Cadet, P., Mantione, K. J. and Stefano, G. B. (2003). "Molecular identification and functional expression of mu 3, a novel alternatively spliced variant of the human mu opiate receptor gene." J Immunol **170**(10): 5118-5123.
Caraceni, A., Hanks, G., Kaasa, S., Bennett, M. I., Brunelli, C., Cherny, N., Dale, O., De Conno, F., Fallon, M., Hanna, M., Haugen, D. F., Juhl, G., King, S., Klepstad, P., Laugsand, E. A., Maltoni, M., Mercadante, S., Nabal, M., Pigni, A., Radbruch, L., Reid, C., Sjogren, P., Stone, P. C., Tassinari, D. and Zeppetella, G. (2012). "Use of opioid analgesics in the treatment of cancer pain: evidence-based recommendations from the EAPC." Lancet Oncol **13**(2): e58-e68.
Caraceni, A., Hanks, G., Kaasa, S., Bennett, M. I., Brunelli, C., Cherny, N., Dale, O., De Conno, F., Fallon, M., Hanna, M., Haugen, D. F., Juhl, G., King, S., Klepstad, P., Laugsand, E. A., Maltoni, M., Mercadante, S., Nabal, M., Pigni, A., Radbruch, L., Reid, C., Sjogren, P., Stone, P. C., Tassinari, D. and Zeppetella, G. (2012). "Use of opioid analgesics in the treatment of cancer pain: evidence-based recommendations from the EAPC." Lancet Oncol **13**(2): e58-68.
Carr, D., Jacox, A., Chapman, C., Fields, H., Heidrich, G. and Hester, N. (1992). Acute pain management: operative or medical procedures and trauma. Rockville, MD, Agency for Health Care Policy and Research.
Carr, D. B. and Goudas, L. C. (1999). "Acute pain." Lancet **353**(9169): 2051-2058.
Carroll, I. R., Angst, M. S. and Clark, J. D. (2004). "Management of perioperative pain in patients chronically consuming opioids." Reg Anesth Pain Med **29**(6): 576-591.
Celerier, E., Rivat, C., Jun, Y., Laulin, J. P., Larcher, A., Reynier, P. and Simonnet, G. (2000). "Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine." Anesthesiology **92**(2): 465-472.
Cepeda, M. S., Africano, J. M., Polo, R., Alcala, R. and Carr, D. B. (2003). "What decline in pain intensity is meaningful to patients with acute pain?" Pain **105**(1-2): 151-157.
Cepeda, M. S., Carr, D. B., Miranda, N., Diaz, A., Silva, C. and Morales, O. (2005). "Comparison of morphine, ketorolac, and their combination for postoperative pain: results from a large, randomized, double-blind trial." Anesthesiology **103**(6): 1225-1232.
Chakrabarti, A., Woody, G. E., Griffin, M. L., Subramaniam, G. and Weiss, R. D. (2010). "Predictors of buprenorphine-naloxone dosing in a 12-week treatment trial for opioid-dependent youth: secondary analyses from a NIDA Clinical Trials Network study." Drug Alcohol Depend **107**(2-3): 253-256.
Chakrabarti, S., Prather, P. L., Yu, L., Law, P. Y. and Loh, H. H. (1995). "Expression of the mu-opioid receptor in CHO cells: ability of mu-opioid ligands to promote alpha-azidoanilido[32P]GTP labeling of multiple G protein alpha subunits." J Neurochem **64**(6): 2534-2543.
Chang, G., Chen, L. and Mao, J. (2007). "Opioid tolerance and hyperalgesia." Med Clin North Am **91**(2): 199-211.
Chaudhary, R. S., Gangwal, S. S., Jindal, K. C. and Khanna, S. (1993). "Reversed-phase high-performance liquid chromatography of ketorolac and its application to bioequivalence studies in human serum." Journal of Chromatography **614**: 180-184.
Chazan, S., Ekstein, M. P., Marouani, N. and Weinbroum, A. A. (2008). "Ketamine for acute and subacute pain in opioid-tolerant patients." J Opioid Manag **4**(3): 173-180.
Chen, A. C., Dworkin, S. F., Haug, J. and Gehrig, J. (1989). "Human pain responsivity in a tonic pain model: psychological determinants." Pain **37**(2): 143-160.
Chen, A. C., Dworkin, S. F., Haug, J. and Gehrig, J. (1989). "Human pain responsivity in a tonic pain model: psychological determinants (Abstract)." Pain **37**(2): 143-160.
Chen, B. T., Hopf, F. W. and Bonci, A. (2010). "Synaptic plasticity in the mesolimbic system: therapeutic implications for substance abuse." Ann N Y Acad Sci **1187**: 129-139.
Chen, Y., Boettger, M. K., Reif, A., Schmitt, A., Uceyler, N. and Sommer, C. (2010). "Nitric oxide synthase modulates CFA-induced thermal hyperalgesia through cytokine regulation in mice." Mol Pain **6**: 13.

Chindalore, V. L., Craven, R. A., Yu, K. P., Butera, P. G., Burns, L. H. and Friedmann, N. (2005). "Adding ultralow-dose naltrexone to oxycodone enhances and prolongs analgesia: a randomized, controlled trial of Oxytrex." J Pain **6**(6): 392-399.
Chong, C., Schug, S. A., Page-Sharp, M., Jenkins, B. and Ilett, K. F. (2009). "Development of a sublingual/oral formulation of ketamine for use in neuropathic pain: Preliminary findings from a three-way randomized, crossover study." Clin Drug Investig **29**(5): 317-324.
Christensen, K., Daniels, S., Bandy, D., Ernst, C. C., Hamilton, D. A., Mermelstein, F. H., Wang, J. and Carr, D. B. (2011). "A double-blind placebo-controlled comparison of a novel formulation of intravenous diclofenac and ketorolac for postoperative third molar extraction pain." Anesth Prog **58**(2): 73-81.
Christie, M. J. (2008). "Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction." Br J Pharmacol **154**(2): 384-396.
Christoph, T., Kogel, B., Schiene, K., Meen, M., De Vry, J. and Friderichs, E. (2005). "Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain." Eur J Pharmacol **507**(1-3): 87-98.
Chu, L. F., Angst, M. S. and Clark, D. (2008). "Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations." Clin J Pain **24**(6): 479-496.
Chu, L. F., D'Arcy, N., Brady, C., Zamora, A. K., Young, C. A., Kim, J. E., Clemenson, A. M., Angst, M. S. and Clark, J. D. (2012). "Analgesic tolerance without demonstrable opioid-induced hyperalgesia: a double-blinded, randomized, placebo-controlled trial of sustained-release morphine for treatment of chronic nonradicular low-back pain." Pain **153**(8): 1583-1592.
Clarke, H., Pereira, S., Kennedy, D., Andrion, J., Mitsakakis, N., Gollish, J., Katz, J. and Kay, J. (2009). "Adding gabapentin to a multimodal regimen does not reduce acute pain, opioid consumption or chronic pain after total hip arthroplasty." Acta Anaesthesiol Scand **53**(8): 1073-1083.
Clements, J. A., Nimmo, W. S. and Grant, I. S. (1982). "Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans." J Pharm Sci **71**(5): 539-542.
Codd, E. E., Shank, R. P., Schupsky, J. J. and Raffa, R. B. (1995). "Serotonin and norepinephrine uptake inhibiting activity of centrally acting analgesics: structural determinants and role in antinociception." Journal of Pharmacology and Experimental Therapeutics **274**(3): 1263-1270.
Coller, J. K., Cahill, S., Edmonds, C., Farquharson, A. L., Longo, M., Minniti, R., Sullivan, T., Somogyi, A. A. and White, J. M. (2011). "OPRM1 A118G genotype fails to predict the effectiveness of naltrexone treatment for alcohol dependence." Pharmacogenet Genomics **21**(12): 902-905.
Coller, J. K., Christrup, L. L. and Somogyi, A. A. (2009). "Role of active metabolites in the use of opioids." Eur J Clin Pharmacol **65**(2): 121-139.
Collett, B. J. (2001). "Chronic opioid therapy for non-cancer pain." Br J Anaesth **87**(1): 133-143.
Colloca, L. and Benedetti, F. (2006). "How prior experience shapes placebo analgesia." Pain **124**(1-2): 126-133.
Comer, S. D., Walker, E. A. and Collins, E. D. (2005). "Buprenorphine/naloxone reduces the reinforcing and subjective effects of heroin in heroin-dependent volunteers." Psychopharmacology (Berl) **181**(4): 664-675.
Compton, M. A. (1994). "Cold-pressor pain tolerance in opiate and cocaine abusers: correlates of drug type and use status." Journal of Pain and Symptom Management **9**(7): 462-473.
Compton, P., Canamar, C. P., Hillhouse, M. and Ling, W. (2012). "Hyperalgesia in Heroin Dependent Patients and the Effects of Opioid Substitution Therapy." J Pain.
Compton, P., Charuvastra, V. C., Kintaudi, K. and Ling, W. (2000). "Pain responses in methadone-maintained opioid abusers." Journal of Pain and Symptom Management **20**(4): 237-245.
Compton, P., Charuvastra, V. C. and Ling, W. (2001). "Pain intolerance in opioid-maintained former opiate addicts: effect of long-acting maintenance agent." Drug Alcohol Depend **63**(2): 139-146.
Compton, P., Charuvastra, V. C. and Ling, W. (2003). "Effect of oral ketorolac and gender on human cold pressor pain tolerance." Clin Exp Pharmacol Physiol **30**(10): 759-763.

Compton, P., Kehoe, P., Sinha, K., Torrington, M. and Ling, W. (2010). "Gabapentin improves cold-pressor pain responses in methadone-maintained clients." Drug and Alcohol Dependence **109**(1-3): 213-219.
Compton, P. A., Ling, W. and Torrington, M. A. (2008). "Lack of effect of chronic dextromethorphan on experimental pain tolerance in methadone-maintained patients." Addict Biol **13**(3-4): 393-402.
Cone, E. J., Gorodetzky, C. W., Darwin, W. D. and Buchwald, W. F. (1984). "Stability of the 6,14-endo-ethanotetrahydrooripavine analgesics: acid-catalyzed rearrangement of buprenorphine." J Pharm Sci **73**(2): 243-246.
Cone, E. J., Gorodetzky, C. W., Yousefnejad, D., Buchwald, W. F. and Johnson, R. E. (1984). "The metabolism and excretion of buprenorphine in humans." Drug Metab Dispos **12**(5): 577-581.
Connor, M. and Christie, M. J. (1999). "Opioid receptor signalling mechanisms." Clinical and Experimental Pharmacology and Physiology **26**(7): 493-499.
Cooper, N., Forrest, K. and Cramp, P. (2006). Essential Guide to Acute Care Second Edition. Sydney, Blackwell Publishing.
Crain, S. M. and Shen, K. F. (1996). "Modulatory effects of Gs-coupled excitatory opioid receptor functions on opioid analgesia, tolerance, and dependence." Neurochem Res **21**(11): 1347-1351.
Crain, S. M. and Shen, K. F. (2000). "Antagonists of excitatory opioid receptor functions enhance morphine's analgesic potency and attenuate opioid tolerance/dependence liability." Pain **84**(2-3): 121-131.
Crain, S. M. and Shen, K. F. (2001). "Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low-dose naltrexone, unmasking potent opioid analgesia." Brain Res **888**(1): 75-82.
Crettol, S., Monnat, M. and Eap, C. B. (2007). "Could pharmacogenetic data explain part of the interindividual sensitivity to methadone-induced respiratory depression?" Crit Care **11**(1): 119.
Cruciani, R. A., Lussier, D., Miller-Saultz, D. and Arbuck, D. M. (2003). "Ultra-low dose oral naltrexone decreases side effects and potentiates the effect of methadone." J Pain Symptom Manage **25**(6): 491-494.
Cushman, P., Jr. (1972). "Methadone maintenance therapy for heroin addiction. Some surgical considerations." Am J Surg **123**(3): 267-270.
Dahan, A., van Dorp, E., Smith, T. and Yassen, A. (2008). "Morphine-6-glucuronide (M6G) for postoperative pain relief." Eur J Pain **12**(4): 403-411.
Dahan, A., Yassen, A., Romberg, R., Sarton, E., Teppema, L., Olofsen, E. and Danhof, M. (2006). "Buprenorphine induces ceiling in respiratory depression but not in analgesia." Br J Anaesth **96**(5): 627-632.
Dahlstrom, B., Tamsen, A., Paalzow, L. and Hartvig, P. (1982). "Patient-controlled analgesic therapy, Part IV: pharmacokinetics and analgesic plasma concentrations of morphine." Clin Pharmacokinet **7**(3): 266-279.
Dang, V. C. and Christie, M. J. (2012). "Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons." Br J Pharmacol **165**(6): 1704-1716.
Darke, S., Ross, J. and Teesson, M. (2007). "The Australian Treatment Outcome Study (ATOS): what have we learnt about treatment for heroin dependence?" Drug Alcohol Rev **26**(1): 49-54.
Dauri, M., Faria, S., Gatti, A., Celidonio, L., Carpenedo, R. and Sabato, A. F. (2009). "Gabapentin and pregabalin for the acute post-operative pain management. A systematic-narrative review of the recent clinical evidences." Curr Drug Targets **10**(8): 716-733.
Davis, A. M. and Inturrisi, C. E. (1999). "d-Methadone blocks morphine tolerance and N-methyl-D-aspartate-induced hyperalgesia." J Pharmacol Exp Ther **289**(2): 1048-1053.
Davis, J. J., Johnson, K. B., Egan, T. D., Vezina, D. P., Snell, T. E. and Swenson, J. D. (2003). "Preoperative fentanyl infusion with pharmacokinetic simulation for anesthetic and perioperative management of an opioid-tolerant patient." Anesth Analg **97**(6): 1661-1662.
Davis, J. J., Swenson, J. D., Hall, R. H., Dillon, J. D., Johnson, K. B., Egan, T. D., Pace, N. L. and Niu, S. Y. (2005). "Preoperative "fentanyl challenge" as a tool to estimate postoperative opioid dosing in chronic opioid-consuming patients." Anesth Analg **101**(2): 389-395, table of contents.
Davis, K. D. (2011). "Neuroimaging of pain: what does it tell us?" Curr Opin Support Palliat Care **5**(2): 116-121.

Daykin, A. P., Bowen, D. J., Saunders, D. A. and Norman, J. (1986). "Respiratory depression after morphine in the elderly. A comparison with younger subjects." Anaesthesia **41**(9): 910-914.
de Leon-Casasola, O. (2008). "Implementing therapy with opioids in patients with cancer." Oncol Nurs Forum **35 Suppl**: 7-12.
de Leon-Casasola, O. A. (1996). "Postoperative pain management in opioid-tolerant patients." Reg Anesth **21**(6 Suppl): 114-116.
de Leon-Casasola, O. A. and Lema, M. J. (1994). "Epidural bupivacaine/sufentanil therapy for postoperative pain control in patients tolerant to opioid and unresponsive to epidural bupivacaine/morphine." Anesthesiology **80**(2): 303-309.
de Leon-Casasola, O. A., Myers, D. P., Donaparthi, S., Bacon, D. R., Peppriell, J., Rempel, J. and Lema, M. J. (1993). "A comparison of postoperative epidural analgesia between patients with chronic cancer taking high doses of oral opioids versus opioid-naive patients." Anesth Analg **76**(2): 302-307.
De Oliveira, G. S., Jr., Agarwal, D. and Benzon, H. T. (2012). "Perioperative single dose ketorolac to prevent postoperative pain: a meta-analysis of randomized trials." Anesth Analg **114**(2): 424-433.
Dean, M. (2004). "Opioids in renal failure and dialysis patients." J Pain Symptom Manage **28**(5): 497-504.
Deb, I., Chakraborty, J., Gangopadhyay, P. K., Choudhury, S. R. and Das, S. (2010). "Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction." J Neurochem **112**(2): 486-496.
Del Favero, A., Tonato, M. and Roila, F. (1992). "Issues in the measurement of nausea." Br J Cancer Suppl **19**: S69-71.
Dickenson, A. H. (2002). "Gate control theory of pain stands the test of time." Br J Anaesth **88**(6): 755-757.
Dietis, N., Rowbotham, D. J. and Lambert, D. G. (2011). "Opioid receptor subtypes: fact or artifact?" Br J Anaesth **107**(1): 8-18.
Dole, V. P., Nyswander, M. E. and Kreek, M. J. (1966). "Narcotic blockade." Arch Intern Med **118**(4): 304-309.
Dole, V. P., Nyswander, M. E. and Kreek, M. J. (1966). "Narcotic blockade--a medical technique for stopping heroin use by addicts." Trans Assoc Am Physicians **79**: 122-136.
Dores, R. M. and Baron, A. J. (2011). "Evolution of POMC: origin, phylogeny, posttranslational processing, and the melanocortins." Ann N Y Acad Sci **1220**: 34-48.
Doverty, M., Somogyi, A. A., White, J. M., Bochner, F., Beare, C. H., Menelaou, A. and Ling, W. (2001b). "Methadone maintenance patients are cross-tolerant to the antinociceptive effects of morphine." Pain **93**(2): 155-163.
Doverty, M., White, J. M., Somogyi, A. A., Bochner, F., Ali, R. and Ling, W. (2001a). "Hyperalgesic responses in methadone maintenance patients." Pain **90**(1-2): 91-96.
Dunbar, S. A., Karamian, I. and Zhang, J. (2007). "Ketorolac prevents recurrent withdrawal induced hyperalgesia but does not inhibit tolerance to spinal morphine in the rat." Eur J Pain **11**(1): 1-6.
Durkin, B., Page, C. and Glass, P. (2010). "Pregabalin for the treatment of postsurgical pain." Expert Opin Pharmacother **11**(16): 2751-2758.
Duster, T. (1970). The Legislation of Morality: Law, Drugs and Moral Judgement. . New York, Free Press.
Duthie, D. J. (1998). "Remifentanil and tramadol." Br J Anaesth **81**(1): 51-57.
Dyer, K. R., Foster, D. J. R., White, J. M., Somogyi, A. A., Menelaou, A. and Bochner, F. (1999). "Steady-state pharmacokinetics and pharmacodynamics in methadone maintenance patients: comparison of those who do and do not experience withdrawal and concentration-effect relationships." Clinical Pharmacology and Therapeutics **65**(6): 685-694.
Dyer, K. R., White, J. M., Foster, D. J., Bochner, F., Menelaou, A. and Somogyi, A. A. (2001). "The relationship between mood state and plasma methadone concentration in maintenance patients." Journal of Clinical Psychopharmacology **21**(1): 78-84.

Eap, C. B., Cuendet, C. and Baumann, P. (1990). "Binding of d-methadone, l-methadone, and dl-methadone to proteins in plasma of healthy volunteers: role of the variants of alpha 1-acid glycoprotein." Clin Pharmacol Ther **47**(3): 338-346.
Eckhardt, K., Li, S., Ammon, S., Schanzle, G., Mikus, G. and Eichelbaum, M. (1998). "Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation." Pain **76**(1-2): 27-33.
Eilers, H., Philip, L. A., Bickler, P. E., McKay, W. R. and Schumacher, M. A. (2001). "The reversal of fentanyl-induced tolerance by administration of "small-dose" ketamine." Anesth Analg **93**(1): 213-214.
Eissenberg, T., Johnson, R. E., Bigelow, G. E., Walsh, S. L., Liebson, I. A., Strain, E. C. and Stitzer, M. L. (1997). "Controlled opioid withdrawal evaluation during 72 h dose omission in buprenorphine-maintained patients." Drug Alcohol Depend **45**(1-2): 81-91.
Elia, N., Lysakowski, C. and Tramer, M. R. (2005). "Does multimodal analgesia with acetaminophen, nonsteroidal antiinflammatory drugs, or selective cyclooxygenase-2 inhibitors and patient-controlled analgesia morphine offer advantages over morphine alone? Meta-analyses of randomized trials." Anesthesiology **103**(6): 1296-1304.
Elkader, A. and Sproule, B. (2005). "Buprenorphine: clinical pharmacokinetics in the treatment of opioid dependence." Clin Pharmacokinet **44**(7): 661-680.
Ellenhorn, M. and Barceloux, D. (1988). Medical Toxicology: Diagnosis and Treatment of Human Poisoning. New York, Elsevier.
Elvir-Lazo, O. L. and White, P. F. (2010). "Postoperative pain management after ambulatory surgery: role of multimodal analgesia." Anesthesiol Clin **28**(2): 217-224.
Enggaard, T. P., Poulsen, L., Arendt-Nielsen, L., Brosen, K., Ossig, J. and Sindrup, S. H. (2006). "The analgesic effect of tramadol after intravenous injection in healthy volunteers in relation to CYP2D6." Anesth Analg **102**(1): 146-150.
Escher, M., Daali, Y., Chabert, J., Hopfgartner, G., Dayer, P. and Desmeules, J. (2007). "Pharmacokinetic and pharmacodynamic properties of buprenorphine after a single intravenous administration in healthy volunteers: a randomized, double-blind, placebo-controlled, crossover study." Clin Ther **29**(8): 1620-1631.
Evers, A., Crowder, C. and Balser, J. (2006). General Anaesthetics. Goodman and Gilman's The Pharmacological Basis of Therapeutics. L. Brunton, J. Lazo and K. Parker. New York, McGraw-Hill.
Fanning, J. J., Stucke, A. G., Christensen, M. A., Cassidy, L. D. and Berens, R. J. (2012). "Perioperative opiate requirements in children with previous opiate infusion." Paediatr Anaesth **22**(3): 203-208.
Farahmand, S., Ahmadi, O., Dehpour, A. and Khashayar, P. (2012). "Does adding low doses of oral naltrexone to morphine alter the subsequent opioid requirements and side effects in trauma patients?" Am J Emerg Med **30**(1): 75-78.
Fareed, A., Vayalapalli, S., Casarella, J. and Drexler, K. (2012). "Effect of buprenorphine dose on treatment outcome." J Addict Dis **31**(1): 8-18.
Farrell, M. (2005). Pain Management. Sydney, Lippincott, Williams and Wilkins.
Fatseas, M. and Auriacombe, M. (2007). "Why buprenorphine is so successful in treating opiate addiction in France." Curr Psychiatry Rep **9**(5): 358-364.
Fay, P. (1975). The Opium War: 1840-1842. Chapel Hill, The University of North Carolina Press.
Fernandes, M., Kluwe, S. and Coper, H. (1977). "The development of tolerance to morphine in the rat." Psychopharmacology (Berl) **54**(2): 197-201.
Filitz, J., Ihmsen, H., Gunther, W., Troster, A., Schwilden, H., Schuttler, J. and Koppert, W. (2008). "Supra-additive effects of tramadol and acetaminophen in a human pain model." Pain **136**(3): 262-270.
Fischer, B. D., Carrigan, K. A. and Dykstra, L. A. (2005). "Effects of N-methyl-D-aspartate receptor antagonists on acute morphine-induced and l-methadone-induced antinociception in mice." J Pain **6**(7): 425-433.
Fishbain, D. A., Cole, B., Lewis, J. E., Gao, J. and Rosomoff, R. S. (2009). "Do opioids induce hyperalgesia in humans? An evidence-based structured review." Pain Med **10**(5): 829-839.
Foley, K. M. (1993). "Opioids." Neurol Clin **11**(3): 503-522.

Foley, K. M. (1995). "Misconceptions and controversies regarding the use of opioids in cancer pain." Anticancer Drugs **6 Suppl 3**: 4-13.
Foster, D. J. R., Somogyi, A. A., Dyer, K. R., White, J. M. and Bochner, F. (2000). "Steady-state pharmacokinetics of (R)- and (S)-methadone in methadone maintenance patients." British Journal of Clinical Pharmacology **50**: 427-440.
Fredheim, O. M., Moksnes, K., Borchgrevink, P. C., Kaasa, S. and Dale, O. (2008). "Clinical pharmacology of methadone for pain." Acta Anaesthesiol Scand **52**(7): 879-889.
Freud, S. (1961). Letters of Sigmund Freud, 1873-1939. London, Hogarth Press.
Freud, S. (1974). Cocaine papers. New York, Stonehill.
Frink, M. C., Hennies, H. H., Englberger, W., Haurand, M. and Wilffert, B. (1996). "Influence of tramadol on neurotransmitter systems of the rat brain." Arzneimittelforschung **46**(11): 1029-1036.
Fudala, P. J., Johnson, R. E. and Jaffe, J. H. (1990). "Outpatient comparison of buprenorphine and methadone maintenance. II. Effects on cocaine usage, retention time in study and missed clinic visits." NIDA Res Monogr **105**: 587-588.
Gandhi, K., Heitz, J. W. and Viscusi, E. R. (2011). "Challenges in acute pain management." Anesthesiol Clin **29**(2): 291-309.
Garzon, J., Martinez-Pena, Y. and Sanchez-Blazquez, P. (1997). "Gx/z is regulated by mu but not delta opioid receptors in the stimulation of the low Km GTPase activity in mouse periaqueductal grey matter." Eur J Neurosci **9**(6): 1194-1200.
Gaveriaux-Ruff, C., Nozaki, C., Nadal, X., Hever, X. C., Weibel, R., Matifas, A., Reiss, D., Filliol, D., Nassar, M. A., Wood, J. N., Maldonado, R. and Kieffer, B. L. (2011). "Genetic ablation of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia." Pain.
Ge, Z. J., Zhang, L. C., Zeng, Y. M., Dai, T. J., Chang, L., Wang, J. K., Cui, G. X., Tan, Y. F., Zhao, Y. P. and Liu, G. J. (2007). "Involvement of local orphanin FQ in the tolerance induced by repeated microinjections of morphine into ventrolateral periaqueductal gray in rats." Pharmacology **80**(4): 261-268.
Gianoulakis, C. (2004). "Endogenous opioids and addiction to alcohol and other drugs of abuse." Curr Top Med Chem **4**(1): 39-50.
Gibson, T. P. (1996). "Pharmacokinetics, efficacy, and safety of analgesia with a focus on tramadol HCl." Am J Med **101**(1A): 47S-53S.
Gieryk, A., Ziolkowska, B., Solecki, W., Kubik, J. and Przewlocki, R. (2010). "Forebrain PENK and PDYN gene expression levels in three inbred strains of mice and their relationship to genotype-dependent morphine reward sensitivity." Psychopharmacology (Berl) **208**(2): 291-300.
Gillis, J. C. and Brogden, R. N. (1997). "Ketorolac. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use in pain management." Drugs **53**(1): 139-188.
Gilson, A. M. and Joranson, D. E. (2008). "Is the DEA's new "prescription series" regulation balanced?" J Pain Palliat Care Pharmacother **22**(3): 218-220.
Gintzler, A. R. and Chakrabarti, S. (2006). "Post-opioid receptor adaptations to chronic morphine; altered functionality and associations of signaling molecules." Life Sci **79**(8): 717-722.
Goeringer, K. E., Logan, B. K. and Christian, G. D. (1997). "Identification of tramadol and its metabolites in blood from drug-related deaths and drug-impaired drivers." J Anal Toxicol **21**(7): 529-537.
Gong, Q. L., Hedner, J., Bjorkman, R. and Hedner, T. (1992). "Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat." Pain **48**(2): 249-255.
Gordon, A., Rashiq, S., Moulin, D., Clark, A., Beaulieu, A., Eisenhoffer, J., Piraino, P., Quigley, P., Harsanyi, Z. and Darke, A. (2010). "Buprenorphine transdermal system for opioid therapy in patients with chronic low back pain." Pain Res Manag **15**(3): 169-178.
Gordon, D., Inturrisi, C. E., Greensmith, J. E., Brennan, T. J., Goble, L. and Kerns, R. D. (2008). "Perioperative pain management in the opioid-tolerant individual." J Pain **9**(5): 383-387.
Gorman, A. L., Elliott, K. J. and Inturrisi, C. E. (1997). "The d- and l-isomers of methadone bind to the non-competitive site on the N-methyl-D-aspartate (NMDA) receptor in rat forebrain and spinal cord." Neurosci Lett **223**(1): 5-8.

Gottrup, H., Bach, F. W., Arendt-Nielsen, L. and Jensen, T. S. (2000). "Peripheral lidocaine but not ketamine inhibits capsaicin-induced hyperalgesia in humans." Br J Anaesth **85**(4): 520-528.
Gottrup, H., Bach, F. W. and Jensen, T. S. (2004). "Differential effects of peripheral ketamine and lidocaine on skin flux and hyperalgesia induced by intradermal capsaicin in humans." Clin Physiol Funct Imaging **24**(2): 103-108.
Gottrup, H., Bach, F. W., Juhl, G. and Jensen, T. S. (2006). "Differential effect of ketamine and lidocaine on spontaneous and mechanical evoked pain in patients with nerve injury pain." Anesthesiology **104**(3): 527-536.
Gourlay, G. K., Willis, R. J. and Lamberty, J. (1986). "A double-blind comparison of the efficacy of methadone and morphine in postoperative pain control." Anesthesiology **64**(3): 322-327.
Gregoretti, C., Moglia, B., Pelosi, P. and Navalesi, P. (2009). "Clonidine in perioperative medicine and intensive care unit: more than an anti-hypertensive drug." Curr Drug Targets **10**(8): 799-814.
Grond, S. and Sablotzki, A. (2004). "Clinical pharmacology of tramadol." Clin Pharmacokinet **43**(13): 879-923.
Guignard, B., Bossard, A. E., Coste, C., Sessler, D. I., Lebrault, C., Alfonsi, P., Fletcher, D. and Chauvin, M. (2000). "Acute opioid tolerance: intraoperative remifentanil increases postoperative pain and morphine requirement." Anesthesiology **93**(2): 409-417.
Gutstein, H. and Akil, H. (2006). Opioid Analgesics and Antagonists. New York, Mc Graw-Hill.
Gwirtz, K. H., Kim, H. C., Nagy, D. J., Young, J. V., Byers, R. S., Kovach, D. A. and Li, W. (1995). "Intravenous ketorolac and subarachnoid opioid analgesia in the management of acute postoperative pain." Reg Anesth **20**(5): 395-401.
Haller, G., Waeber, J. L., Infante, N. K. and Clergue, F. (2002). "Ketamine combined with morphine for the management of pain in an opioid addict." Anesthesiology **96**(5): 1265-1266.
Halliday, A. J., Bartlett, S. E., Colditz, P. and Smith, M. T. (1999). "Brain region-specific studies of the excitatory behavioral effects of morphine-3-glucuronide." Life Sci **65**(2): 225-236.
Hamann, S. and Sloan, P. (2007). "Oral naltrexone to enhance analgesia in patients receiving continuous intrathecal morphine for chronic pain: a randomized, double-blind, prospective pilot study." J Opioid Manag **3**(3): 137-144.
Hara, K., Minami, K. and Sata, T. (2005). "The effects of tramadol and its metabolite on glycine, gamma-aminobutyric acidA, and N-methyl-D-aspartate receptors expressed in Xenopus oocytes." Anesth Analg **100**(5): 1400-1405, table of contents.
Harris, D. S., Jones, R. T., Welm, S., Upton, R. A., Lin, E. and Mendelson, J. (2000). "Buprenorphine and naloxone co-administration in opiate-dependent patients stabilized on sublingual buprenorphine." Drug Alcohol Depend **61**(1): 85-94.
Hay, J. L., La Vincente, S. F., Somogyi, A. A., Chapleo, C. B. and White, J. M. (2011). "Potentiation of buprenorphine antinociception with ultra-low dose naltrexone in healthy subjects." Eur J Pain **15**(3): 293-298.
Hay, J. L., White, J. M., Bochner, F., Somogyi, A. A., Semple, T. J. and Rounsefell, B. (2008). "Hyperalgesia in Opioid-Managed Chronic Pain and Opioid-Dependent Patients." J Pain.
Hay, J. L., White, J. M., Bochner, F., Somogyi, A. A., Semple, T. J. and Rounsefell, B. (2009). "Hyperalgesia in Opioid-Managed Chronic Pain and Opioid-Dependent Patients." J Pain **3**: 316-322.
Heel, R. C., Brogden, R. N., Speight, T. M. and Avery, G. S. (1979). "Buprenorphine: a review of its pharmacological properties and therapeutic efficacy." Drugs **17**(2): 81-110.
Hempenstall, K., Nurmikko, T. J., Johnson, R. W., A'Hern, R. P. and Rice, A. S. (2005). "Analgesic therapy in postherpetic neuralgia: a quantitative systematic review." PLoS Med **2**(7): e164.
Hennies, H. H., Friderichs, E. and Schneider, J. (1988). "Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids." Arzneimittelforschung **38**(7): 877-880.
Hernandez-Avila, C. A., Oncken, C., Van Kirk, J., Wand, G. and Kranzler, H. R. (2002). "Adrenocorticotropin and cortisol responses to a naloxone challenge and risk of alcoholism." Biol Psychiatry **51**(8): 652-658.
Hernandez-Avila, C. A., Wand, G., Luo, X., Gelernter, J. and Kranzler, H. R. (2003). "Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1)." Am J Med Genet B Neuropsychiatr Genet **118B**(1): 60-65.

Hernandez-Delgadillo, G. P., Ventura Martinez, R., Diaz Reval, M. I., Dominguez Ramirez, A. M. and Lopez-Munoz, F. J. (2002). "Metamizol potentiates morphine antinociception but not constipation after chronic treatment." Eur J Pharmacol **441**(3): 177-183.
Hijazi, Y. and Boulieu, R. (2002). "Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes." Drug Metab Dispos **30**(7): 853-858.
Ho, A. and Dole, V. P. (1979). "Pain perception in drug-free and in methadone-maintained human ex-addicts." Proc Soc Exp Biol Med **162**(3): 392-395.
Hoflich, A. S., Langer, M., Jagsch, R., Bawert, A., Winklbaur, B., Fischer, G. and Unger, A. (2011). "Peripartum pain management in opioid dependent women." Eur J Pain.
Hollt, V. (1986). "Opioid peptide processing and receptor selectivity." Annu Rev Pharmacol Toxicol **26**: 59-77.
Hummel, T., Cramer, O., Mohammadian, P., Geisslinger, G., Pauli, E. and Kobal, G. (1997). "Comparison of the antinociception produced by two oral formulations of ibuprofen: ibuprofen effervescent vs ibuprofen tablets." Eur J Clin Pharmacol **52**(2): 107-114.
Hutchinson, M. R. (2004). "Thesis. Opioids and immune function: the role of non-classical opioid receptors and the association with pain perception."
Huxtable, C. A., Roberts, L. J., Somogyi, A. A. and MacIntyre, P. E. (2011). "Acute pain management in opioid-tolerant patients: a growing challenge." Anaesth Intensive Care **39**(5): 804-823.
Ing Lorenzini, K., Besson, M., Daali, Y., Salomon, D., Dayer, P. and Desmeules, J. (2011). "A randomized, controlled trial validates a peripheral supra-additive antihyperalgesic effect of a paracetamol-ketorolac combination." Basic Clin Pharmacol Toxicol **109**(5): 357-364.
International Association for the Study of Pain. (2011). "IASP Taxonomy." from http://www.iasp-pain.org/AM/Template.cfm?Section=Pain_Defi...isplay.cfm&ContentID=1728.
Inturrisi, C. E. (2005). "Pharmacology of methadone and its isomers." Minerva Anestesiol **71**(7-8): 435-437.
Inturrisi, C. E., Colburn, W. A., Kaiko, R. F., Houde, R. W. and Foley, K. M. (1987). "Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain." Clin Pharmacol Ther **41**(4): 392-401.
Inverarity, J., Lauderdale, P. and Field, B. (1983). Law and Society: Sociological Perspective on Criminal Law. Boston, Little, Brown.
Iribarne, C., Dreano, Y., Bardou, L. G., Menez, J. F. and Berthou, F. (1997). "Interaction of methadone with substrates of human hepatic cytochrome P450 3A4." Toxicology **117**(1): 13-23.
Iribarne, C., Picart, D., Dreano, Y., Bail, J. P. and Berthou, F. (1997). "Involvement of cytochrome P450 3A4 in N-dealkylation of buprenorphine in human liver microsomes." Life Sci **60**(22): 1953-1964.
Jaffe, J. (1995). Opioid-related disorders. Comprehensive Textbook of Psychiatry. Sixth Edition. H. Kaplan and B. Sadock. Baltimore, MD, Williams and Wilkins**:** 842-863.
Jaffe, J. and Martin, W. (1990). Opioid Analgesics and Antagonists. New York, Pergamon Press.
Jamison, R. N., Kauffman, J. and Katz, N. P. (2000). "Characteristics of methadone maintenance patients with chronic pain." J Pain Symptom Manage **19**(1): 53-62.
Jennings, W. (1901). "On the physiological cure of the morphia habit." Lancet: 363.
Jensen, M. L., Foster, D. J., Upton, R. N., Kristensen, K., Hansen, S. H., Jensen, N. H., Nielsen, B. N., Skram, U., Villesen, H. H. and Christrup, L. (2007). "Population pharmacokinetics of buprenorphine following a two-stage intravenous infusion in healthy volunteers." Eur J Clin Pharmacol **63**(12): 1153-1159.
Johnson, E. E., Christie, M. J. and Connor, M. (2005). "The role of opioid receptor phosphorylation and trafficking in adaptations to persistent opioid treatment." Neurosignals **14**(6): 290-302.
Johnson, R. E., Fudala, P. J. and Payne, R. (2005). "Buprenorphine: considerations for pain management." J Pain Symptom Manage **29**(3): 297-326.
Johnson, R. E., Jaffe, J. H. and Fudala, P. J. (1992). "A controlled trial of buprenorphine treatment for opioid dependence." Jama **267**(20): 2750-2755.
Johnstone, E. C., Yudkin, P. L., Hey, K., Roberts, S. J., Welch, S. J., Murphy, M. F., Griffiths, S. E. and Walton, R. T. (2004). "Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch." Pharmacogenetics **14**(2): 83-90.

Jones, H. E. (2004). "Practical considerations for the clinical use of buprenorphine." Sci Pract Perspect **2**(2): 4-20.
Jones, J. (1700). The Mysteries of Opium Reveal'd. London, Printed for Richard Smith.
Kalso, E. (2005). "Improving opioid effectiveness: from ideas to evidence." Eur J Pain **9**(2): 131-135.
Kantor, T. G., Cantor, R. and Tom, E. (1980). "A study of hospitalized surgical patients on methadone maintenance." Drug Alcohol Depend **6**(3): 163-173.
Katzung, B. (2007). Basic and clinical pharmacology, McGraw Hill Medical.
Khan, M. I., Walsh, D. and Brito-Dellan, N. (2011). "Opioid and adjuvant analgesics: compared and contrasted." Am J Hosp Palliat Care **28**(5): 378-383.
Kissin, I., Bright, C. A. and Bradley, E. L., Jr. (2000). "The effect of ketamine on opioid-induced acute tolerance: can it explain reduction of opioid consumption with ketamine-opioid analgesic combinations?" Anesth Analg **91**(6): 1483-1488.
Knaggs, R. D., Crighton, I. M., Cobby, T. F., Fletcher, A. J. and Hobbs, G. J. (2004). "The pupillary effects of intravenous morphine, codeine, and tramadol in volunteers." Anesth Analg **99**(1): 108-112.
Knapp, C., Ciraulo, D. and Jaffe, J. (2003). Opiates: Clinical Aspects. Substance Abuse. A Comprehensive Textbook. Fourth Edition. J. Lowinson, P. Ruiz, R. Millman and J. Langrod. Philadelphia, Lippincott, Williams and Wilkins.
Kobayashi, K., Yamamoto, T., Chiba, K., Tani, M., Shimada, N., Ishizaki, T. and Kuroiwa, Y. (1998). "Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4." Drug Metab Dispos **26**(8): 818-821.
Kocabas, S., Karaman, S., Uysallar, E. and Firat, V. (2005). "The use of tramadol and morphine for pain relief after abdominal hysterectomy." Clin Exp Obstet Gynecol **32**(1): 45-48.
Koltzenburg, M., Pokorny, R., Gasser, U. E. and Richarz, U. (2006). "Differential sensitivity of three experimental pain models in detecting the analgesic effects of transdermal fentanyl and buprenorphine." Pain **126**(1-3): 165-174.
Koob, G. and Le Moal, M. (2006). Opioids.
Koppert, W. (2006). "Response to Dr. Russo's letter: Anti-hyperalgesia properties of buprenorphine." Pain **122**(216).
Koppert, W., Dern, S. K., Sittl, R., Albrecht, S., Schuttler, J. and Schmelz, M. (2001). "A new model of electrically evoked pain and hyperalgesia in human skin: the effects of intravenous alfentanil, S(+)-ketamine, and lidocaine." Anesthesiology **95**(2): 395-402.
Koppert, W., Ihmsen, H., Korber, N., Wehrfritz, A., Sittl, R., Schmelz, M. and Schuttler, J. (2005). "Different profiles of buprenorphine-induced analgesia and antihyperalgesia in a human pain model." Pain **118**(1-2): 15-22.
Koppert, W., Sittl, R., Scheuber, K., Alsheimer, M., Schmelz, M. and Schuttler, J. (2003). "Differential modulation of remifentanil-induced analgesia and postinfusion hyperalgesia by S-ketamine and clonidine in humans." Anesthesiology **99**(1): 152-159.
Kramer, J. C. (1979). "Opium rampant: medical use, misuse and abuse in Britain and the West in the 17th and 18th centuries." Br J Addict Alcohol Other Drugs **74**(4): 377-389.
Kreek, M. J., Borg, L., Ducat, E. and Ray, B. (2010). "Pharmacotherapy in the treatment of addiction: methadone." J Addict Dis **29**(2): 200-216.
Kreek, M. J. and Reisinger, M. (1997). The addict as patient. Substance Abuse: A Comprehensive Textbook. Lowinson J.H., Ruiz P., Millan R.B. and L. J.D. Baltimore, USA, Williams and Wilkins. **3:** 563-589.
Kress, H. G. (2008). "Clinical update on the pharmacology, efficacy and safety of transdermal buprenorphine." Eur J Pain.
Kuhlman, J. J., Jr., Lalani, S., Magluilo, J., Jr., Levine, B. and Darwin, W. D. (1996). "Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine." J Anal Toxicol **20**(6): 369-378.
La Vincente, S. F., White, J. M., Somogyi, A. A., Bochner, F. and Chapleo, C. B. (2008). "Enhanced buprenorphine analgesia with the addition of ultra-low-dose naloxone in healthy subjects." Clin Pharmacol Ther **83**(1): 144-152.

Lamberts, J. T., Jutkiewicz, E. M., Mortensen, R. M. and Traynor, J. R. (2011). "Mu-opioid receptor coupling to Galpha(o) plays an important role in opioid antinociception." Neuropsychopharmacology **36**(10): 2041-2053.
Langenfeld, S., Birkenfeld, L., Herkenrath, P., Muller, C., Hellmich, M. and Theisohn, M. (2005). "Therapy of the neonatal abstinence syndrome with tincture of opium or morphine drops." Drug Alcohol Depend **77**(1): 31-36.
Larson, M. D. (2008). "Mechanism of opioid-induced pupillary effects." Clin Neurophysiol **119**(6): 1358-1364.
Laskowski, K., Stirling, A., McKay, W. P. and Lim, H. J. (2011). "A systematic review of intravenous ketamine for postoperative analgesia." Can J Anaesth **58**(10): 911-923.
Latimer, D. and Goldberg, J. (1981). Flowers in the Blood. New York, Franklin Watts.
Laugwitz, K. L., Offermanns, S., Spicher, K. and Schultz, G. (1993). "mu and delta opioid receptors differentially couple to G protein subtypes in membranes of human neuroblastoma SH-SY5Y cells." Neuron **10**(2): 233-242.
Laulin, J. P., Maurette, P., Corcuff, J. B., Rivat, C., Chauvin, M. and Simonnet, G. (2002). "The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance." Anesth Analg **94**(5): 1263-1269, table of contents.
Lee, M., Silverman, S. M., Hansen, H., Patel, V. B. and Manchikanti, L. (2011). "A comprehensive review of opioid-induced hyperalgesia." Pain Physician **14**(2): 145-161.
Lehmann, K. A., Kratzenberg, U., Schroeder-Bark, B. and Horrichs-Haermeyer, G. (1990). "Postoperative patient-controlled analgesia with tramadol: analgesic efficacy and minimum effective concentrations (abstract)." Clin J Pain **6**(3): 212-220.
Leung, A., Wallace, M. S., Ridgeway, B. and Yaksh, T. (2001). "Concentration-effect relationship of intravenous alfentanil and ketamine on peripheral neurosensory thresholds, allodynia and hyperalgesia of neuropathic pain." Pain **91**(1-2): 177-187.
Levine, J. D., Gordon, N. C. and Fields, H. L. (1978). "The mechanism of placebo analgesia." Lancet **2**(8091): 654-657.
Lewis, J., Mansour, A., Khachaturian, H., Watson, S. and Akil, H. (1987). Opioids and pain regulation. Neurotransmitters and pain control, pain and headache. H. Akil and J. W. Lewis. Basel, S. Karger. **9:** 129-159.
Lewis, J. W. (1985). "Buprenorphine." Drug Alcohol Depend **14**(3-4): 363-372.
Liebman, P. M., Lehofer, M., Schonauer-Cejpek, M., Legl, T., Pernhaupt, G., Moser, M. and Schauenstein, K. (1994). "Pain sensitivity in former opioid addicts." Lancet **344**(8928): 1031-1032.
Liebmann, P. M., Lehofer, M., Moser, M., Hoehn-Saric, R., Legl, T., Pernhaupt, G. and Schauenstein, K. (1997). "Persistent analgesia in former opiate addicts is resistant to blockade of endogenous opioids." Biol Psychiatry **42**(10): 962-964.
Liebmann, P. M., Lehofer, M., Schonauer-Cejpek, M., Legl, T., Pernhaupt, G., Moser, M. and Schauenstein, K. (1994). "Pain sensitivity in former opioid addicts." Lancet **344**(8928): 1031-1032.
Limongelli, V., Bonomi, M., Marinelli, L., Gervasio, F. L., Cavalli, A., Novellino, E. and Parrinello, M. (2010). "Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition." Proc Natl Acad Sci U S A **107**(12): 5411-5416.
Ling, W., Charuvastra, C., Collins, J. F., Batki, S., Brown, L. S., Jr., Kintaudi, P., Wesson, D. R., McNicholas, L., Tusel, D. J., Malkerneker, U., Renner, J. A., Jr., Santos, E., Casadonte, P., Fye, C., Stine, S., Wang, R. I. and Segal, D. (1998). "Buprenorphine maintenance treatment of opiate dependence: a multicenter, randomized clinical trial." Addiction **93**(4): 475-486.
Ling, W., Wesson, D. R., Charuvastra, C. and Klett, C. J. (1996). "A controlled trial comparing buprenorphine and methadone maintenance in opioid dependence." Arch Gen Psychiatry **53**(5): 401-407.
Lintz, W., Barth, H., Becker, R., Frankus, E. and Schmidt-Bothelt, E. (1998). "Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 2nd communication: drops with ethanol." Arzneimittelforschung **48**(5): 436-445.
Lintz, W., Barth, H., Osterloh, G. and Schmidt-Bothelt, E. (1998). "Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 3rd Communication: suppositories." Arzneimittelforschung **48**(9): 889-899.

Lintz, W., Becker, R., Gerloff, J. and Terlinden, R. (2000). "Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 4th communication: drops (without ethanol)." Arzneimittelforschung **50**(2): 99-108.
Lintz, W., Beier, H. and Gerloff, J. (1999). "Bioavailability of tramadol after i.m. injection in comparison to i.v. infusion." Int J Clin Pharmacol Ther **37**(4): 175-183.
Loeser, J. D. and Melzack, R. (1999). "Pain: an overview." Lancet **353**(9164): 1607-1609.
Loftus, R. W., Yeager, M. P., Clark, J. A., Brown, J. R., Abdu, W. A., Sengupta, D. K. and Beach, M. L. (2010). "Intraoperative ketamine reduces perioperative opiate consumption in opiate-dependent patients with chronic back pain undergoing back surgery." Anesthesiology **113**(3): 639-646.
Lowinson, J., Marion, I., Joseph, H., Langrod, J., Salsitz, E., Thomas Payte, J. and Dole, V. (2003). Methadone Maintenance. Substance Abuse. A Comprehensive Textbook. Fourth Edition. J. Lowinson, P. Ruiz, R. Millman and J. Langrod. Philadelphia, Lippincott, Williams and Wilkins**:** 618.
Luginbuhl, M., Gerber, A., Schnider, T. W., Petersen-Felix, S., Arendt-Nielsen, L. and Curatolo, M. (2003). "Modulation of remifentanil-induced analgesia, hyperalgesia, and tolerance by small-dose ketamine in humans." Anesth Analg **96**(3): 726-732, table of contents.
Lugo, R. A., Satterfield, K. L. and Kern, S. E. (2005). "Pharmacokinetics of methadone." J Pain Palliat Care Pharmacother **19**(4): 13-24.
Macht, D. (1915). "The history of opium and some of its preparations and alkaloids." Journal of the American Medical Association **64**: 477-481.
Macintyre, P. (2005). Acute pain management in "non-standard" adult patient groups (patients who are elderly, have obstructive sleep apnea, or are opioid-tolerant). Pain 2005-An updated review: Refresher course syllabus. D. M. Justins. Seattle, IASP press**:** 272-274.
Macintyre, P., Schug, S. and Scott, D. (2010). "Acute Pain Management. Scientific Evidence. Third Edition." Retrieved February 6, 2012.
Macintyre, P., Schug, S., Scott, D., EJ, V. and SM, W. (2010). "Acute Pain Management. Scientific Evidence. 3rd Edition." Retrieved Accessed 2012 February 6th, from www.anzca.edu.au/resources/books-and-publications.
Manchikanti, L., Ailinani, H., Koyyalagunta, D., Datta, S., Singh, V., Eriator, I., Sehgal, N., Shah, R., Benyamin, R., Vallejo, R., Fellows, B. and Christo, P. J. (2011). "A systematic review of randomized trials of long-term opioid management for chronic non-cancer pain." Pain Physician **14**(2): 91-121.
Mandema, J. and Stanski, D. (1996). "Population pharmacodynamic model for ketorolac analgesia." Clin Pharm Ther **60**: 619-635.
Manfredi, P. L., Gonzales, G. R., Cheville, A. L., Kornick, C. and Payne, R. (2001). "Methadone analgesia in cancer pain patients on chronic methadone maintenance therapy." J Pain Symptom Manage **21**(2): 169-174.
Mao, J. (1999). "NMDA and opioid receptors: their interactions in antinociception, tolerance and neuroplasticity." Brain Research Reviews **30**(3): 289-304.
Mao, J. (2002). "Opioid-induced abnormal pain sensitivity: implications in clinical opioid therapy." Pain **100**(3): 213-217.
Mao, J., Price, D. D. and Mayer, D. J. (1995). "Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain." Pain **61**(3): 353-364.
Mao, J., Price, D. D. and Mayer, D. J. (1995). "Mechanisms of hyperalgesia and opiate tolerance: a current view of their possible interactions." Pain **62**: 259-274.
Marinangeli, F., Ciccozzi, A., Aloisio, L., Colangeli, A., Paladini, A., Bajocco, C., Coaccioli, S. and Varrassi, G. (2007). "Improved cancer pain treatment using combined fentanyl-TTS and tramadol." Pain Pract **7**(4): 307-312.
Martin, J. E. and Inglis, J. (1965). "Pain tolerance and narcotic addiction." The British Journal of Social and Clinical Psychology. **4**(3): 224-229.
Martin, W. R. (1967). "Opioid antagonists." Pharmacol Rev **19**(4): 463-521.
Martin, W. R. (1983). "Pharmacology of opioids." Pharmacol Rev **35**(4): 283-323.

Martin, W. R., Jasinski, D. R., Haertzen, C. A., Kay, D. C., Jones, B. E., Mansky, P. A. and Carpenter, R. W. (1973). "Methadone--a reevaluation." Arch Gen Psychiatry **28**(2): 286-295.
Mattioli, T. A., Milne, B. and Cahill, C. M. (2010). "Ultra-low dose naltrexone attenuates chronic morphine-induced gliosis in rats." Mol Pain **6**: 22.
Maund, E., McDaid, C., Rice, S., Wright, K., Jenkins, B. and Woolacott, N. (2011). "Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: a systematic review." Br J Anaesth **106**(3): 292-297.
May, A. (2007). "Neuroimaging: visualising the brain in pain." Neurol Sci **28 Suppl 2**: S101-107.
Mayer, D. J., Mao, J., Holt, J. and Price, D. D. (1999). "Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions." Proceedings of the National Academy of Sciences USA **96**(14): 7731-7736.
Mayet, S., Gossop, M., Lintzeris, N., Markides, V. and Strang, J. (2011). "Methadone maintenance, QTc and torsade de pointes: who needs an electrocardiogram and what is the prevalence of QTc prolongation?" Drug Alcohol Rev **30**(4): 388-396.
McAleer, S. D., Mills, R. J., Polack, T., Hussain, T., Rolan, P. E., Gibbs, A. D., Mullins, F. G. and Hussein, Z. (2003). "Pharmacokinetics of high-dose buprenorphine following single administration of sublingual tablet formulations in opioid naive healthy male volunteers under a naltrexone block." Drug Alcohol Depend **72**(1): 75-83.
McCarthur, J., Kennedy, T., Semple, T., Brougham, L., Compton, P., de Crespigny, C. and Athanasos, P. (2007). Postoperative opioid loading requirements following major surgery for opioid tolerant and other drug dependent patients. Australian Professional Society for Alcohol and Other Drugs/Cutting Edge, Auckland, New Zealand.
McCarthur, J., Kennedy, T., Semple, T., Brougham, L., Compton, P., de Crespigny, C. and Athanasos, P. (2008). Postoperative recovery of opioid tolerant patients. Australian Professional Society for Alcohol and Other Drugs Sydney.
McNaull, B., Trang, T., Sutak, M. and Jhamandas, K. (2007). "Inhibition of tolerance to spinal morphine antinociception by low doses of opioid receptor antagonists." Eur J Pharmacol **560**(2-3): 132-141.
Megarbane, B., Decleves, X., Bloch, V., Bardin, C., Chast, F. and Baud, F. J. (2007). "Case report: quantification of methadone-induced respiratory depression using toxicokinetic/toxicodynamic relationships." Crit Care **11**(1): R5.
Megarbane, B., Hreiche, R., Pirnay, S., Marie, N. and Baud, F. J. (2006). "Does high-dose buprenorphine cause respiratory depression?: possible mechanisms and therapeutic consequences." Toxicol Rev **25**(2): 79-85.
Melzack, R. (2005). "Evolution of the neuromatrix theory of pain. The Prithvi Raj Lecture: presented at the third World Congress of World Institute of Pain, Barcelona 2004." Pain Pract **5**(2): 85-94.
Mendelson, J., Upton, R. A., Everhart, E. T., Jacob, P., 3rd and Jones, R. T. (1997). "Bioavailability of sublingual buprenorphine (abstract)." J Clin Pharmacol **37**(1): 31-37.
Menelaou, A., Doverty, M. and Somogyi, A. (2001). Development of a sensitive assay for the quantification of (S)-ketamine in plasma using HPLC with UV detection. Australian Society of Clinical and Experimental Pharmacologists and Toxicologists, Dunedin, New Zealand.
Menelaou, A., Hay, J. and Somogyi, A. (2002). Development of an assay for the quantification of tramadol in plasma using HPLC with fluorescence detection. Australian Society of Clinical and Experimental Pharmacologists and Toxicologists, Sydney, National Health and Medical Research Council.
Meresaar, U., Nilsson, M. I., Holmstrand, J. and Anggard, E. (1981). "Single dose pharmacokinetics and bioavailability of methadone in man studied with a stable isotope method." Eur J Clin Pharmacol **20**(6): 473-478.
Meyer, R., Schaper, S., Kieser, S., Magerl, W., Roehrig, J. and Duenges, B. (2005). Methadone and levomethadone similarly reduce pain and hyperalgesia in a human pain model. IASP abstract book of the 2005 World Congress of Pain.

Mistry, M. and Houston, J. B. (1987). "Glucuronidation in vitro and in vivo. Comparison of intestinal and hepatic conjugation of morphine, naloxone, and buprenorphine." Drug Metab Dispos **15**(5): 710-717.
Mitchell, J. and Condon, J. (2005). Pain Management. Lewis's Medical Surgical Nursing, Assessment and Management of Clinical Problems. D. Bown and H. Edwards. Sydney, Elsevier**:** 132-140.
Mitchell, T. B., White, J. M., Somogyi, A. A. and Bochner, F. (2006). "Switching between methadone and morphine for maintenance treatment of opioid dependence: impact on pain sensitivity and mood status." Am J Addict **15**(4): 311-315.
Mitra, S. (2008). "Opioid-induced hyperalgesia: pathophysiology and clinical implications." J Opioid Manag **4**(3): 123-130.
Mitra, S. and Sinatra, R. S. (2004). "Perioperative management of acute pain in the opioid-dependent patient." Anesthesiology **101**(1): 212-227.
Modesto-Lowe, V., Brooks, D. and Petry, N. (2010). "Methadone deaths: risk factors in pain and addicted populations." J Gen Intern Med **25**(4): 305-309.
Montoya, I. D., Gorelick, D. A., Preston, K. L., Schroeder, J. R., Umbricht, A., Cheskin, L. J., Lange, W. R., Contoreggi, C., Johnson, R. E. and Fudala, P. J. (2004). "Randomized trial of buprenorphine for treatment of concurrent opiate and cocaine dependence." Clin Pharmacol Ther **75**(1): 34-48.
Moody, D. E., Fang, W. B., Morrison, J. and McCance-Katz, E. (2011). "Gender differences in pharmacokinetics of maintenance dosed buprenorphine." Drug Alcohol Depend **118**(2-3): 479-483.
Moore, R. A., Straube, S., Wiffen, P. J., Derry, S. and McQuay, H. J. (2009). "Pregabalin for acute and chronic pain in adults." Cochrane Database Syst Rev(3): CD007076.
Moss, J. and Rosow, C. E. (2008). "Development of peripheral opioid antagonists' new insights into opioid effects." Mayo Clin Proc **83**(10): 1116-1130.
Musacchio, J. M. (1990). "The psychotomimetic effects of opiates and the sigma receptor." Neuropsychopharmacology **3**(3): 191-200.
Nath, R. P., Upton, R. A., Everhart, E. T., Cheung, P., Shwonek, P., Jones, R. T. and Mendelson, J. E. (1999). "Buprenorphine pharmacokinetics: relative bioavailability of sublingual tablet and liquid formulations." J Clin Pharmacol **39**(6): 619-623.
Nesher, N., Ekstein, M. P., Paz, Y., Marouani, N., Chazan, S. and Weinbroum, A. A. (2009). "Morphine with adjuvant ketamine vs higher dose of morphine alone for immediate postthoracotomy analgesia." Chest **136**(1): 245-252.
Nesher, N., Serovian, I., Marouani, N., Chazan, S. and Weinbroum, A. A. (2008). "Ketamine spares morphine consumption after transthoracic lung and heart surgery without adverse hemodynamic effects." Pharmacol Res **58**(1): 38-44.
Nestler, E. J. (1997). "Molecular mechanisms of opiate and cocaine addiction." Curr Opin Neurobiol **7**(5): 713-719.
New York Academy of Science Committee on Public Health (1990). "Methadone Management of Heroin Addiction." Bulletin of the New York Academy of Medicine **46**(391).
Niesters, M., Dahan, A., Swartjes, M., Noppers, I., Fillingim, R. B., Aarts, L. and Sarton, E. Y. (2011). "Effect of ketamine on endogenous pain modulation in healthy volunteers." Pain **152**(3): 656-663.
Nikoshkov, A., Drakenberg, K., Wang, X., Horvath, M. C., Keller, E. and Hurd, Y. L. (2008). "Opioid neuropeptide genotypes in relation to heroin abuse: dopamine tone contributes to reversed mesolimbic proenkephalin expression." Proc Natl Acad Sci U S A **105**(2): 786-791.
Nilsson, M. I., Meresaar, U. and Anggard, E. (1982). "Clinical pharmacokinetics of methadone." Acta Anaesthesiol Scand Suppl **74**: 66-69.
Niscola, P., Scaramucci, L., Vischini, G., Giovannini, M., Ferrannini, M., Massa, P., Tatangelo, P., Galletti, M. and Palumbo, R. (2010). "The use of major analgesics in patients with renal dysfunction." Curr Drug Targets **11**(6): 752-758.
O'Brien, R. and Cohen, S. (1984). Encyclopaedia of Drug Abuse. New York, Facts on File.
Ohtani, M., Kotaki, H., Sawada, Y. and Iga, T. (1995). "Comparative analysis of buprenorphine- and norbuprenorphine-induced analgesic effects based on pharmacokinetic-pharmacodynamic modeling." J Pharmacol Exp Ther **272**(2): 505-510.

Ohtani, M., Kotaki, H., Uchino, K., Sawada, Y. and Iga, T. (1994). "Pharmacokinetic analysis of enterohepatic circulation of buprenorphine and its active metabolite, norbuprenorphine, in rats." Drug Metab Dispos **22**(1): 2-7.
Ossipov, M. H., Lai, J., King, T., Vanderah, T. W., Malan, T. P., Jr., Hruby, V. J. and Porreca, F. (2004). "Antinociceptive and nociceptive actions of opioids." J Neurobiol **61**(1): 126-148.
Ossipov, M. H., Lai, J., Vanderah, T. W. and Porreca, F. (2003). "Induction of pain facilitation by sustained opioid exposure: relationship to opioid antinociceptive tolerance." Life Sci **73**(6): 783-800.
Pain, I. A. f. T. S. o. (2007). "IASP Pain Terminology [online]." 2007.
Pan, Y. X., Xu, J., Mahurter, L., Xu, M., Gilbert, A. K. and Pasternak, G. W. (2003). "Identification and characterization of two new human mu opioid receptor splice variants, hMOR-1O and hMOR-1X." Biochem Biophys Res Commun **301**(4): 1057-1061.
Park, K. M., Max, M. B., Robinovitz, E., Gracely, R. H. and Bennett, G. J. (1995). "Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects." Pain **63**(2): 163-172.
Paronis, C. A. and Woods, J. H. (1997). "Ventilation in morphine-maintained rhesus monkeys. II: Tolerance to the antinociceptive but not the ventilatory effects of morphine." J Pharmacol Exp Ther **282**(1): 355-362.
Pasricha, P. (2006). Treatment of Disorders of Bowel Motility and Water Flux; antiemetics; Agents Used in Biliary and Pancreatic Disease. New York, Mc Graw-Hill.
Patanwala, A. E., Jarzyna, D. L., Miller, M. D. and Erstad, B. L. (2008). "Comparison of opioid requirements and analgesic response in opioid-tolerant versus opioid-naive patients after total knee arthroplasty." Pharmacotherapy **28**(12): 1453-1460.
Peles, E., Schreiber, S., Gordon, J. and Adelson, M. (2005). "Significantly higher methadone dose for methadone maintenance treatment (MMT) patients with chronic pain." Pain **113**(3): 340-346.
Penson, R. T., Joel, S. P., Bakhshi, K., Clark, S. J., Langford, R. M. and Slevin, M. L. (2000). "Randomized placebo-controlled trial of the activity of the morphine glucuronides." Clin Pharmacol Ther **68**(6): 667-676.
Pergolizzi, J., Aloisi, A. M., Dahan, A., Filitz, J., Langford, R., Likar, R., Mercadante, S., Morlion, B., Raffa, R. B., Sabatowski, R., Sacerdote, P., Torres, L. M. and Weinbroum, A. A. (2010). "Current knowledge of buprenorphine and its unique pharmacological profile." Pain Pract **10**(5): 428-450.
Pergolizzi, J. and Will, L. (2006). Multimodal analgesic therapy. Postoperative Pain Management: An Evidence-Based Guide to Practice. G. Shorten, D. B. Carr, D. Harmon, M. Puig and J. Browne. Philadelphia, Pennsylvania, Saunders Elsevier**:** 182-196.
Perneger, T. V. (1999). "Adjusting for multiple testing in studies is less important than other concerns." Bmj **318**(7193): 1288.
Petrenko, A. B., Yamakura, T., Baba, H. and Shimoji, K. (2003). "The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review." Anesth Analg **97**(4): 1108-1116.
Petrovic, P., Kalso, E., Petersson, K. M., Andersson, J., Fransson, P. and Ingvar, M. (2010). "A prefrontal non-opioid mechanism in placebo analgesia." Pain **150**(1): 59-65.
Petry, N. M., Bickel, W. K. and Badger, G. J. (1999). "A comparison of four buprenorphine dosing regimens in the treatment of opioid dependence." Clin Pharmacol Ther **66**(3): 306-314.
Petry, N. M., Bickel, W. K. and Badger, G. J. (2000). "A comparison of four buprenorphine dosing regimens using open-dosing procedures: is twice-weekly dosing possible?" Addiction **95**(7): 1069-1077.
Pickworth, W. B., Johnson, R. E., Holicky, B. A. and Cone, E. J. (1993). "Subjective and physiologic effects of intravenous buprenorphine in humans." Clin Pharmacol Ther **53**(5): 570-576.
Pilcher, W. H., Joseph, S. A. and McDonald, J. V. (1988). "Immunocytochemical localization of pro-opiomelanocortin neurons in human brain areas subserving stimulation analgesia." J Neurosurg **68**(4): 621-629.
Pinto, H., Maskrey, V., Swift, L., Rumball, D., Wagle, A. and Holland, R. (2010). "The SUMMIT trial: a field comparison of buprenorphine versus methadone maintenance treatment." J Subst Abuse Treat **39**(4): 340-352.

Portenoy, R. and Payne, R. (1997). Acute and chronic pain. Substance Abuse: A Comprehensive Textbook. Lowinson J.H., Ruiz P., Millan R.B. and L. J.D. Baltimore, USA, Williams and Wilkins. **3:** 563-589.
Price, T. J., Cervero, F., Gold, M. S., Hammond, D. L. and Prescott, S. A. (2009). "Chloride regulation in the pain pathway." Brain Res Rev **60**(1): 149-170.
Pud, D., Cohen, D., Lawental, E. and Eisenberg, E. (2006). "Opioids and abnormal pain perception: New evidence from a study of chronic opioid addicts and healthy subjects." Drug Alcohol Depend **82**(3): 218-223.
Quiding, H., Lundqvist, G., Boreus, L. O., Bondesson, U. and Ohrvik, J. (1993). "Analgesic effect and plasma concentrations of codeine and morphine after two dose levels of codeine following oral surgery." Eur J Clin Pharmacol **44**(4): 319-323.
Raffa, R., Nayak, R. and Liao, S. (1995). "The mechanism of action and pharmacokintetics of tramadol hydrochloride." Review of Contemporary Pharmacotherapies **6**: 485-497.
Raffa, R. B., Friderichs, E., Reimann, W., Shank, R. P., Codd, E. E. and Vaught, J. L. (1992). "Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic." J Pharmacol Exp Ther **260**(1): 275-285.
Raffa, R. B., Friderichs, E., Reimann, W., Shank, R. P., Codd, E. E., Vaught, J. L., Jacoby, H. I. and Selve, N. (1993). "Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol." J Pharmacol Exp Ther **267**(1): 331-340.
Raghavendra, V., Tanga, F., Rutkowski, M. D. and DeLeo, J. A. (2003). "Anti-hyperalgesic and morphine-sparing actions of propentofylline following peripheral nerve injury in rats: mechanistic implications of spinal glia and proinflammatory cytokines." Pain **104**(3): 655-664.
Rakhman, E., Shmain, D., White, I., Ekstein, M. P., Kollender, Y., Chazan, S., Dadia, S., Bickels, J., Amar, E. and Weinbroum, A. A. (2011). "Repeated and escalating preoperative subanesthetic doses of ketamine for postoperative pain control in patients undergoing tumor resection: a randomized, placebo-controlled, double-blind trial." Clin Ther **33**(7): 863-873.
Rapp, S. E., Ready, L. B. and Nessly, M. L. (1995). "Acute pain management in patients with prior opioid consumption: a case-controlled retrospective review." Pain **61**(2): 195-201.
Ready, L., Oden, R., Chadwick, H., Benedetti, C., Rooke, G. and Caplan, R. e. a. (1988). "Development of an anesthesiology-based postoperative pain management service." Anesthesiology **68**: 100-106.
Reed, L. J., Glasper, A., de Wet, C. J., Bearn, J. and Gossop, M. (2007). "Comparison of buprenorphine and methadone in the treatment of opiate withdrawal: possible advantages of buprenorphine for the treatment of opiate-benzodiazepine codependent patients?" J Clin Psychopharmacol **27**(2): 188-192.
Reisine, T. and Pasternak, G. (1996). Opioid Analgesics and Antagonists. New York, McGraw-Hill.
Remy, C., Marret, E. and Bonnet, F. (2005). "Effects of acetaminophen on morphine side-effects and consumption after major surgery: meta-analysis of randomized controlled trials." Br J Anaesth **94**(4): 505-513.
Rhodin, A., Stridsberg, M. and Gordh, T. (2010). "Opioid endocrinopathy: a clinical problem in patients with chronic pain and long-term oral opioid treatment." Clin J Pain **26**(5): 374-380.
Rich, B. A. (2000). "An ethical analysis of the barriers to effective pain management." Camb Q Healthc Ethics **9**(1): 54-70.
Richebe, P. and Beaulieu, P. (2009). "Perioperative pain management in the patient treated with opioids: continuing professional development." Can J Anaesth **56**(12): 969-981.
Richebe, P., Cahana, A. and Rivat, C. (2012). "Tolerance and opioid-induced hyperalgesia. Is a divorce imminent?" Pain **153**(8): 1547-1548.
Rivat, C., Laulin, J. P., Corcuff, J. B., Celerier, E., Pain, L. and Simonnet, G. (2002). "Fentanyl enhancement of carrageenan-induced long-lasting hyperalgesia in rats: prevention by the N-methyl-D-aspartate receptor antagonist ketamine." Anesthesiology **96**(2): 381-391.
Rogers, A. G. (1989). "Management of postoperative pain in patients on methadone maintenance." J Pain Symptom Manage **4**(3): 161-162.
Rohdewald, P., Granitzki, H. W. and Neddermann, E. (1988). "Comparison of the analgesic efficacy of metamizole and tramadol in experimental pain." Pharmacology **37**(4): 209-217.

Romberg, R., Olofsen, E., Sarton, E., den Hartigh, J., Taschner, P. E. and Dahan, A. (2004). "Pharmacokinetic-pharmacodynamic modeling of morphine-6-glucuronide-induced analgesia in healthy volunteers: absence of sex differences." Anesthesiology **100**(1): 120-133.
Romundstad, L., Stubhaug, A., Niemi, G., Rosseland, L. A. and Breivik, H. (2006). "Adding propacetamol to ketorolac increases the tolerance to painful pressure." Eur J Pain **10**(3): 177-183.
Rook, E. J., Huitema, A. D., van den Brink, W., van Ree, J. M. and Beijnen, J. H. (2006). "Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature." Curr Clin Pharmacol **1**(1): 109-118.
Rosen, M. I., Wallace, E. A., McMahon, T. J., Pearsall, H. R., Woods, S. W., Price, L. H. and Kosten, T. R. (1994). "Buprenorphine: duration of blockade of effects of intramuscular hydromorphone." Drug Alcohol Depend **35**(2): 141-149.
Rosenblum, A., Joseph, H., Fong, C., Kipnis, S., Cleland, C. and Portenoy, R. K. (2003). "Prevalence and characteristics of chronic pain among chemically dependent patients in methadone maintenance and residential treatment facilities." Jama **289**(18): 2370-2378.
Rosenblum, A., Parrino, M., Schnoll, S. H., Fong, C., Maxwell, C., Cleland, C. M., Magura, S. and Haddox, J. D. (2007). "Prescription opioid abuse among enrollees into methadone maintenance treatment." Drug Alcohol Depend **90**(1): 64-71.
Rosenthal, M. H. and Bayait, F. (1988). "Buprenorphine: a cost-effective alternative to Schedule II analgesics for moderate to severe pain relief." Hosp Formul **23**(1): 57-60, 62, 71.
Rossi, S. (2012). "Australian Medicines Handbook." from http://www.amh.hcn.net.au/.
Rubenstein, R. B., Spira, I. and Wolff, W. I. (1976). "Management of surgical problems in patients on methadone maintenance." Am J Surg **131**(5): 566-569.
Ruscheweyh, R., Stumpenhorst, F., Knecht, S. and Marziniak, M. (2010). "Comparison of the cold pressor test and contact thermode-delivered cold stimuli for the assessment of cold pain sensitivity." J Pain **11**(8): 728-736.
Russo, M. A. (2006). "Anti-hyperalgesia properties of buprenorphine." Pain **122**(1-2): 216; author reply 216.
San, L., Cami, J., Fernandez, T., Olle, J. M., Peri, J. M. and Torrens, M. (1992). "Assessment and management of opioid withdrawal symptoms in buprenorphine-dependent subjects." Br J Addict **87**(1): 55-62.
Sator-Katzenschlager, S., Deusch, E., Maier, P., Spacek, A. and Kress, H. G. (2001). "The long-term antinociceptive effect of intrathecal S(+)-ketamine in a patient with established morphine tolerance." Anesth Analg **93**(4): 1032-1034, table of contents.
Savage, S. R. (1998). Principles of pain treatment in the addicted patient. Principles of Addiction Medicine. A. Graham and T. Schultz. Chevy Chase, Maryland, USA, American Society of Addiction Medicine, Inc.
Schall, U., Katta, T., Pries, E., Kloppel, A. and Gastpar, M. (1996). "Pain perception of intravenous heroin users on maintenance therapy with levomethadone." Pharmacopsychiatry **29**(5): 176-179.
Schmid, R. L., Sandler, A. N. and Katz, J. (1999). "Use and efficacy of low-dose ketamine in the management of acute postoperative pain: a review of current techniques and outcomes." Pain **82**(2): 111-125.
Schulz, J. E. (1997). "The integration of medical management with recovery." J Psychoactive Drugs **29**(3): 233-237.
Schumacher, M., Basbaum, A. and Way, W. (2007). Opioid Analgesics and Antagonists. Basic and Clinical Pharmacology. B. Katzung. New York, McGraw-Hill.
Scimeca, M. M., Savage, S. R., Portenoy, R. and Lowinson, J. (2000). "Treatment of pain in methadone-maintained patients." Mt Sinai Journal of Medicine **67**(5-6): 412-422.
Scott, D., Ed. (2008). Molecular imaging studies of pain processing. Pain 2008. An updated review. Refresher course syllabus. Seattle, IASP Press.
Scott, L. J. and Perry, C. M. (2000). "Tramadol: a review of its use in perioperative pain." Drugs **60**(1): 139-176.
Shiran, M. R., Lennard, M. S., Iqbal, M. Z., Lagundoye, O., Seivewright, N., Tucker, G. T. and Rostami-Hodjegan, A. (2009). "Contribution of the activities of CYP3A, CYP2D6, CYP1A2 and other potential covariates to the disposition of methadone in patients undergoing methadone maintenance treatment." Br J Clin Pharmacol **67**(1): 29-37.

Sigtermans, M., Dahan, A., Mooren, R., Bauer, M., Kest, B., Sarton, E. and Olofsen, E. (2009). "S(+)-ketamine effect on experimental pain and cardiac output: a population pharmacokinetic-pharmacodynamic modeling study in healthy volunteers." Anesthesiology **111**(4): 892-903.
Sills, G. J. (2006). "The mechanisms of action of gabapentin and pregabalin." Curr Opin Pharmacol **6**(1): 108-113.
Sindrup, S. H. and Brosen, K. (1995). "The pharmacogenetics of codeine hypoalgesia." Pharmacogenetics **5**(6): 335-346.
Sinha, V. R., Kumar, R. V. and Singh, G. (2009). "Ketorolac tromethamine formulations: an overview." Expert Opin Drug Deliv **6**(9): 961-975.
Sinner, B. and Graf, B. M. (2008). "Ketamine." Handb Exp Pharmacol(182): 313-333.
Smith, M. T. (2000). "Neuroexcitatory effects of morphine and hydromorphone: evidence implicating the 3-glucuronide metabolites." Clin Exp Pharmacol Physiol **27**(7): 524-528.
Song, P. and Zhao, Z. Q. (2001). "The involvement of glial cells in the development of morphine tolerance." Neurosci Res **39**(3): 281-286.
Staahl, C., Olesen, A. E., Andresen, T., Arendt-Nielsen, L. and Drewes, A. M. (2009). "Assessing efficacy of non-opioid analgesics in experimental pain models in healthy volunteers: an updated review." Br J Clin Pharmacol **68**(3): 322-341.
Stine, S. and Kosten, T. (1999). Opioids. Addictions. A Comprehensive Guidebook. B. McCrady and E. Epstein. New York, Oxford University Press.
Strain, E. C., Preston, K. L., Liebson, I. A. and Bigelow, G. E. (1995). "Buprenorphine effects in methadone-maintained volunteers: effects at two hours after methadone." J Pharmacol Exp Ther **272**(2): 628-638.
Straube, S., Derry, S., Moore, R. A., Wiffen, P. J. and McQuay, H. J. (2010). "Single dose oral gabapentin for established acute postoperative pain in adults." Cochrane Database Syst Rev(5): CD008183.
Subrahmanyam, V., Renwick, A. B., Walters, D. G., Young, P. J., Price, R. J., Tonelli, A. P. and Lake, B. G. (2001). "Identification of cytochrome P-450 isoforms responsible for cis-tramadol metabolism in human liver microsomes." Drug Metab Dispos **29**(8): 1146-1155.
Subramaniam, K., Subramaniam, B. and Steinbrook, R. A. (2004). "Ketamine as adjuvant analgesic to opioids: a quantitative and qualitative systematic review." Anesth Analg **99**(2): 482-495, table of contents.
Swanson, R. and Klein, D. (2005). Comfort and Sedation. New York, Elsevier.
Terry, C. and Pellens, M. (1928). The Opium Problem. New York, The Committee on Drug Addictions in Collaboration with the Bureau of Social Hygeine
Tetrault, J. M. and Fiellin, D. A. (2012). "Current and potential pharmacological treatment options for maintenance therapy in opioid-dependent individuals." Drugs **72**(2): 217-228.
Thomas, D. A., Williams, G. M., Iwata, K., Kenshalo, D. R., Jr. and Dubner, R. (1992). "Effects of central administration of opioids on facial scratching in monkeys." Brain Res **585**(1-2): 315-317.
Tiippana, E. M., Hamunen, K., Kontinen, V. K. and Kalso, E. (2007). "Do surgical patients benefit from perioperative gabapentin/pregabalin? A systematic review of efficacy and safety." Anesth Analg **104**(6): 1545-1556, table of contents.
Trang, T., Sutak, M., Quirion, R. and Jhamandas, K. (2002). "The role of spinal neuropeptides and prostaglandins in opioid physical dependence." Br J Pharmacol **136**(1): 37-48.
Treister, R., Eisenberg, E., Lawental, E. and Pud, D. (2012). "Is opioid-induced hyperalgesia reversible? A study on active and former opioid addicts and drug naive controls." J Opioid Manag **8**(6): 343-349.
Tremblay, J. and Hamet, P. (2010). "Genetics of pain, opioids, and opioid responsiveness." Metabolism **59 Suppl 1**: S5-8.
Trescot, A. M., Datta, S., Lee, M. and Hansen, H. (2008). "Opioid pharmacology." Pain Physician **11**(2 Suppl): S133-153.
Trevor, A. and White, P. (2007). General Anaesthetics. Basic and Clinical Pharmacology. Tenth Edition. B. Katzung. New York, McGraw-Hill.
Troster, A., Sittl, R., Singler, B., Schmelz, M., Schuttler, J. and Koppert, W. (2006). "Modulation of remifentanil-induced analgesia and postinfusion hyperalgesia by parecoxib in humans." Anesthesiology **105**(5): 1016-1023.

Tucker, A., Kim, Y. I., Nadeson, R. and Goodchild, C. (1999). The antinociceptive effect of ketamine in acute pain models in man. Proceedings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists.
Tucker, C. (1990). "Acute pain and substance abuse in surgical patients." J Neurosci Nurs **22**(6): 339-349.
Tukey, J. W. (1977). "Some thoughts on clinical trials, especially problems of multiplicity." Science **198**(4318): 679-684.
Uhart, M. and Wand, G. S. (2009). "Stress, alcohol and drug interaction: an update of human research." Addict Biol **14**(1): 43-64.
Unlugenc, H., Tetiker, S., Buyukkurt, S., Guler, T. and Isik, G. (2009). "Comparison of the effect of adding remifentanil to patient-controlled tramadol or morphine for postoperative analgesia after major abdominal surgery." J Opioid Manag **5**(5): 247-255.
Urban, M. K., Ya Deau, J. T., Wukovits, B. and Lipnitsky, J. Y. (2008). "Ketamine as an adjunct to postoperative pain management in opioid tolerant patients after spinal fusions: a prospective randomized trial." HSS J **4**(1): 62-65.
Van Dyke, C. and Byck, R. (1982). "Cocaine." Sci Am **246**(3): 128-141.
Van Elstraete, A. C., Sitbon, P., Mazoit, J. X. and Benhamou, D. (2008). "Gabapentin prevents delayed and long-lasting hyperalgesia induced by fentanyl in rats." Anesthesiology **108**(3): 484-494.
Van Elstraete, A. C., Sitbon, P., Trabold, F., Mazoit, J. X. and Benhamou, D. (2005). "A single dose of intrathecal morphine in rats induces long-lasting hyperalgesia: the protective effect of prior administration of ketamine." Anesth Analg **101**(6): 1750-1756.
Vanderah, T. W., Ossipov, M. H., Lai, J., Malan, T. P., Jr. and Porreca, F. (2001a). "Mechanisms of opioid-induced pain and antinociceptive tolerance: descending facilitation and spinal dynorphin." Pain **92**(1-2): 5-9.
Vanderah, T. W., Suenaga, N. M., Ossipov, M. H., Malan, T. P., Jr., Lai, J. and Porreca, F. (2001b). "Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced abnormal pain and antinociceptive tolerance." Journal of Neuroscience **21**(1): 279-286.
Verebely, K., Volavka, J., Mule, S. and Resnick, R. (1975). "Methadone in man: pharmacokinetic and excretion studies in acute and chronic treatment." Clin Pharmacol Ther **18**(2): 180-190.
von Zastrow, M. (2010). "Regulation of opioid receptors by endocytic membrane traffic: Mechanisms and translational implications." Drug Alcohol Depend.
Vree, T. B., van Dongen, R. T. and Koopman-Kimenai, P. M. (2000). "Codeine analgesia is due to codeine-6-glucuronide, not morphine." Int J Clin Pract **54**(6): 395-398.
Vree, T. B. and Verwey-van Wissen, C. P. (1992). "Pharmacokinetics and metabolism of codeine in humans." Biopharm Drug Dispos **13**(6): 445-460.
Wall, P. D. (1978). "The gate control theory of pain mechanisms. A re-examination and re-statement." Brain **101**(1): 1-18.
Wallace, M. S., Braun, J. and Schulteis, G. (2002). "Postdelivery of alfentanil and ketamine has no effect on intradermal capsaicin-induced pain and hyperalgesia." Clin J Pain **18**(6): 373-379.
Walsh, S. L. and Eissenberg, T. (2003). "The clinical pharmacology of buprenorphine: extrapolating from the laboratory to the clinic." Drug Alcohol Depend **70**(2 Suppl): S13-27.
Walsh, S. L., Preston, K. L., Bigelow, G. E. and Stitzer, M. L. (1995). "Acute administration of buprenorphine in humans: partial agonist and blockade effects." J Pharmacol Exp Ther **274**(1): 361-372.
Walter, D. and Inturrissi, C., Eds. (1995). Absorption, distribution, metabolism and excretion of buprenorphine in animals and humans. Buprenorphine: combating drug abuse with a unique opioid. New York, Wiley-Liss.
Wang, Y. H., Sun, J. F., Tao, Y. M., Chi, Z. Q. and Liu, J. G. (2010). "The role of kappa-opioid receptor activation in mediating antinociception and addiction." Acta Pharmacol Sin **31**(9): 1065-1070.
Watkins, L. R., Hutchinson, M. R., Ledeboer, A., Wieseler-Frank, J., Milligan, E. D. and Maier, S. F. (2007). "Norman Cousins Lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids." Brain Behav Immun **21**(2): 131-146.

Way, E. L., Loh, H. H. and Shen, F. H. (1969). "Simultaneous quantitative assessment of morphine tolerance and physical dependence." J Pharmacol Exp Ther **167**(1): 1-8.
Webb, A. R., Leong, S., Myles, P. S. and Burn, S. J. (2002). "The addition of a tramadol infusion to morphine patient-controlled analgesia after abdominal surgery: a double-blinded, placebo-controlled randomized trial." Anesth Analg **95**(6): 1713-1718, table of contents.
Wei, L. N. and Loh, H. H. (2011). "Transcriptional and epigenetic regulation of opioid receptor genes: present and future." Annu Rev Pharmacol Toxicol **51**: 75-97.
Weinbroum, A. A. (2003). "A single small dose of postoperative ketamine provides rapid and sustained improvement in morphine analgesia in the presence of morphine-resistant pain." Anesth Analg **96**(3): 789-795, table of contents.
Weinbroum, A. A. (2012). "Non-opioid IV adjuvants in the perioperative period: Pharmacological and clinical aspects of ketamine and gabapentinoids." Pharmacol Res.
White, J. and Hay, J. L. (2007). Opioids, pain and addiction: Cause and consequence. Translation of Addictions Science into Practice. P. Miller and D. Kavanagh. San Diego, California, Elsevier Pergamon.
White, J. M. and Irvine, R. J. (1999). "Mechanisms of fatal opioid overdose." Addiction **94**(7): 961-972.
White, P. F. (2008). "Multimodal analgesia: its role in preventing postoperative pain." Curr Opin Investig Drugs **9**(1): 76-82.
White, P. F., Raeder, J. and Kehlet, H. (2012). "Ketorolac: its role as part of a multimodal analgesic regimen." Anesth Analg **114**(2): 250-254.
White, P. F., Way, W. L. and Trevor, A. J. (1982). "Ketamine--its pharmacology and therapeutic uses." Anesthesiology **56**(2): 119-136.
White, W. (1998). Slaying the Dragon. The History of Addiction Treatment and Recovery in America. Bloomington, Indiana, Chestnut Health Systems.
Wiffen, P. J. and McQuay, H. J. (2007). "Oral morphine for cancer pain." Cochrane Database Syst Rev(4): CD003868.
Woods, J. H. and Winger, G. (1987). "Behavioral characterization of opioid mixed agonist-antagonists." Drug Alcohol Depend **20**(4): 303-315.
Wright, C. (1874). "On the Action of Organic Acids and their Anhydrides on the Natural Alkaloids." Journal of the Chemical Society **27**: 1031-1043.
Yanagihara, Y., Ohtani, M., Kariya, S., Uchino, K., Hiraishi, T., Ashizawa, N., Aoyama, T., Yamamura, Y., Yamada, Y. and Iga, T. (2003). "Plasma concentration profiles of ketamine and norketamine after administration of various ketamine preparations to healthy Japanese volunteers." Biopharm Drug Dispos **24**(1): 37-43.
Yassen, A., Olofsen, E., Romberg, R., Sarton, E., Danhof, M. and Dahan, A. (2006). "Mechanism-based pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of buprenorphine in healthy volunteers." Anesthesiology **104**(6): 1232-1242.
Yassen, A., Olofsen, E., Romberg, R., Sarton, E., Teppema, L., Danhof, M. and Dahan, A. (2007). "Mechanism-based PK/PD modeling of the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers." Clin Pharmacol Ther **81**(1): 50-58.
Yilmaz, P., Diers, M., Diener, S., Rance, M., Wessa, M. and Flor, H. (2010). "Brain correlates of stress-induced analgesia." Pain **151**(2): 522-529.
Young, G. (1753). A Treatise on Opium. London, A. Miller.
Zacny, J. P., Conley, K. and Galinkin, J. (1997). "Comparing the subjective, psychomotor and physiological effects of intravenous buprenorphine and morphine in healthy volunteers." J Pharmacol Exp Ther **282**(3): 1187-1197.
Zakine, J., Samarcq, D., Lorne, E., Moubarak, M., Montravers, P., Beloucif, S. and Dupont, H. (2008). "Postoperative ketamine administration decreases morphine consumption in major abdominal surgery: a prospective, randomized, double-blind, controlled study." Anesth Analg **106**(6): 1856-1861.
Zhang, L., Yu, Y., Mackin, S., Weight, F. F., Uhl, G. R. and Wang, J. B. (1996). "Differential mu opiate receptor phosphorylation and desensitization induced by agonists and phorbol esters." J Biol Chem **271**(19): 11449-11454.