AN INVESTIGATION OF THE ACTIVITY OF SOME LACTAMS IN THE CENTRAL NERVOUS SYSTEM

by

Elisabeth Breuker, B.Sc. Hons. (Adel.)

A thesis submitted for the degree of
Master of Science
Department of Human Physiology and Pharmacology,
University of Adelaide.

Awarded December 1977

1977

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief contains no material previously published by another person, except where due reference is made in the text.

Part of the work (see Appendices 9-10) has been published in the Journal of Neurochemistry, 1975, Vol. 25, pp 903-904; and in Brain Research, 1967, Vol. 110, pp 413-416.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr D.I.B. Kerr, for his help and advice throughout the course of this study.

My thanks are also due to Dr R. Prager and Dr A.D. Ward for help with the chemical aspects of this work, as as well as for the synthesis of the compounds I used. I am grateful to members of the department who assisted me in any way.

Finally I wish to thank all those people who so kindly helped me when I visited them in Cambridge, Bristol, London and Canberra.

INDEX

	page
DECLARATION	
ACKNOWLEDGEMENTS	
SUMMARY	
ABBREVIATIONS	
INTRODUCTION	1
METHODS	
Compounds	8
Animals	8
Administration of drugs	8
Preliminary screening of compounds	8
Median effective dose (ED_{50}) estimation	9
Log P	9
Titration Experiment	9
RESULTS	
Preliminary screening	11
Median effective dose (ED_{50})	12
Titration experiment	13
DISCUSSION	14
Substitutions	15
Substitution at C-2	15
Substitution at C-3	16
Substitution at C-4	16
Substitution at C-5	17
Substitution at C-6	17
Substitution at C-7	17
Substitution at N	18

page

Saturation of lactam ring 18

Increasing ring size 19

Log P and biological activity 20

Activity in the whole animal 22

Titration experiment 24

CONCLUSIONS 26

APPENDIX 1 28

APPENDIX 2 29

APPENDIX 3 33

APPENDIX 4 35

APPENDIX 5 38

APPENDIX 6 40

APPENDIX 7 42

APPENDIX 8 43

APPENDIX 9 46

APPENDIX 10 48

BIBLIOGRAPHY 52

SUMMARY

1. Lactams and several other compounds were injected intraperitoneally in mice and changes in activity were observed.
2. The ED_{50} of compounds active in mice, i.e. causing convulsions or loss of righting reflex was estimated.
3. The effect of various substitutions on the lactam ring and resultant changes in the log P value is discussed in relation to changes in behaviour of mice after administration of the lactams.
4. Two lactams, 7-nC_4H_9-caprolactam and 4,6,6-$(CH_3)_3$-caprolactam were titrated against each other and were found to antagonize one another.
5. Various experiments were carried out on isolated preparations to determine if the lactams had any effect on putative amino acid transmitters.

ABBREVIATIONS

CNS	central nervous system
ED_{50}	median effective dose
GABA	gamma-aminobutyric acid
GAD	glutamate decarboxylase
$HD_{50}A$	ED_{50} of 7-B in presence of 4-M
log P	1-octanol-water partition coefficient
L-GLU	L-glutamate
NADH	nicotinamide adenine dinucleotide diphosphate
PRO	proline
VHS	Van Harreveld's solution
μ	dipole moment
7-B	7-nC_4H_9-caprolactam
4-M	4,6,6-$(CH_3)_3$-caprolactam

INTRODUCTION

The aims of this thesis are:

1. To observe the activity of the whole animal (mouse) upon administration of caprolactam and its derivatives. (A number of other unrelated compounds were also screened).
2. To quantitatively analyse this activity and determine if there is any correlation between the biological activity of the compound and its log P value.
3. To determine what effect the various substitutents on the lactam ring have in relation to the activity in the mouse upon administration of the compound.
4. To look at a few systems whereby it may be possible to establish whether the lactams are GABA antagonists.
5. To determine whether two lactams displaying opposite effects in the mouse antagonize each other.

Several studies have already been made on a few lactams in the past years (Lien, Lien and Tong, 1971; Elison, Lien, Zinger, Hussain, Tong and Golden, 1971; Goldblatt, Farquharson, Bennett and Askew, 1954). In this thesis, part of the work deals with a particular lactam, caprolactam, which is screened for any activity in the CNS and also examines how this activity changes with certain substitutions.

Caprolactam, when administered intravenously has an excitatory action on the CNS which results in convulsions at a rather high dose (≃ 600 mg/Kg) (Elison *et al.*, 1971). This activity stems from the resonating structure $>N-\overset{\overset{A}{\|}}{C}- \rightarrow >N^{+}=\overset{\overset{A^{-}}{|}}{C}^{-}$ where A=O or S (Lien and Kumler, 1967), a factor found to be common in central nervous system depressants and stimulants. Elison *et al.* (1971) also showed that the longer the chain of the ω-amino acid from which the lactams are obtained, the greater

the lipid solubility and the more effective the compound is as a stimulant. As well as this, a lactam lacking N-substitution is a fairly potent CNS stimulant, as are the thio-derivatives of the compounds.

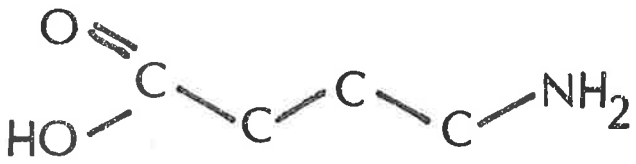
These cyclic compounds have an advantage over their parent ω-amino acids in that their ability to cross the blood-brain barrier is greatly enhanced by the reduction of polarity during the cyclization process. ε-aminocaproic acid, the amino acid from which caprolactam is derived by ring closure, augments the local responses evoked from the cerebral cortex but shows no significant effect on cerebellar activity (Purpura, Girado, Smith, Callan and Grundfest, 1959). When this amino acid is cyclized, it's synaptic action is enhanced. The cyclization of these acids increases the lipid solubility of the molecule (by reducing the polarity) thus enabling the molecule to penetrate the blood-brain barrier and enter the CNS.

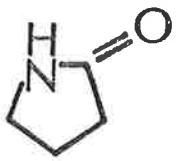
Although caprolactam had a stimulant effect on the CNS, it is thought that this is not due to a direct excitatory effect on the CNS. When applied directly to the spinal cord neurones, caprolactam did not produce any activity (Curtis and Watkins, 1960). ε-aminocaproic acid, however, did show some depression of the spinal cord neurones. On the other hand, longer chain ω-amino acids showed no activity; it was only ω-amino acids with a chain length of less than six carbons that showed any depression. But when applied topically to the cerebral cortex, the longer-chain ω-amino acids augmented responses evoked from the cortex while the short-chain ω-amino acids had a depressant effect on the cerebral cortex (Purpura *et al.*,).

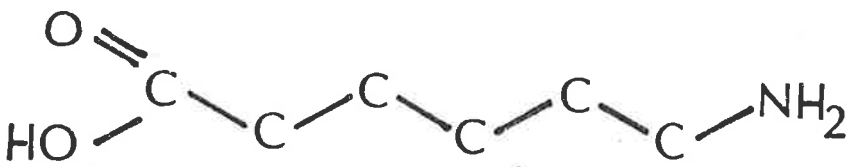
Supported by this evidence Kerr, Dennis, Breuker, Prager, Ward and Duong (1976) thought it most likely that the activity displayed by ε-aminocaproic acid and caprolactam was mediated by a blockade of some

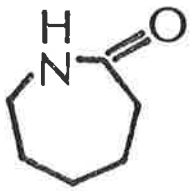
FIGURE 1

Structures of γ-aminobutyric acid (GABA), γ-butyrolactam, ε-aminocaproic acid, and ε-caprolactam.


O
C
HO
C
C
C
NH2


H
N
O


O
C
HO
C
C
C
C
C
NH2


H
N
O

form of CNS inhibition, rather than by direct excitation of CNS neurones. And since GABA-mediated inhibition predominates over glycinergic-mediated inhibition in higher CNS centres (Curtis and Johnston, 1974), it appeared most likely that the inhibition by caprolactam would be GABA-mediated. Kerr *et al.* (1976) further showed that this was in fact the case with caprolactam and two of its derivatives when they used the strionigral pathway. GABA is the recognised transmitter in this pathway and picrotoxin, a known GABA antagonist depresses the strionigral responses. The same result occurred with caprolactam and the two derivatives.

Apart from screening caprolactam and its derivatives, a further part of this thesis endeavours to determine whether these lactams antagonised GABA in other systems (Appendices 4-8). This was compiled from work carried out in different research units I visited and worked in in Canberra, Cambridge, London and Bristol as well as some research carried out in California using one of the lactams.

Returning to the screening of lactam activity in the CNS, whole animal studies only were carried out. This meant that many factors had to be considered; physical characteristics of the chemical molecule, elimination and/or inactivation of the compound in the body as well as concentration changes in different parts of the animal.

The screening of the compounds resulted in an investigation of the relationship between chemical structure and biological activity which provides information about which moieties in the molecule are essential to obtain potent and specific drugs as well as providing more ideas for the mechanisms of action of drugs (van Rossum, 1963; Smythies, 1974).

Most drugs activate biological tissue by interacting with receptive sites whose molecular structure and physical and chemical properties are

suitable for the attachment of the drug there. To isolate a system as such may be difficult, but even so, the interaction of an isolated receptor with a drug would not be truly representative of that found in the tissue. Consideration has not been given to any steady state processes which control the chemical environment of the receptors. Events such as route of administration, absorption, distribution, metabolism and excretion are all involved in the activity elicited by a drug in the whole animal.

The basic step in drug action is to determine how the molecules reach the receptive site and attain a sufficient concentration after administering the drug in order that the drug causes an effect. The route of administration and the rate of absorption are the main factors in determining the time course of onset of action and the drug dosage needed to produce the desired effect. But other factors e.g. distribution and metabolism are also of significant importance. The rate, extent and pattern of initial distribution of a drug are determined by the physico-chemical characteristics of the drug. Lipid-soluble drugs that readily cross membranes are distributed throughout all fluid compartments, whereas lipid-insoluble drugs have more difficulty in doing so (Brodie and Hogben, 1957). These drugs normally pass through pores in the membranes if they are to cross the membranes. Another factor to consider is the binding of drugs to plasma proteins, inhibiting their passage across cellular membranes.

The termination of the drug effect and thus its duration of action is usually brought about by metabolism or excretion or both, but it may also result from redistribution of the drug from its site of action into other tissues or sites. Many drugs are lipid-soluble weak organic acids or bases that are not readily eliminated from the body due to tubular

reabsorption of the undissociated molecules. To be excreted more rapidly, they are metabolized or transformed into more polar compounds. These metabolites are usually less lipid soluble, more ionized at physiological pH, less bound to plasma and tissue proteins, less stored in fat and less able to penetrate cell membranes. Thus metabolism not only fosters drug inactivation but also often results in elimination of the drug.

The most important route of excretion for most drugs is the kidney. Many drugs are also excreted into the bile but are usually recycled through the intestine, making this route quantitatively unimportant.

Although all tissues of the body may be exposed to virtually the same concentration of a drug, it is common knowledge that some tissues are affected much more than others. The selective effect of many drugs is best explained by postulating the existence of specific receptive molecules within cells or on their surfaces. These molecules or sites are referred to as "receptors". The most compelling reasons for postulating the receptor hypothesis originates from quantitative studies on drug effects and their inhibition by antagonists (van Rossum, 1963; Schild, 1947; Albert, 1975).

Because the biological activity of a compound is determined by its chemical structure, if the chemical structure of a compound sets values for various physico-chemical parameters, then these parameters may also correlate with the biological activity of the compound. Elison *et al.* (1971) observed that, in mice, the acute lethal toxicity of some CNS stimulants with a lactam structure was found to be correlated with the log P value of the compound where P is the 1-octanol-water partition coefficient. Hansch and Anderson (1967) have also noticed this in a series of barbiturates. From a study of the structure-activity relationships of barbiturates in four biochemical systems, inhibition

of *Arbacia* egg cell division, inhibition of rat brain oxygen consumption, hypnotic activity, and inhibition of NADH oxidation, they found that very good structure-activity correlations could be obtained considering only the log P values of the various barbiturate derivatives. Hansch *et al.* (1967) also observed that steric and electronic effects played a role but that this was only a minor role. These factors are more specifically concerned with the behaviour of the compound molecule at the responsive site. The steric factor is determined by the shape of the ring of the lactam (Elison *et al.*, 1971) or barbiturate (Hansch *et al.*, 1967); whether it is flat or has a "chair" or "boat" formation, and also by the different substituents on the ring and how these may effect the binding of the compound molecule to the responsive sites and thus produce an effect. The electronic factor describes the electron distribution of the compound and this varies with different substituents on the ring. Lien *et al.* (1971) used the dipole moment (μ) to bring these factors into their discussion of lactams. It appears to play a significant role in determining the convulsant activity and the acute lethal toxicity of the lactams they studied. They found that a higher dipole moment causes the >NH hydrogen to be more electron deficient, and the >CS grouping to have a higher electron density; these two effects provide stronger hydrogen bonding which is associated with increased convulsant activity and acute lethal toxicity.

But Hansch, Steward, Anderson and Bently (1967) point out that although the relative activity of drugs in a series of congeners is highly dependent on their lipophilic character, this correlation does not hold indefinitely as the lipophilic character continues to increase. This is thought to be due to a decrease in mobility of drug movement through biological material when one departed in either direction from

ideal lipophilic character. By measuring other parameters such as the dipole moment, we can make more conclusions about structure-activity relationships.

Nevertheless, with the lactams provided, and being given the log P values for some of them, it was decided that a correlation study of biological activity and log P would still be carried out.

As well as the above variations in biological activity due to changes in chemical structure, another field of study in structure-activity relationships is that of studying the biological activity of a number of closely related compounds, i.e. where there is a basic structure and what in fact is being studied, is the effect of different substituents situated on various parts of the basic structure. This aspect of structure-activity relationships was tackled in this thesis. The basic structure was the caprolactam molecule.

Another part of the thesis deals with the titration of two lactams against each other. These two lactams were observed to have opposite effects on the whole animals, in this case the mouse. Where, with one lactam, the compound caused mice to convulse, the other lactam had a depressant effect. Thus it was thought that the two compounds might antagonize each other. This idea was prompted by the work of Shulman and Laycock (Pt 2, 1967). They suggested that analeptics and hypnotics produced their opposed effects at the same site by modifying the liberation or action of a transmitter substance. Although their work was not entirely conclusive since they only used whole animals, it was nevertheless thought to be an interesting exercise to undertake.

METHODS

Compounds

Fresh stock solutions of all the compounds (Appendix 1) were made up each day. Saline at room temperature was used wherever possible to dissolve and dilute the drugs. Those drugs not soluble in saline were dissolved in propylene glycol or varying mixtures of saline and propylene glycol so that needles as small as possible could be used when administering the drug. Propylene glycol injected on its own did not have any effect on mice.

Animals

White mice, weighing between 15-40 gm were used in all experiments. Although not ideal, in some cases the mice had to be reused up to four times. However they were only reused after at least a seven day interval following the previous administration. Because the activity observed was short-acting, there is not much chance that enzyme induction would occur. This has been observed with short-acting barbiturates. No enzyme induction takes place for these barbiturates whereas for longer-acting barbiturates, enzyme induction does occur (Brian Priestly, private communication).

Administration of drugs

All drugs were administered intraperitoneally in volumes ranging from 0.25 ml to 0.5 ml, using needles as small as possible. The mice were identified by coloured markings on their tail.

Preliminary screening of compounds

All compounds were initially screened by injecting mice intraperitoneally at doses ranging up to 200 mg/Kg body weight. Generally the doses were 10, 20, 60, 100 and 200 mg/Kg or until a definite response was observed. Occasionally fewer doses were injected to cover

the same range, depending on the quantity of drug available at the time. If, for any of the compounds given, definite convulsant or depressant behaviour was seen in the animal, no higher doses were given and the compound was further tested in more mice to determine a median effective dose. The mice were observed for at least thirty minutes. Signs of any other CNS or autonomic effects were also looked for.

Median effective dose (ED_{50}) estimation

To determine the median effective dose, groups of five mice were injected with varying doses such that there were at least two doses where the number of mice showing CNS activity, i.e. convulsions or loss of righting reflex, lay between one and five. Observation time was at least thirty minutes. The ED_{50} was then calculated by the method of Litchfield and Wilcoxon (1948) using 95% confidence limits.

Log P

As well as calculating the ED_{50} of a compound, the log P value was also determined. This was measured by the Department of Organic Chemistry at the University of Adelaide.

Following this, a graph of log ($1/ED_{50}$) against log P (James, 1974) was plotted. Appendix 2 describes the algorithm used to estimate the curve as well as another algorithm which was used to determine if there was any significant variation between groups of lactams used.

Titration experiment

Following the method set out by Shulman and Laycock (Pt 1, 1967), groups of ten mice were used for each dose of the depressant lactam 7-nC_4H_9-caprolactam (7-B). A concentration of this lactam (ED_{50}A) was determined such that when titrated against a fixed dose of the convulsant lactam 4,6,6-$(CH_3)_3$-caprolactam (4-M), the quantity of both drugs present simultaneously at their sites of action during a specified

titration interval, resulted in a depressant titration effect equivalent to that produced by the ED_{50} of 7-B alone. Groups of mice were tested at each dose of 4-M until there were at least two groups of mice whose effects quantitatively lay between 0% and 100%. The $ED_{50}A$ for that particular dose of 4-M was then determined by the method of Litchfield and Wilcoxon (1968) using 95% confidence limits.

This procedure was continued so that the lactam 4-M had been titrated at increasing concentrations up to approximately twice the ED_{50} of 4-M which had been calculated previously.

The time interval between administering the depressant and convulsant lactam was determined by the time taken for the compounds to react individually on the mice. On the basis of this information, the administration of the two lactams was spaced such that both compounds produced maximal effects at approximately the same time.

The mice were observed closely for at least fifteen minutes and then at intervals of five to ten minutes until the mice had recovered, this latter period of observation lasting up to three hours.

A titration curve was constructed by plotting mean $ED_{50}A$ values against related doses of the lactam 4-M. A curve of best fit was estimated using least squares regression analysis (Appendix 3).

The mice used in this titration were not reused.

RESULTS

Preliminary screening

Of the eighty-five compounds screened, twenty-four lactams produced some distinctive behaviour in the mice. All the remaining compounds did not have any significant effect on the animals at the doses used. This latter group also included some pyrrolidinone compounds but none of these showed any effect on the mice at doses up to 200 mg/Kg body weight.

One of the active compounds, 7-nC_4H_9-caprolactam had a partially depressant effect on the mice tested. About two to three minutes after administration of this lactam, the mice first became hyperactive, walking with an unsteady gait and occasionally displaying a Straub tail or even had mild clonic convulsions. But this lasted no more than one minute. After this initial behaviour, the mice developed ataxia or muscle incoordination and then lost their righting reflex, the duration being approximately four minutes where-upon recovery was complete. The actual time for the loss of righting reflex was about three minutes.

With all the other lactams that produced any change in behaviour in the mice, the change was of an excitatory nature. Within one to ten minutes after administration of the compound, the mice became hyperactive running around in their container and displaying a Straub tail. This would last about one to two minutes and was followed by clonic convulsions. The Straub tail was persistent throughout the convulsions in all mice. Accelerated respiration and in some cases defaecation were also observed. In several cases the clonic convulsions were followed by tonic seizures which were also accompanied by urination. When tonic seizures occurred, death generally resulted. If death did not occur (with or without tonic seizures), the convulsions persisted for one to three minutes, whereupon the mice became very quiet and often slept. It is not thought

that this phase following the convulsions is due to a neuro-muscular blocking effect caused by the lactams since the lactams had no effect on the toad sciatic-sartorius preparation (Max King, private communication).

There was no evidence of pilo-erection, salivation, bradycardia or antinociceptive action.

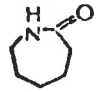
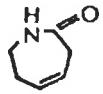
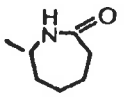
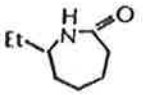
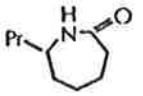
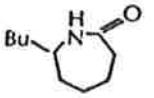
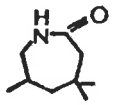
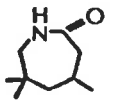
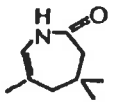
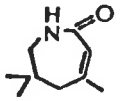
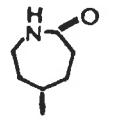
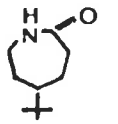
Median effective dose (ED_{50})

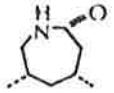
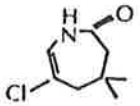
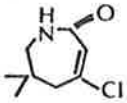
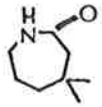
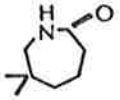
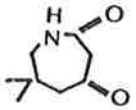
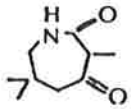
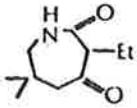
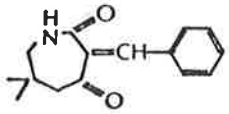
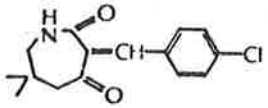
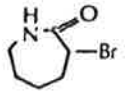
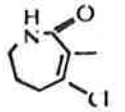
Table 1 shows the structure and molecular weight of all the compounds screened as well as the ED_{50}, in mg/Kg and mM/Kg, and log P values for those compounds which produced significant activity in the mice. Compounds 18, 19 and 25 although active do not have an assigned log P value. This was due to the difficulty in evaluating the log P values for these compounds.

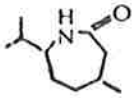
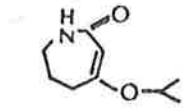
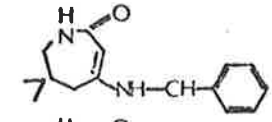
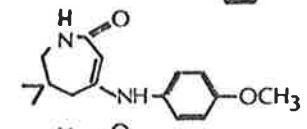
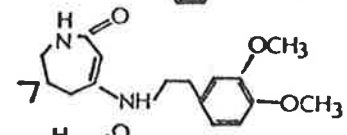
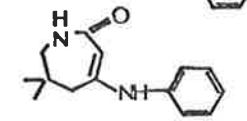
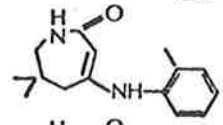
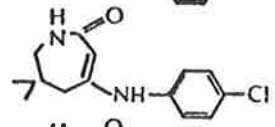
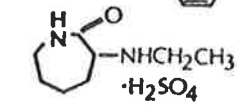
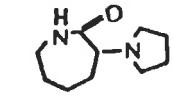
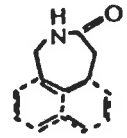
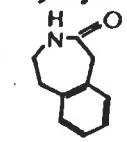
The graph of log P against log ($1/ED_{50}$) of the active caprolactams is seen in Fig. 2. 7-nC_4H_9-caprolactam was not included because its activity was of a depressant nature whereas the activity of the other lactams is of an excitatory nature. The numbers in the graph refer to the compound numbers in Table 1. A Hansch analysis was carried out on the twenty compounds in the graph and this showed that there was no significant correlation between biological activity and log P values (Appendix 2). Furthermore F-ratios were carried out between three groups of lactams: the thiocaprolactams (compounds No. 53, 54, 55, 56, 57, 61, see Table 1), the monosubstituted caprolactams (compounds No. 1, 4, 5, 13, see Table 1), and the disubstituted caprolactams (compounds No. 7, 8, 9, 10, 14, 15, 17, 38, 39, 41, see Table 1). In all cases the F-ratio tests showed that there was no significant difference between these groups to suggest that this might be one reason why there was no correlation between biological activity and log P value in the Hansch analysis (Appendix 2).

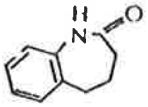
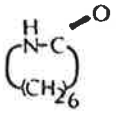
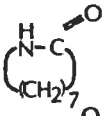
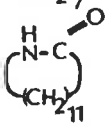
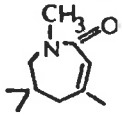
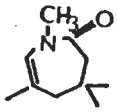
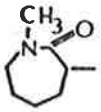
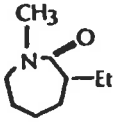
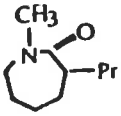
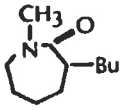
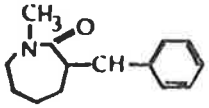
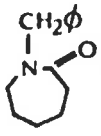
TABLE 1

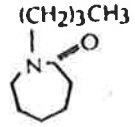
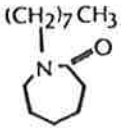
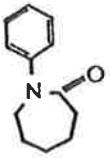
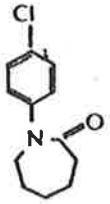
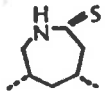
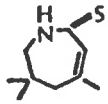
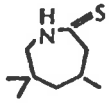
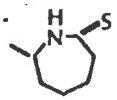
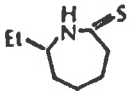
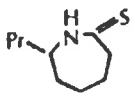
A list of the structures of compounds which were screened on mice with their molecular weights and log P values and ED_{50} values calculated in mg/Kg and mM/Kg with 95% confidence limits in brackets.

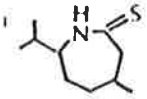
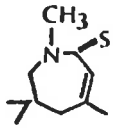
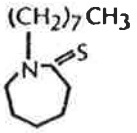
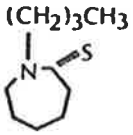
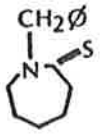
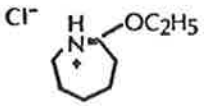
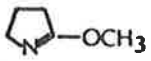
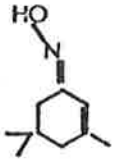
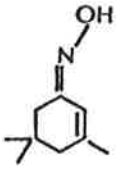
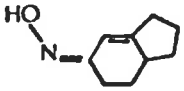
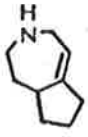
NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
1		113	-0.1	580	5.133
2		111			
3		127	0.2		
4		141	0.7	34(31.3-33.7)	0.241(0.222-0.262)
5		151	1.2	60(51.8-69.4)	0.387(0.334-0.448)
6		169	1.7	111(105.6-116.7)	0.657(0.625-0.753)
7		155	1.0	9(8.1-10.0)	0.058(0.052-0.064)
8		155	1.0	6.2(5.3-7.3)	0.04(0.037-0.047)
9		152	1.06	17(16.4-17.7)	0.112(0.108-0.116)
10		152	1.0	6.7(6.1-7.3)	0.044(0.04-0.048)
11		127	0.2		
12		169	1.58		

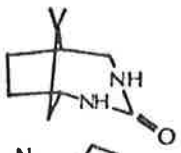
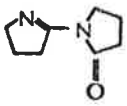
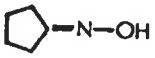
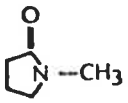
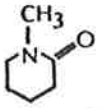
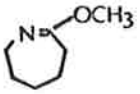
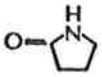
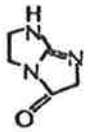
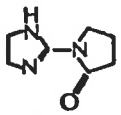
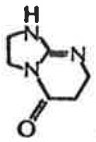
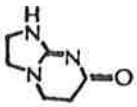
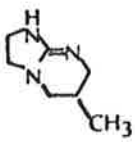
NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
13		127	0.2	62(59.7-64.4)	0.488(0.47-0.507)
14		172	1.79	29(24.6-34.2)	0.169(0.143-0.199)
15		172	1.26	4.1(3.5-4.8)	0.024(0.020-0.028)
16		141	0.7		
17		141	0.7	6.4(5.1-7.9)	0.045(0.036-0.056)
18		155		84(75.2-93.8)	0.542(0.485-0.605)
19		169		41.5(32.5-53.0)	0.245(0.192-0.314)
20		183			
21		244			
22		278			
23		192			
24		114			

NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
25		159		66(60-72.6)	0.415(0.377-0.457)
26		192			
27		244	1.71		
28		260	1.16		
29		319	-0.68		
30		230			
31		244			
32		265			
33		254			
34		182	0.25		
35		261			
36		161			

NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
37		161			
38		127	0.24	100(92-108)	0.787(0.724-0.85)
39		141	0.67	88(79.2-97.7)	0.624(0.562-0.693)
40		187			
41		168	1.32	41(34.2-49.2)	0.244(0.203-0.293)
42		168			
43		141			
44		155			
45		169			
46		183			
47		229			
48		203			

NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
49		171			
50		225			
51		177			
52		211			
53		129	0.72	36(32.8-39.5)	0.279(0.254-0.306)
54		144	1.02	16(13.1-14.5)	0.111(0.091-0.135)
55		168	1.55	11.5(7.4-17.9)	0.068(0.044-0.106)
56		171	1.94	7.2(6.6-7.9)	0.042(0.038-0.046)
57		143	0.98	33.5(29.6-37.9)	0.234(0.207-0.265)
58		157	1.45		
59		171	1.77		

NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
60		185			
61		182	2.06	82(80.3-83.8)	0.45(0.441-0.46)
62		241			
63		187			
64		219			
65		142			
66		127			
67		153			
68		153			
69		151			
70		139			

NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
71		182			
72		152			
73		87			
74		99			
75		113			
76		99			
77		85			
78		99			
79		153			
80		125			
81		139			
82		139			
83		153			

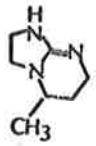
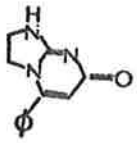
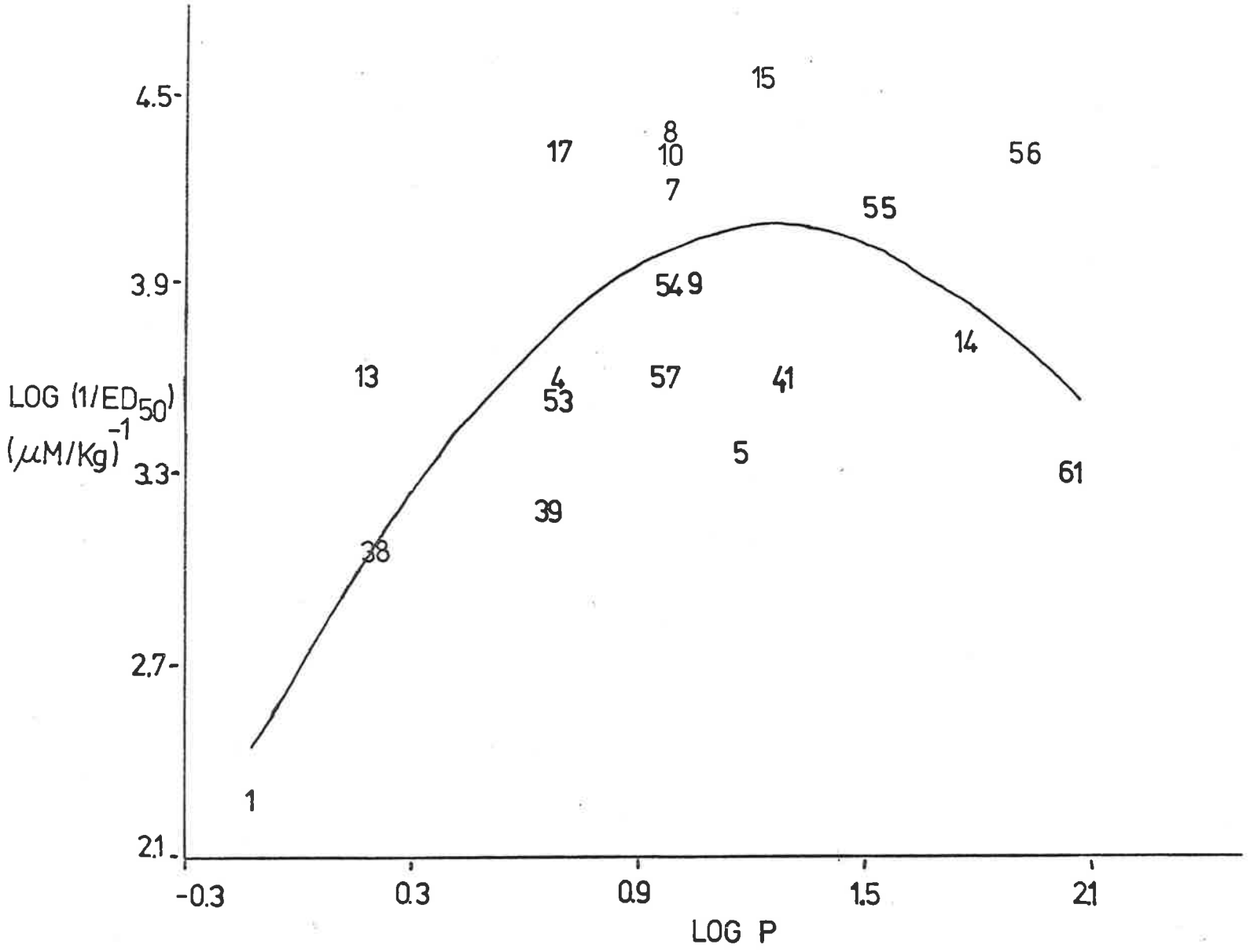
NO.	STRUCTURE	MOLECULAR WEIGHT	LOG P	ED_{50} (mg/Kg)	ED_{50} (mM/Kg)
84		153			
85		214			

FIGURE 2

The estimated curve of log P-log $(1/ED_{50})$ $(\mu M/Kg)^{-1}$ of active caprolactams. The numbers on the curve refer to the number of the compound in Table 1. A table of values, observed and calculated, of log $(1/ED_{50})$ in $(\mu M/Kg)^{-1}$ is listed in Appendix 3.


4.5
3.9
3.3
2.7
2.1
LOG (1/ED50)
(μM/Kg)-1
-0.3
0.3
0.9
1.5
2.1
LOG P
15
8
17
10
56
7
55
54 9
14
13
4
57
41
53
5
61
39
38
1

Titration experiment

There were only a few cases where the mice showed preliminary convulsive activity or hyperactivity in the presence of both 7-nC_4H_9-caprolactam (7-B) and 4,6,6-$(CH_3)_3$-caprolactam (4-M). When these and other mice lost their righting reflex, they did not appear very relaxed as they did when only 7-B was given. In all cases where the mice lost their righting reflex, the onset time for this to occur was five minutes. The duration of loss of righting reflex ranged from three to nineteen minutes.

In all cases, 7-B was administered first because its onset of actions is longer than that for 4-M. The second lactam was given three minutes later. Table 2 and Fig. 3 show the titration values and the regression line estimated according to the Polyanna curve-fitting programme (Appendix 3).

FIGURE 3

Regression line representing the titration of 7-B by 4-M. Each titration point is the mean of at least 3 extimations.

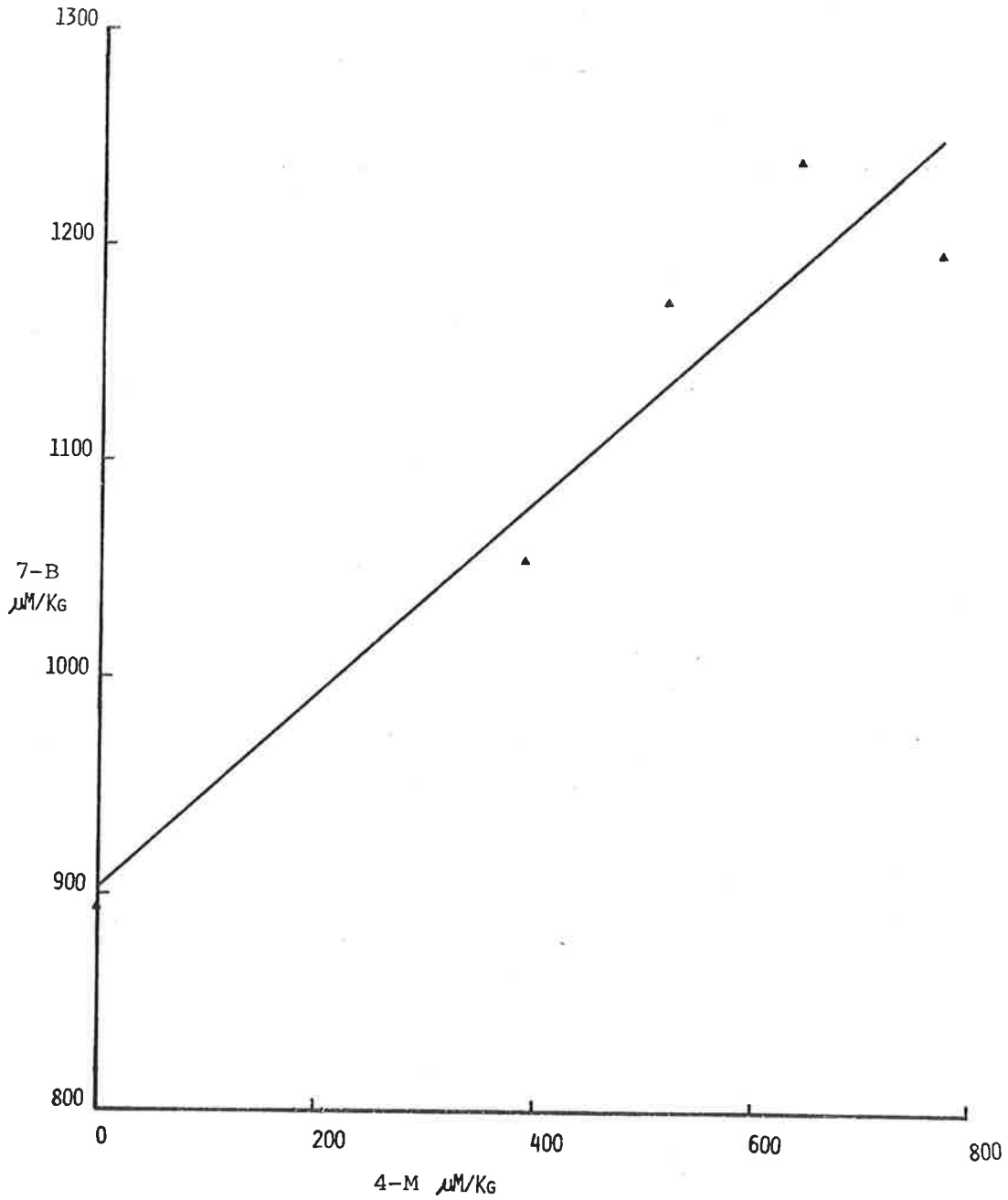

1300
1200
1100
1000
900
800
7-B
μM/Kg
0
200
400
600
800
4-M μM/Kg

TABLE 2

Titration values* ($ED_{50}A$) in μM/Kg) and regression line † representing the titration of 4-methyl-6-dimethylcaprolactam (4M) against 7-butyl-caprolactam (7B).

4M (μM/Kg) (x titrant)	7B (μM/Kg) (y titrant)
0	893
39	1053
52	1172
64	1237
77	1195

Equation of regression line:

$$y = 4.497x + 901.33$$

* the titration end-point is hypnosis in 50% of a group of mice.

† regression line estimated using the Polyanna curve-fitting programme - Appendix 3.

DISCUSSION

There are two major groups of factors which influence the actions of a compound in an animal. They are:

(i) factors related to the compound itself such as lipid and aqueous solubility, molecular size, charge, rate of dissolution, structure etc., and

(ii) factors related to the barriers in the body. These include such elements as absorbing surface areas, enzymatic activity, bowel actions, stomach emptying, pH of body fluids, and vascularity and blood flow in various parts of the animal.

To investigate the effect of all these factors in relation to the convulsant activity of the lactams in whole mice would be an enormous task. However, much can be inferred from these whole animal studies by considering how various substitutions on the lactam ring alter its convulsant activity. Related with this is the solubility of the compound, particularly lipid solubility, and for this, the log P values of the lactams will be considered along with the discussion on the effect of substitutions on the lactam ring.

For those compounds which were screened on mice and did not produce any CNS activity in the animal, the following reasons might explain their lack of behaviour in the whole animal.

(i) the compound never reached the CNS, i.e. it did not cross the blood-brain barrier. Since log P values were only determined for the active compounds, there is at present no information to determine if this is the reason. But another method which could be used to determine if the drugs did cross the blood-brain barrier but yet were without any effect on the mice is by administering the compound via the intra-cisternal route

(Lockett and Davis, 1958). This method could also give some information about the transport of the compound, i.e. whether any of the compound might be metabolized on inactivated en route.

(ii) the compound reached the CNS, but did not react with any responsive site and thus no CNS activity was seen. By studying *in vitro* experiments which are sensitive to drugs in the CNS, it might be possible to determine whether the CNS has a responsive site(s) for these lactams. However this encompasses such an enormous range of experiments that it was not attempted at this stage.

(iii) the compound was metabolized and/or eliminated too quickly for a sufficient concentration of the compound to cause any significant effect. Admittedly the route of administration used gives no information about this at all. The intracisternal route described by Lockett and Davis (1958) would give some information about what occurs to the animal in the CNS alone, and from this we might be able to deduce whether the drug is being deactivated in any sense.

(iv) the amount of compound administered was not sufficient to cause any CNS activity. In this thesis it was decided to study compounds which were active at relatively low doses and thus those compounds not active at doses below 200 mg/Kg were set aside.

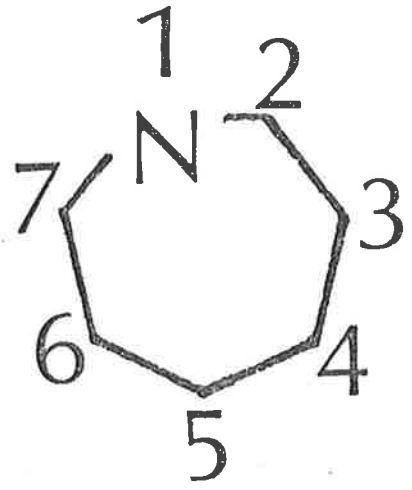
Substitutions

In this section the lactams were considered with respect to the substitutions on the ring and how this alters the potency of the lactam. Log P values, where available, have been used in the discussion.

Substitution at C-2: In all cases the log P values were increased with sulphur substitution for oxygen at C-2. In general the ED_{50} was decreased by replacing oxygen with sulphur at C-2, i.e. the compound has

FIGURE 4

Basic structure of a 7-sided lactam ring.


1
2
N
7
3
6
4
5

become more potent, e.g. compounds with no substitution at all except for that at C-2, or a methyl group at C-4 or C-6 (the compound screened being a 1:1 mixture of the two lactams). However, the ED_{50} increased where there was a methyl group at C-4 and two methyl groups at C-6 (Table 3), presumably the log P value may have become too high for optimal activity.

The last pair of compounds in Table 3, 7-CH_3-caprolactam and 7-CH_3-thiocaprolactam, did not have any effect on the mouse where there was an oxygen substituent at C-2 but did cause convulsions when the substituent was a sulphur atom.

Substitution at C-3: In general, the substitutions at C-3 did not result in very potent lactams (Table 4). The only interesting lactams with a C-3 substitution also had an oxygen atom substituted at C-4, and two methyl groups at C-6. With a methyl group at C-3 plus the other substitutions at C-4 and C-6, the lactam had a greater potency than if there was no substitution at C-3. However, if the size of the substituent at C-3 increased beyond CH_3 no activity was seen in the mouse when these compounds were given at low doses. This may be due to interference of the C-3 substitutuion with the amide group, thus preventing any bonding from taking place at the responsive site. However, it cannot be assumed in this study that we are only looking at the response of one site to the compounds. Therefore the above can only be taken as pure speculation.

Substitution at C-4: There was only one lactam which had a substitution at C-4 alone and this did not produce any convulsant activity in the mouse in doses up to 200 mg/Kg. However, in conjunction with a substitution at C-6, when this lactam is administered to mice, convulsant activity resulted. From Table 5, it is interesting to note that the potency of the lactam is less when the substitution on C-4 consists of two methyl groups, and the

TABLE 3

Substitution at C-2

A list of lactams comparing values of ED_{50} (μM/Kg and log P when the substituent at C-2 is either an oxygen or a sulphur atom.

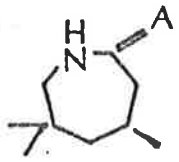
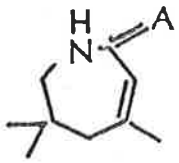
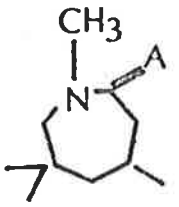
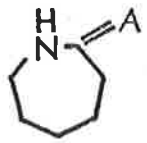
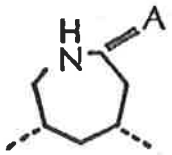
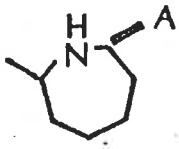
	LOG P		ED_{50} (mM/Kg)	
	A=O	A=S	A=O	A=S
	1.0	1.94	0.04(0.037-0.047)	0.042(0.38-0.046)
	1.0	1.55	0.044(0.04-0.048)	0.068(0.044-0.106)
	1.32	2.06	0.244(0.203-0.293)	0.45(0.441-0.46)
	-0.1	0.72	5.133	0.279(0.254-0.306)
	0.2	1.02	0.488(0.47-0.507)	0.111(0.091-0.135)
	0.2	0.98		0.234(0.207-0.265)

TABLE 4

Substitution at C-3

A list of lactams with substituents at C-3 (except the first lactam) including values for ED_{50} (μM/Kg).

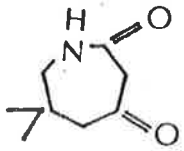
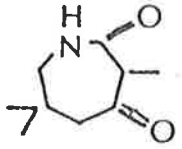
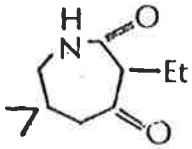
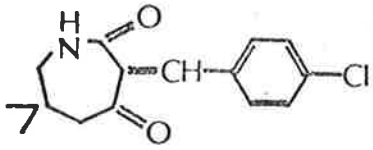
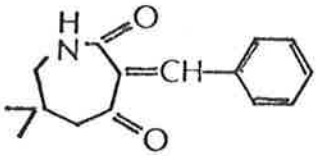
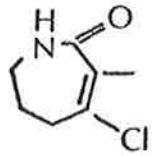
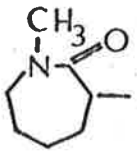
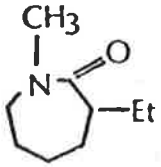
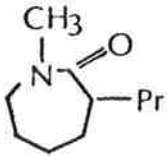
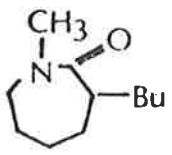
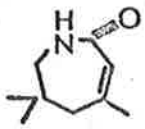
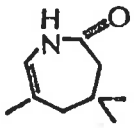
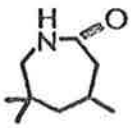
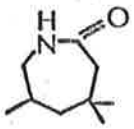
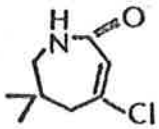
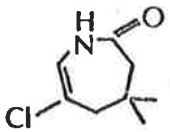
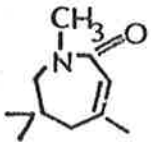
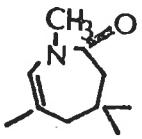
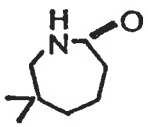
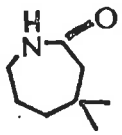
	ED_{50} (mM/Kg)
	0.542(0.485-0.605)
	0.245(0.192-0.314)
	
	
	
	
	
	
	
	

TABLE 5

Substitution at C-4

A list of lactams with substituents at C-4 (except 6-dimethylcaprolactam) including values for ED_{50} (μM/Kg) and log P where these have been measured.

	LOG P	ED_{50} (mM/Kg)		LOG P	ED_{50} (mM/Kg)
	1.0	0.044(0.04-0.048)		1.06	0.112(0.108-0.116)
	1.0	0.04(0.037-0.047)		1.0	0.058(0.052-0.064)
	1.26	0.024(0.020-0.028)		1.79	0.169(0.143-0.199)
	1.32	0.244(0.203-0.293)			
	0.7	0.045(0.036-0.056)		0.7	

substitution on C-6 is either a methyl or chloro group than if these substitutions were reversed for C-4 and C-6. The log P values either remained the same for each pair or varied such that the lactam with the two methyl groups at C-4 had a larger log P value than when the substitutions were reversed at C-4 and C-6. The significance of this reversal is not clear; it may be related to the fact that there is a conformational change in the ring due to conjugation of the p orbital of the substituent with a p orbital nearby.

Substitution at C-5: Only two lactams were screened with substitutions at C-5 - 5-CH_3-caprolactam and 5-tC_4H_9-caprolactam. Neither caused any activity when administered to the intact mouse (Fig. 5). The log P values were not too extreme, being 0.20 and 1.58, respectively. It may be possible that substitutions at C-5 interfer with bonding of the lactams with responsive sites and thus prevent any activity.

Substitution at C-6: Substitution of this carbon has a strong influence on the potency of the lactam. When two methyl groups are placed on the C-6 atom, the caprolactam becomes a very potent convulsant (Table 6). If, as well as this, a methyl group is substituted at C-4, the potency of the lactam remains approximately the same ~~is enhanced still further.~~ ~~However,~~ When the ring is unsaturated, the potency of the compound ~~decreases,~~ remains the same while it increases when the unsaturated ring has a chloro rather than methyl substituent at C-4. The activity of these lactams appears to be related to their respective log P values. As the potency of the lactam increases, the log P value increases from -0.1 to 0.26. This may be a parabolic relationship as proposed by Hansch *et al.* (1967) but there are insufficient numbers of compounds to be certain of this.

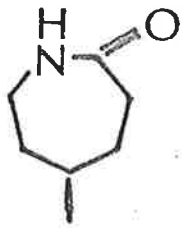
Substitution at C-7: Alkyl substitution at this position showed some interesting results (Table 7). A methyl group at C-7 on the lactam ring had no effect on the mice below a concentration of 200 mg/Kg. Ethyl and propyl substitutions at this carbon caused mice to convulse, the

FIGURE 5

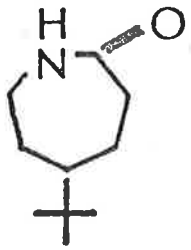
Substitution at C-5

The two lactams with substituents at C-5 which were screened in the whole mouse (including log P values).

LOG P



0.20

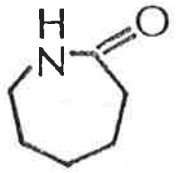
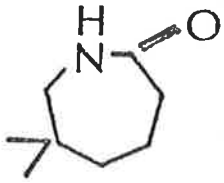
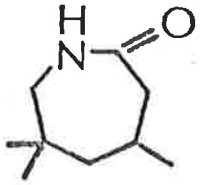
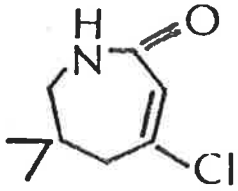
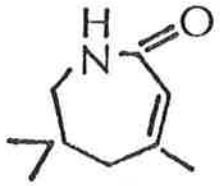


1.58

TABLE 6

Substitution at C-6

A list of lactams with substituents at C-6 (except caprolactam) including values for ED_{50} (μM/Kg) and log P.

	LOG P	ED_{50} (mM/Kg)
	-0.1	5.133
	0.70	0.045(0.036-0.056)
	1.0	0.04(0.037-0.047)
	1.26	0.024(0.020-0.028)
	1.0	0.044(0.04-0.048)

C_2H_5-caprolactam being more potent than 7-C_3H_7-caprolactam. But with a butyl group on C-7, the activity observed in the mice after administration of this compound was a mixture of excitation followed by depression. At first the mice displayed hyperactivity and ataxia and than a loss of righting reflex was observed. However, when the first three compounds had their oxygen atom at C-2 replaced with a sulphur atom, only 7-CH_3-thiocaprolactam showed any convulsive activity in the mice below 200 mg/kg.

The only other compound with a substitution at C-7 is an isopropyl group (Table 7) but it has a lower potency than the other excitatory lactams in this category. The log P values for these compounds, where available, were found to increase with an increase in molecular weight of the compound. Otherwise, there appeared to be no clear relation between the ED_{50} and the log P value of each compound.

Substitution at N: When a methyl group was placed on the nitrogen atom, as with 4,6,6-$(CH_3)_3$ Δ-3-caprolactam and 4,6,6-$(CH_3)_3$ Δ-6-caprolactam (Fig. 6), the potency of the compound was reduced considerably with the former compound, while no response was seen in the mouse below 200 mg/Kg with the latter compound log P also increased with N substitution. All other compounds with nitrogen substitution did not have any effect on the mice for the doses tested. It is obviously important that there is no substitution on the nitrogen atom if the potency of the lactam is to be maintained or increased.

Saturation of the lactam ring: An unsaturated lactam ring in comparison to the related saturated lactam is less potent (Table 8). This difference may be due either to the change in electron density at different sites and/or to changes in the conformation of the ring.

Saturated caprolactams generally adopt a buckled "chair or boat like" conformation. Introduction of unsaturation has the effect of

FIGURE 6

Substitution at N

Comparing values of ED_{50} (μM/Kg) and log P where measured for lactams with or without substituents at N (other than H).

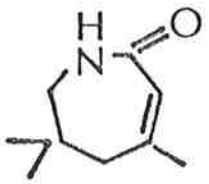
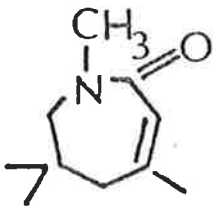
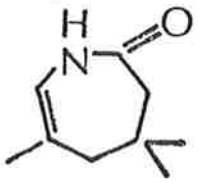
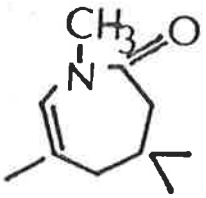
	LOG P	ED_{50} (mM/Kg)
	1.0	0.044(0.04-0.048)
	1.32	0.244(0.203-0.293)
	1.06	0.112(0.108-0.116)
		

TABLE 7

Substitution at C-7

A list of lactams with substituents at C-7 including values for ED_{50} (μM/Kg) and Log P where these have been measured.

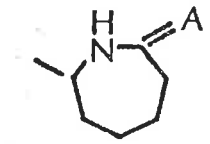
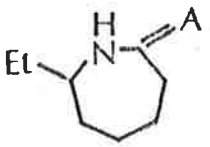
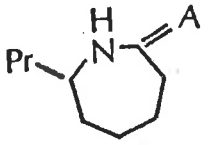
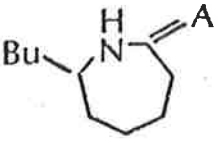
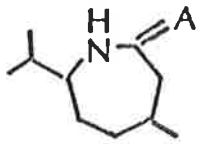
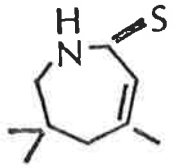
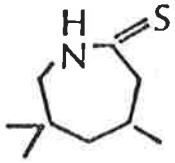
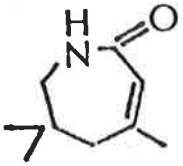
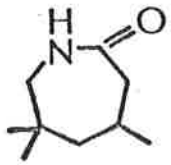
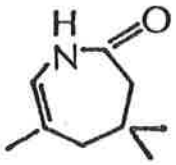
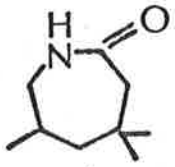
	LOG P		ED_{50} (mM/Kg)	
	A=O	A=S	A=O	A=S
	0.20	0.98		0.234(0.207-0.265)
	0.7		0.241(0.222-0.262)	
	1.2		0.387(0.334-0.448)	
	1.7		0.657(0.625-0.753)	
			0.415(0.377-0.457)	

TABLE 8

Saturation of ring

A list of lactams comparing values of ED_{50} (μM/Kg) and log P when the ring is saturated or unsaturated.

	LOG P	ED_{50} (mM/Kg)
	1.55	0.068(0.044-0.106)
	1.94	0.042(0.038-0.046)
	1.0	0.044(0.04-0.048)
	1.0	0.04(0.037-0.047)
	1.06	0.112(0.108-0.116)
	1.0	0.058(0.052-0.064)

flattening the ring, which in turn effects the binding of the compound at the responsive sites. There does not appear to be any direct correlation between log P values and ED_{50} values or for log P values for saturation lactams and those for unsaturated lactams.

Increasing ring size: When the number of carbons in the ring is increased (Table 1, Nos 38, 39, 40), the compound may cause convulsions in the mice, e.g. when there are seven or eight carbons in the ring. But when the ring size is increased still further, i.e. twelve carbons, convulsions do not occur in doses up to 200 mg/Kg. Lien, Lien and Tong (1971) also noticed this, and observed that the toxicity of the lactams (with seven or eight carbon atoms in the ring) increased for the related thiocaprolactams. The log P values also increased for the thiocaprolactams with respect to the related caprolactams. This is also seen with the lactams used in this study.

From the discussion this far, the position and type of substitutions on the lactam ring play an important role in determining the activity of the lactam in the intact animal.

The strategic parts of the lactam molecule as seen in relation to these substitutions are:

(i) the amide or thioamide group

(ii) C-6

(iii) C-4

The first group, the amide or thioamide group, is also found to be important in the work done by Lien and Kumler (1967). It is also thought that substitutions on the ring provide regions for the hydrophobic interaction and lipoid solubility. Although not much more can be said on this aspect while only studying the reaction of the lactams in whole

animals, this may be a sufficient basis on which to turn to *in vitro* experiments and hence try to determine the mechanism(s) of action of the lactams in the animal which would result in the activity observed when the lactams are administered to the intact animal.

Log P and biological activity: The solubility of a drug in lipid and aqueous media plays an important role in determining the rate at which it penetrates into the brain and cerebrospinal fluid (CSF).

Drugs that act upon the CNS after intraperitoneal administration must have a suitable combination of those properties that confer ready penetration, namely low ionization at plasma pH, low binding to plasma protein, and a suitable lipid/water partition coefficient (or log P). This has already been discussed in the introduction.

The increase in biological activity to a maximum is represented by a parabolic curve, and occurs as compounds with low water solubilities are not able to cross aqueous barriers and those with high aqueous solubilities are not able to cross lipophilic barriers. Somewhere between these limits there is an optimum balance between hydrophilic and hydrophobic properties which corresponds to the maximum point in the curve. The fall-off in biological activity at this point occurs because the compound is too slow reaching the biophase for an equilibrium to occur before a biological response is recorded.

In studies with intact animals, such as those described here, no blood, cerebrospinal fluid or tissue determinations have been made and thus any conclusions made are based on inference. An unknown fraction of the administered drug reaches the site of action if conditions are favourable enough. There may be drug retention by plasma proteins and other body constituents as well as some impedence due to depots or barriers to the free distribution of the drug. But as well as this, inactivation of the

lactam may also occur through metabolism and/or elimination. It is not possible to determine what factors are definitely involved in affecting the access of an injected substance to the CNS in an intact animal. *In vitro* studies would have to be done to gain anymore understanding about this aspect of accessibility.

Although all the active compounds that had log P values assigned were used to estimate the log P - log ($1/ED_{50}$) curve (Fig. 2), it is fairly obvious to see that the curve did not fit very well.

The F-ratios discarded the idea that this might be due to various groups of lactams being significantly different from each other or the rest of the active lactams. One of the reasons that the thiocaprolactams might not be significantly different from the caprolactams is that they may be easily hydrolysed to the respective caprolactams once inside the brain (David Ward, private communication). From this and from previous discussions, this is due to log P not being the sole factor which determines the activity of the compound in the whole mouse. Log P is a factor which is mainly concerned with the biotransportation and metabolism of the compound rather than its behaviour at the responsive site(s) although it does determine the concentration and availability of the compound at this site. Thus, in fact, other parameters would have to be measured and incorporated into this type of analysis (Purcell, Bass and Clayton, 1973; Taft, 1956). This ill-fitting curve implies that for this range of compounds, log P is inadequate for use in a Hansch analysis (Purcell *et al.*, 1973). A further suggested correlation of a physico-chemical parameter with biological activity is that of the chromatographic parameter R_m, to approximate the transport process (David Ward, private communication). There are also numerous other physico-chemical parameters that could be used in this range of compounds in an attempt to

find a better correlation between the physico-chemical parameter(s) and the biological activity. Once a good correlation has been found, we can then proceed with further experiments to determine how and why that particular physico-chemical parameter or group of physico-chemical parameters causes the variation in biological activity in that range of compounds. But on the other hand, *in vitro* experiments should also be attempted to determine where the responsive site(s) are that cause the activity observed in the intact animal experiments.

Activity in the whole animal: Since all the lactams were administered intraperitoneally, for the compound to cause any effect on the CNS of the mouse, it must first be absorbed from the peritoneal cavity into the systemic bloodstream, and so be transported to the CNS. The rate of absorption of a compound in the peritoneal cavity will depend not only on its log P value but also on the vascularity of the peritoneal cavity. Once in the bloodstream, the rate of blood flow is important in setting the time for the onset of action of the drug. But as well as this, metabolism and elimination must also be considered as these may play an important part in determining the concentration of the compound at the responsive site(s). As mentioned before, *in vitro* experiments may give some indication as to their importance in this aspect. But even by changing the route of administration, e.g. intra[C]asternally, we may also get some indication of the significance of these factors on the biological activity of the compound.

But there are still two more factors to consider which determine whether a compound will have an effect or not. One is whether the compound is able to cross the blood-brain barrier. This is determined by the lipid solubility of the compound. After crossing this barrier, the compound must then be able to react with a responsive site(s) and

thus cause a response. But the compound must also attain a threshold concentration at the responsive site before a response can be observed. Hence, neutralization of the lactam to stop the response must also be considered. This also determines the ~~length~~ of duration of the response. Once again metabolism and elimination may be important here, but we must also consider such factors as endogenous antagonists, or any revelant homeostatic mechanisms which may inhibit the activity of the lactam in the intact animal. However, it is not possible to infer any such particular factor as being the mainspring with these studies on the intact animal. Further studies on isolated preparations would be necessary.

Once the compound has crossed the blood-brain barrier, if it is to have any effect on the CNS it must be able to react with a responsive site. This is determined by the molecular structure of the compound in relation to the responsive site or "receptor" molecule. In the case of the active lactams discussed here, it is important that there is a resonating amide or thioamide group and also, to further enhance the potency of the lactams, substitutions at C-4 and C-6.

Thus, if a compound is active in the CNS, its onset of action is determined by the rate of transportation to the responsive site and the duration of action is determined by the elimination or neutralization of the compound. In the lactams, there is a relatively rapid onset of action (1-10 minutes) and brief duration of action (1-2 minutes). But the mechanism of action has still to be considered. The convulsant lactams may cause their effect in the CNS in one or more of the following ways (where the depressant 7-nC_4H_9-caprolactam may have exactly the opposite mechanisms of action).

1. Increasing the presynaptic release of the excitatory transmitter.
2. Decreasing the presynaptic release of the inhibitory transmitter.

3. Increasing the postsynaptic chemosensitivity of the excitatory transmitter.
4. Decreasing the postsynaptic chemosensitivity of the inhibitory transmitter.
5. Antagonizing one of the precursors to the inhibitory transmitter.
6. Inhibiting the synthesis of the inhibitory transmitter.
7. Inhibiting the breakdown of the excitatory transmitter at the synapse.
8. Antagonizing the inhibitory transmitter.
9. Mimicking the excitatory transmitter.
10. Affecting the intra- and extra-cellular ionic concentrations.

However, due to the similarity of the lactams to the putative amino acid transmitters, it is most likely that the lactams are reacting with the responsive sites of these amino acids. The cyclization is probably only necessary for transportation across the blood-brain barrier and the various substitutions are essential for directing the amide or thioamide group in the appropriate position on the responsive site. But to verify this, isolated tissue experiments would have to be carried out. Appendices 4-8 show some isolated tissue experiments which have already been tried and future work will be directed along this course.

Titration experiment: In this set of experiments, from the estimated curve, it is clear that the two lactams antagonize each other in some way. Because of the similarity in structure, it is possible that the lactams may be acting at the same site(s). However, individually both lactams cause CNS activity in the mouse and this may imply that, because the activity varied, the lactams acted at different sites or else acted at the same site(s) but in opposing manners. It is also feasible to consider the suggestion that the lactams act at more than one responsive site since

their actions are of a mixed nature. However, further evidence can only be made available when isolated tissue experiments have been carried out.

CONCLUSIONS

From the lactams studied in this thesis, slight changes in the substitutions on the lactam ring resulted in variation in the biological activity in the intact mouse. This variation was both quantitative and qualitative.

It was shown that the quantitative variation is due to the partition coefficient or log P to some extent. Furthermore it was suggested that the substitutions were important in the binding of the compound at the responsive site(s) and also in their neutralization. However, further experiments using isolated preparations would be needed to confirm this suggestion.

From the titration experiments with 7-B and 4-M, it was observed that the two antagonized one another but that more experiments needed to be done to see what type of antagonism occurred.

The last part of the thesis (Appendices 4-8), looked at the effect of some of the lactams on isolated preparations which are sensitive to various putative amino acid transmitters especially GABA.

The lactams used were 7-nC_4H_9-caprolactam 7-nC_3H_7-caprolactam, 7-C_2H_5-caprolactam, 4,6,6-$(CH_3)_3$ Δ-3-caprolactam, 4,6,6-$(CH_3)_3$-caprolactam and 6,6-$(CH_3)_2$-4Cl-Δ-3-caprolactam. The experiments were designed to test several things:

1. The effect of lactams on the uptake of several amino acids in the rat cerebral cortex (Appendix 4).
2. The effect of lactams on the release of GABA on the rat cerebral cortex (Appendix 4).
3. The displacement of GABA by lactams on the human cerebellum (Appendix 5).
4. The effect of lactams on GABA and β-alanine on the isolated

toad spinal cord (Appendix 6).

5. The effect of lactams on the depolarizing action of GABA on the superior cervical ganglion of the rat (Appendix 7).
6. The effect of 4,6,6-$(CH_3)_3$-caprolactam on GABA uptake in brain homogenates, on GABA binding to brain membrane fractions and on the GABA stimulated Cl^- flux in crayfish muscle (Appendix 8).

The results from experiments 1, 2, 3, 5 and 6 were not very significant in any positive manner. But with experiment 4, there was some antagonism of a lactam against β-alanine.

Overall, however, further isolated experiments would have to be done to determine whether the lactams had any effect on putative amino acid transmitters. These could be done in a number of ways. For example, one transmitter could be tested against the active lactams using a wide range of experiments which are sensitive to the amino acid transmitter and which covers the field as much as possible as to where the lactams could possibly have any effect on the amino acid. Or else a number of putative amino acids for which a particular experiment or set of experiments are sensitive could be used to determine which amino acid(s) is (are) being antagonized by the active lactam. All in all, the work presented in this thesis is in fact only the beginning of our investigation of the lactams in the CNS.

APPENDIX 1

Drugs and chemicals

All the compounds listed in Table 1 were synthesized by the Department of Organic Chemistry at the Univeristy of Adelaide.

Paraldehyde was purchased from Ajax Chemicals Limited.

APPENDIX 2

The Hansch analysis to establish whether log P and log $(1/ED_{50})$ and hence biological activity are correlated was carried out using the folowing algorithm.

This algorithm computes the orthogonal polynomials defined by the distribution of $\{x_i\}$, thus:

$$Q_o = (x_i) \equiv 1$$

$$Q_{k+1}(x_i) = (x_i - a_{k+1})\, Q_k(x_i) - b_{k+1}\, Q_{k-1}(x_i)$$

where $a_{k+1} = J_{k+1}/I_{k+1}$, $b_{k+1} = I_{k+1}/I_k$

$$I_{k+1} = \sum_i w_i [Q_k(x_i)]^2$$

$$J_{k+1} = \sum_i w_i \cdot x_i [Q_k(x_i)]^2$$

$$b_1 = Q_{-1}(x_i) \equiv 0;$$

and if $Q_{k+1}(x) = \sum_{j=0}^{k+1} C_j^{k+1} x^j$

then $C_j^{k+1} = C_{j-1}^k - a_{k+1} C_j^k - b_{k+1} C_j^{k-1}$

where $0 \leqslant k$

$$0 \leqslant j \leqslant k+1$$

and $C_o^o = 1$

$C_m^n \equiv 0$ if $n > m$ or $n < 0$ or $m < 0$

then $\sum_i w_i Q_k(x_i) Q_j(x_i) = \delta_{jk} I_{k+1}$

and the required polynomials are $P_k(x)$

where $P_k(x) = \sum_{j=0}^{k} d_j Q_j(x)$

$$d_j = K_{j+1}/I_{j+1}$$

$$K_{j+1} = \sum_i w_i y_i Q_j(x_i)$$

The iterative formula for the sum of squares of the deviations is:

$$E_{-1} = \sum_i w_i y_i^2$$

$$E_j = E_{j-1} - (K_{j+1})^2/I_{j+1}$$

The polynomial was computed to be:

$$Y = -0.85x^2 + 2.18x + 2.7$$

with a standard deviation = 0.372

For the F-ratio test, the following algorithm was used:

Let $\hat{y} = \hat{b}_o + \hat{b}_1 x_1$ and $\hat{\hat{y}} = \hat{\beta}_o + \hat{\beta}_1 x_1$ } are these different?

1. Pool samples $\Rightarrow (n_1 + n_2)$ observations

$$\hat{y}_p = \hat{a}_o + \hat{a}_1 x$$

and get $\Sigma e_p^2 = \Sigma y_p^2 - \Sigma \hat{y}_p^2$ with

$(n_1 + n_2 - k)$ degrees of freedom (df)

$k = \Sigma b_i$ including b_o

2. Get regressions separately

$$\hat{y}_1 = \hat{b}_o + \hat{b}_1 x_1$$

and $\Sigma e_1^2 = \Sigma y_1^2 - \Sigma \hat{y}_1^2$ $(n_1 - k)$ df

$$\hat{y}_2 = \hat{\beta}_o + \hat{\beta}_1 x$$

and $\Sigma e_2^2 = \Sigma y_2^2 - \Sigma \hat{y}_2^2$ $(n_2 - k)$ df

3. Add $\Sigma e_1^2 + \Sigma e_2^2$ $(n_1 + n_2 - 2k)$ df

4. Σe_p^2 - (3) k df

5. $$F^* = \frac{[\Sigma e_p^2 - (\Sigma e_1^2 + \Sigma e_2^2)]/k}{(\Sigma e_1^2 + \Sigma e_2^2)/(n_1 + n_2 - 2k)}$$

compare with theoretical $F_{0.05}$ with

$$V_1 = k \text{ and } V_2 = n_1 + n_2 - 2k$$

if $F^* > F_{0.05}$ then the distributions are signicantly different.

F-ratios were calculated for the following six sets of data:

1. the disubstituted and thiocaprolactams
2. the monosubstituted and thiocaprolactams
3. the mono- and disubstituted caprolactams
4. the monosubstituted and the remainder of the active lactams
5. the disubstituted and the remainder of the active lactams
6. the thiocaprolactams and the caprolactams.

The results obtained are as follows.

	V_1	V_2	F^*	$F_{0.05}$
1	3	10	0.69	6.55
2	3	4	1.23	16.69
3	3	8	1.10	7.59
4	3	14	1.32	3.59
5	3	14	0.79	3.59
6	3	14	0.73	3.59

In all cases, $F^* > F_{0.05}$, i.e. the distribution of points between any two groups of data are not significantly different.

APPENDIX 3

Polyanna curve-fitting package

A computer programme written by Mr C. Martin of the Department of Applied Mathematics at the University of Adelaide, designed to fit curves to sets of data. The data may be fitted to a straight line (as in the case of the titration experiment), an orthogonal polynomial of a fixed degree, or an orthogonal polynomial whose degree, subject to being less than a certain maximum, is such that the error is minimized, i.e. a polynomial of optimal degree.

The programme may list the data values in a table, the fitted values to the function of the curve, the coefficients of the polynomial approximation, as well as plotting the observed values and the estimated curve.

All the above choices are governed by the control cards the user uses with the data deck.

For the data used in the estimation of the curve in Fig. 2 the equation for the estimated curve was:

$$Y = -0.85x^2 + 2.18x + 2.70$$

A table of $\log (1/ED_{50})$ in $(\mu M/Kg)^{-1}$ for the observed and calculated data is given on the next page.

COMPOUND NO.	LOG P	LOG $(1/ED_{50})$ $(\mu M/Kg)^{-1}$	
		OBSERVED	CALCULATED
1	-0.1	2.29	2.47
4	0.7	3.62	3.80
5	1.2	3.41	4.08
7	1.0	4.24	4.02
8	1.0	4.41	4.02
9	1.06	3.95	4.05
10	1.0	3.61	4.09
13	0.2	3.61	3.10
14	1.79	3.77	3.87
15	1.26	4.61	4.09
17	0.7	4.33	3.80
38	0.24	3.10	3.17
39	0.67	3.20	3.77
41	1.32	3.61	4.09
53	0.72	3.55	3.82
54	1.02	3.95	4.03
55	1.55	4.16	4.03
56	1.94	4.38	3.72
57	0.98	3.63	4.01
61	2.06	3.35	3.57

APPENDIX 4

This series of experiments were carried out at the Department of Neurochemistry, Australian National Univeristy, Canberra under the guidance of Dr G.A.R. Johnston. 4,6,6-$(CH_3)_3$ Δ-3-caprolactam and 7-nC_3H_7-caprolactam were tested to see if they inhibited the uptake of GABA, L-glutamate (L-GLU), L-glutamic acid decaroboxylase (GAD) and L-proline (PRO).

Rats (13 weeks old) were decapitated and the cerebral grey matter removed. After weighing, the grey matter was sliced (0.1 x 0.1 x 2 mm) and suspended in a volume of tissue medium (1 ml/100 mg tissue)*. One hundred µl aliquots were distributed into the incubation vessels each of which contained 10 ml of tissue medium and were placed on ice. The lactam was then added at a concentration of 10^{-4}M. Experiments were done in quadruplicate and each array of four vessels was placed in the incubation bath at 37°C to be preincubated for 15 minutes. The required radioactive amino acid (GABA, L-GLU, PRO or GAD at 0.5 µCi/vessel) was then added and the tissue was collected by filtration after a further 10 minutes incubation. The contents of each array of four vessels was filtered concurrently and washed with 10 ml NaCl at room temperature.

The filters were transferred to scintillation vials and extracted for one hour at room temperature with 1 ml of water. Scintillator§ (10 ml) was then added and radioactivity measured in a scintillation counter.

---

* The control Krebs Ringer buffer (or tissue medium) solution used for uptake and release consisted of NaCl 118.5 mM; KCl 4.75 mM; $CaCl_2$ 1.77 mM; $MgSO_4$ 1.18 mM; D-glucose 5.8 mM, and NaH_2PO_4 15.4 mM, at pH 7.4. The solution was oxygenated before incubation of slices and during the course of release experiments.

§ Scintillator was made up as follows: 12.0 g PPO; 1600 xylene and 800 ml teric N-8.

When L-GLU was used, the incubation period was reduced to 8 minutes and the incubation temperature was lowered to 25°C since uptake had been found to be better at this lower temperature. PRO was only incubated for 4 min at 37°C. In the GAD experiment, preincubation time was 5 minutes and incubation time 30 minutes, both carried out at 37°C.

In all cases, the lactams did not have any significant effect on the uptake of the amino acids. Thus if the lactams were to have any effect on the putative amino acid transmitters it would not be by inhibiting the uptake of these putative transmitters to cause a response.

In the next experiment 4,6,6-$(CH_3)_3$ Δ-3-caprolactam and 7-nC_3H_7-caprolactam were tested to see whether it achieved its action by preventing the release of GABA from the presynaptic terminal.

As before with the uptake experiments, rats (13 weeks old) were decapitated and the cerebral grey matter chopped into slices (0.1 x 0.1 x 1.0 mm) and suspended in cold tissue medium (1 ml/100 mg tissue). Aliquots of 500 µl were distributed into incubation bottles containing 5 ml of cold oxygenated buffer plus one of the lactams at 1×10^{-4}M. Slices were preincubated with shaking at 37°C for 10 minutes before the addition of aminooxyacetic acid (2×10^{-5}M) and [U-^{14}C] GABA (0.5 µCi). The slices were incubated for a further 20 min, then collected by vacuum filtration on glass fibre filter discs and washed with 10 ml of warm buffer (37°C). The filter discs were transferred rapidly to filter holders and perfusion commenced. The apparatus consisted of up to 7 Swinnex filter holders held by spring tool clips on a 37°C water bath. A multichannel peristaltic pump was used to pump oxygenated Krebs Ringer buffer medium through a heating coil contained in the bath and into the filter holders at a rate of 0.5 ml/min. Fractions (1.4 ml) were collected using an automatic fraction collector modified to collect up to 7 fractions

at once directly into scintillation vials. Slices were perfused with normal buffer for different times before switching to "high K-buffer"* for 10 min. Perfusion was continued with normal buffer, then the system was pumped dry and the filter discs with tissue were removed and transferred to scintillation vials containing 1 ml of water. Scintillator (the same as in the previous experiment) was added and the vials were counted.

After calculations had been carried out, it was obvious that 4,6,6-$(CH_3)_3$ Δ-3-caprolactam did not have any effect on GABA release from presynaptic terminals. However, 7-nC_3H_7-caprolactam was found to potentiate the release of GABA in a similar manner to that of bicuculline, though to a lesser degree. Unfortunately, lack of time prevented us from continuing further along this line.

---

* For "high K-buffer", KCl was increased from 4.75 mM to 44.75 mM.

APPENDIX 5

This experiment was carried out in the MRC Neuropharmacology Unit in Cambridge. The lactams used were 7-C_2H_5-caprolactam, 6,6-$(CH_3)_2$-4Cl-Δ-3-caprolactam, 4,6,6-$(CH_3)_3$-caprolactam and 7-nC_4H_9-caprolactam. The aim of the experiment was to determine whether the lactams antagonized the GABA transmitter at the postsynaptic membrane by displacing radioactive GABA.

The human cerebellum was prepared as described by Zukin, Young and Snyder (1974) and kept at -20°C for at least a week. The assay was carried out according to Enna and Snyder (1975).

The frozen crude synaptic membranes (20 ml aliquots) were resuspended in 20 ml of buffer (Tris-citrate buffer at pH 7.1) and centrifuged for 20 min at 48,000 x g. The supernatant was discarded and the pellet was resuspended in 20 ml of buffer. 1 ml aliquots of the "suspension" were incubated in duplicate at 0°C for 15 min with 10 µl [^{3}H] GABA (33 µC/ml) along with 10 µl of 1 mM GABA or varying concentrations of the four lactams. After incubation, the reaction was terminated by transferring two 0.35 ml samples from each tube into plastic microfuge tubes and spinning for 2½ min. The supernatant was discarded and the pellet resuspended in 100 µl H_2O and vortexed. The suspensions were transferred to scintillation vials, 4 ml ethoxyethanol and 10 ml butyl P.B.D. (0.4%) added and radioactivity was assayed by liquid scintillation spectrometry.

Specific [^{3}H] GABA binding was obtained by subtracting from the total bound radioactivity the amount not displaced by a high concentration of GABA (1 mM). The difference between the total bound and the non-specifically bound [^{3}H] GABA represents the amount of [^{3}H] GABA displaced by the unlabelled lactams and is termed the specifically bound

GABA.

Of the four lactams studied in this assay, only one of them had any significant effect on GABA binding. This lactam 4,6,6-$(CH_3)_3$-caprolactam at a concentration of 10^{-3}M displaced the [^{3}H]GABA by 30%. When one compares this with bicuculline where at a concentration of 8×10^{-6}M, the [^{3}H] GABA was displaced by 50%, it appears that the lactams do not have any great effect on GABA binding at postsynaptic membranes.

APPENDIX 6

The toad spinal cord experiment was carried out in the Department of Pharmacology at the University of Bristol. This preparation represents a useful system for the quantitative examination of the pharmacology of neuronal amino acid receptors. 4,6,6-$(CH_3)_3$-caprolactam was used in the experiment described here to determine if it has any effect on responses recorded after application of GABA or β-alanine.

The spinal cord of the frog Rana temporia was removed and placed in a Ringer solution*. The cord was then divided into two equal parts down the midline and the dorsal and ventral roots were also separated. The preparation was set up and superfused at a rate of 3 ml/min with Ringer and left until the responses being recorded had stabilized. The lactam and the amino acids were dissolved in the perfusion medium and added to the preparation by injecting a known quantity into the perfusion medium at a distance from the preparation to avoid any disturbance on the preparation. The ventral root was passed through a mixture of liquid paraffin and petroleum jelly and placed in contact with a silver chloride electrode. Potentials were recorded between this electrode and a similar one placed in contact with the Ringer solution containing the cord. To stimulate the preparation, the dorsal root was passed through liquid paraffin and petroleum jelly and placed in contact with bipolar silver electrodes through which were passed supramaximal square wave pulses. The preparation was superfused for 1 hr with the lactam at a concentration of 5 mM. GABA 1 mM was injected into the medium and showed little reduction in response due to the presence of the lactam. The response

---

* Ringer solution (or perfusion medium). The solution consisted of NaCl 111 mM; KCl 2 mM; NaH_2PO_4 1 mM; $CaCl_2$ 2 mM; $NaHCO_3$ 10 mM; glucose 12 mM, and tris base 10 mM, pH 7.9 adjusted with 11.3 M HCl.

to β-alanine (2 mM) however, was reduced by approximately 70% while the preparation was perfused with the lactam.

These results indicate that the lactam does not cause its convulsant activity by antagonizing the action of GABA, but may do so by antagonizing β-alanine which is also a putative transmitter (Curtis and Johnston, 1974).

APPENDIX 7

The effect of the lactams 4,6,6-$(CH_3)_3$-caprolactam and 6,6-$(CH_3)_3$-4Cl-Δ-3-caprolactam was observed on the depolarizing action of the superior cervical ganglion of the rat in the Department of Pharmacology at St. Thomas's Hospital, London. The method used was the vertical superfusion technique developed by Bowery and Brown (1974). Briefly, rats were anaesthetized with urethane and the superior cervical ganglia were removed together with suitable lengths of pre- and post- ganglionic trunks. The connective tissue sheath was stripped off and the ganglion immersed in Krebs solution* and left overnight at 0°C. The ganglion was then set up. Two Ag/AgCl electrodes were placed in connection with the tissue, one via the thread attached to the post ganglionic trunk and the other directly on the ganglion body close to the preganglionic trunk. The potential difference between the two electrodes was monitored continuously on a Bryans 28000 potentiometric recorder. The tissue was superfused with Krebs solution at a rate of 1 ml/min and the lactams together with GABA (30 μM) were added by addition to this solution. The lactams were screened at concentrations up to 100 μg/ml. Neither showed any antagonism reponses of GABA (30 μM) in the ganglion. This is contrasted with the greater than 50% depression obtained with bicuculline 10 μg/ml in the same preparation.

In conclusion, these experiments suggest that neither of the two compounds owes its convulsant activity to an inhibitory action at GABA receptors.

---

* Krebs solution NaCl 118 mM; KCl 4.8 mM; $CaCl_2$ 2.52 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.18 mM; $MgSO_4$ 1.19 mM, and glucose 11 mM.

APPENDIX 8

The following three experiments were carried out in the Department of Biochemistry at the University of California in Riverside, California. The lactam 4,6,6-$(CH_3)_3$-caprolactam was used in all three experiments.

1. GABA uptake by brain homogenates

The uptake of GABA by mouse brain particulate fractions was assayed according to the method described by Olsen, Bayless and Ban (1975). Fraction P_2, protein concentration 0.25 ± 0.05 mg/ml, was incubated for 30 min at 0°C in 0.1 M NaCl, 0.1 mM aminooxyacetic acid, and 0.01 M Tris-HCl, pH 7.7, containing 0.1 µCi/ml of radioactive [^{3}H] GABA. Triplicate samples contained either (a) no addition; (b) 10^{-3}M non-radioactive GABA (the blank); or (c) 10^{-4}M 4,6,6-$(CH_3)_3$-caprolactam. Samples were then filtered under vacuum, washed twice with 2 ml of the buffer, dried, and radioactivity was determined.

No activity was observed due to the lactam, i.e. it did not differ from the control.

2. GABA binding to brain membrane fractions

The binding of radio labelled GABA to mouse brain homogenates was determined by the technique of Snyder *et al.* (1975) with modifications by Olsen and Greenlee (1976). Fresh mouse brain was homogenized at 0°C in 10 ml of 0.32 M sucrose and centrifuged 10 min at 1,000 x g and the pellet P_1 discarded. The supernatant was centrifuged at 100,000 x g for 1 hr and the resulting supernatant discarded. The pellet (P_2 + P_3) was rehomogenized in 20 volumes of cold distilled H_2O and recentrifuged at 100,000 x g for 1 hr. This pellet was washed once in 10 volumes of H_2O and repelleted, then rehomogenized once more in H_2O and frozen at -20°C (for 1-60 days).

On the day of assay, the tissue was thawed and centrifuged (30 min

at 40,000 r.p.m.). The pellet was rehomogenized in 10 volumes of 50 mM Tris-citrate buffer (sodium-free), ph 7.2, and centrifuged again. The pellet was washed once more in this buffer solution and then resuspended in roughly 25 volumes of the same buffer containing 200 mM NaCl for assay. Final protein concentration was roughly 1.0 mg/ml.

Plastic 5 ml vials were prepared containing 0.1 ml of H_2O with labelled and unlabelled GABA and 4,6,6-$(CH_3)_3$-caprolactam at 10^{-4}M. Each reaction tube contained 0.3 µCi [G-^{3}H] GABA, final concentration 10 mM. To each tube was added 0.9 ml of the tissue, the mixture was agitated (vortex), then incubated 5 min at 0°C, followed by centrifugation for 15 min at 20,000 r.p.m. at 0°C (50,000 x g). The supernatant was discarded and the tubes were superficially rinsed twice with 2 ml buffer at 0°C to remove H_2O (and unbound labelled ligand) from the sides of the vial. The pellets were not disturbed or washed in this process. The vials were decanted, and set upside-down for 10 min. The entire pellet was then dissolved overnight at room temperature in 0.30 ml Soluene-100 and counted in 5 ml of toluene-PPO. Background trapped ligand was taken as that bound in the presence of excess (1 mM) non-radioactive GABA, and subtracted from each point. Both background and specific binding were of the order of 1,500 c.p.m.

The 4,6,6-$(CH_3)_3$-caprolactam was found to cause a 10% inhibition on the GABA binding to the membrane fractions in the presence of NaCl. However this was not a very significant inhibition.

3. GABA-stimulated Cl^- flux in crayfish muscle

This test-tube assay of GABA post-synaptic response was carried out according to the method of Ticku and Olsen (1977, in press). Abdominal muscles were removed from crayfish (Procambarus clarkii) and placed in Tris-buffered Van Harreveld's solution (VHS: 205 mM NaCl;

5.45 mM KCl; 13.6 mM $CaCl_2$; 2.6 mM $MgCl_2$; 5 mM Tris-HCl, ph 7.7). Small segments of the muscle were dissected and thin muscle strips, each about 0.5-1 cm in length and less than 0.1 cm in thickness (about 15 mg), were cut out, tied with thread on one end, and allowed to equilibrate for 60 min in VHS solution. Tissue was continuously flushed with 5% CO_2 - 95% O_2. All experiments were done at 22 ± 2°C.

Following equilibration, muscle strips were transferred to vials containing 5 ml of VHS containing Cl^- (0.1 µCi/ml) solution, with or without GABA (2×10^{-4}M) and 4,6,6-$(CH_3)_3$-caprolactam (10^{-4}M). The muscle strips (5-8 per experimental condition) were withdrawn from the tracer solution at exactly 15 sec, blotted gently on a filter paper, weighed, and solubilized. Radioactivity was measured as described for the GABA binding assay. Also counted were tissue blanks and 100 µl of the Cl^--VHS incubating medium. Counting efficiency of all samples was greater than 98%. Cl^- space was determined as follows

$$\frac{\text{CPM/Kg wet weight tissue}}{\text{CPM/ml incubation medium}} = \text{ml/Kg wet weight tissue}$$

Effects of the lactam on background and GABA-stimulated chloride uptake were compared to controls by students's t-test. As with the GABA uptake experiment, 4,6,6-$(CH_3)_3$-caprolactam did not have any effect on the GABA-stimulated Cl^- flux in the crayfish muscle.

APPENDIX 9

Journal of Neurochemistry, 1975, Vol. 25. pp. 903–904. Pergamon Press. Printed in Great Britain.

SHORT COMMUNICATION

Inhibition of acetylcholinesterase by bicuculline and related alkaloids

(*Received* 14 *April* 1975. *Accepted* 20 *May* 1975)

THE PHTHALIDE isoquinoline alkaloid bicuculline antagonises the inhibitory action of GABA at certain synapses in the mammalian CNS (CURTIS & JOHNSTON, 1974). There is much evidence that the convulsant action of bicuculline and a number of structurally related alkaloids is directly related to antagonism of GABA-mediated synaptic inhibition. In addition, bicuculline has been suggested to influence cholinergic transmission: systemically administered bicuculline increases the rate of release of acetylcholine from the surface of the cerebral cortex of anaesthetised rats (GARDNER & WEBSTER, 1973), and elevates the acetylcholine content of mouse brain (SVENNEBY & ROBERTS, 1974). The competitive inhibition by bicuculline of mouse brain acetylcholinesterase activity *in vitro*, may be the basis for the above effects of bicuculline on acetylcholine release and content (SVENNEBY & ROBERTS, 1973). In the present study the inhibition of rat brain acetylcholinesterase activity by bicuculline is compared to that by 3 other phthalide isoquinoline alkaloids: the optical enantiomer of bicuculline, (−)-bicuculline, and the pair of enantiomers (+)- and (−)-adlumine (Fig. 1). These alkaloids represent the 4 possible configurations about the $C_{(1)}$–$C_{(9)}$ bond in the phthalide isoquinoline series.

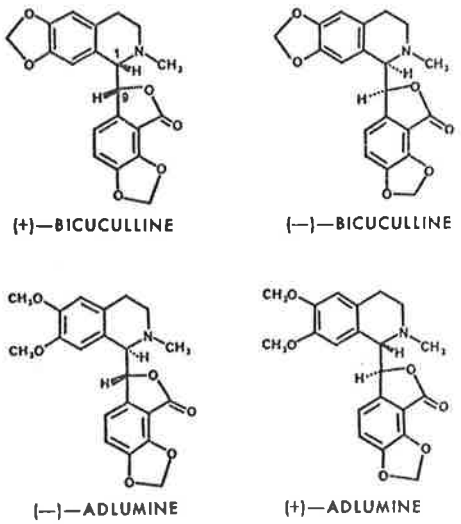


FIG. 1. Structures of the 4 isoquinoline alkaloids tested as inhibitors of acetylcholinesterase activity.

Extracts of rat brain membranes were prepared as described by VARELA (1973), and acetylcholinesterase activity was assayed by the method of ELLMAN *et al.* (1961) using *S*-acetylthiocholine as substrate. Enzymic reactions were followed for 5 min at 25°C measuring absorption at 412 nm in an automatic multi-sample recording spectrophotometer (Varian Techtron model 635 enzyme kinetics system). The alkaloids were tested as freshly prepared solutions of their hydrochlorides, unless stated otherwise, at a final concentration of 100 μM, except for physostigmine which was tested at 0·1 μM. Initial velocities of enzymic hydrolysis of acetylthiocholine in the presence and absence of inhibitors were calculated from the recorded changes in absorbance, and the inhibition parameters computed using the procedures of CLELAND (1963). Chemicals were obtained from the following sources: (+)-bicuculline (Pierce), (+)-bicuculline methochloride (prepared as described by JOHNSTON *et al.*, 1972), (−)-bicuculline (gift from Dr S. Teitel, Nutley, New Jersey), (+)- and (−)-adlumine (gifts from Dr R. H. F. Manske, Guelph, Ontario), physostigmine salicylate (B.D.H.), GABA, *S*-acetylthiocholine iodide and 5,5′-dithiobis-(2-nitrobenzoic acid) (Calbiochem).

All 4 phthalide isoquinoline alkaloids inhibited rat brain acetylcholinesterase activity under the conditions employed, and all except (−)-adlumine acted in a manner consistent with competitive inhibition. The kinetic con-

TABLE 1. INHIBITION OF RAT BRAIN ACETYLCHOLINESTERASE BY SOME PHTHALIDE ISOQUINOLINE ALKALOIDS

Alkaloid	Configuration at $C_{(1)}$, $C_{(9)}$	Type of inhibition	Inhibition constants (μM) K_{is}	K_{ii}
(+)-Bicuculline methochloride	S,R	Comp.	17 ± 2	—
(+)-Bicuculline hydrochloride	S,R	Comp.	70 ± 10	—
(−)-Bicuculline hydrochloride	R,S	Comp.	365 ± 95	—
(−)-Adlumine hydrochloride	R,R	Non-comp.	57 ± 24	75 ± 21
(+)-Adlumine hydrochloride	S,S	Comp.	218 ± 52	—

The alkaloids were tested at 100 μM against rat brain acetylcholinesterase activity assayed using *S*-acetylthiocholine as substrate (30–240 μM). The inhibition constants (K_{is}, the slope inhibition constant; K_{ii} the intercept inhibition constant) were calculated using the procedures of CLELAND (1963), and are means ± S.E.M.

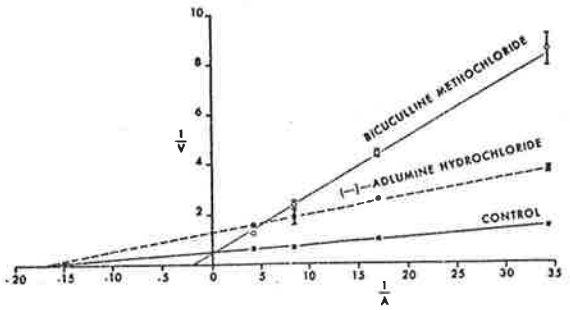


FIG. 2. Double reciprocal plots of the initial velocity of the hydrolysis of *S*-acetylthiocholine (v, μmol.min^{-1}) as a function of the substrate concentration (A, mM). The points are means ± S.E. of 4 experimental values. The lines represent the computed fit (CLELAND, 1963) of the individual experimental values to the equation $v = VA/[K(1 + I/K_{is}) + A]$ for competitive inhibition in the case of bicuculline methochloride, and to the equation $v = VA/[K(1 + I/K_{is}) + A(1 + I/K_{ii})]$ for noncompetitive inhibition in the case of (−)-adlumine hydrochloride. In both cases the inhibitor concentration (I) was 100 μM.

stants for these inhibitions, computed from data of the type illustrated for bicuculline methochloride and (−)-adlumine hydrochloride in Fig. 2, are given in Table 1. Bicuculline methochloride was the most potent inhibitor. The values of the inhibition constants for bicuculline hydrochloride and methochloride are close to those reported (65 and 25 μM respectively) for these compounds as inhibitors of mouse brain acetylcholinesterase (SVENNEBY & ROBERTS, 1973). Compounds with the R-configuration at $C_{(9)}$ were more potent inhibitors than the corresponding compounds with the *S*-configuration. GABA in concentrations up to 1 mM did not influence acetylcholinesterase activity or the inhibition of this enzymic activity by 100 μM bicuculline methochloride. The ultraviolet spectrum of bicuculline methochloride was essentially unchanged during incubation in the presence or absence of the brain enzyme preparation under the standard assay conditions in which *S*-acetylthiocholine and 5,5′-dithio-bis-(2-nitrobenzoic acid) were omitted. Thus bicuculline methochloride appears to be stable under the conditions of measurement and is not a substrate for the rat brain enzyme.

None of the phthalide isoquinoline alkaloids were as effective as physostigmine as an inhibitor of acetylcholinesterase activity. The K_{is} for physostigmine was 0·031 ± 0·006 μM, which is close to the value of 0·06 μM found by AUGUSTINSSON & NACHMANSOHN (1949) for physostigmine as an inhibitor of the enzyme from electric eel. Thus physostigmine is some three orders of magnitude more potent than the phthalide isoquinoline alkaloids as an acetylcholinesterase inhibitor. The latter alkaloids appear to be similar in potency to strychnine, a competitive inhibitor of the rat brain enzyme with a K_{is} of 70 μM (ALID *et al.*, 1974). The relatively weak anticholinesterase activities of bicuculline and strychnine are unlikely to be the basis of the selective effects of these convulsant alkaloids on certain synaptic inhibitions in the CNS (CURTIS *et al.*, 1974; ALID *et al.*, 1974). The effective doses of bicuculline and strychnine administered intravenously to antagonise central synaptic inhibitions would result in concentrations of 0·025–0·5 μM if uniformly distributed in body fluids (CURTIS *et al.*, 1974). Much higher concentrations are needed *in vitro* for inhibition of acetylcholinesterase by bicuculline and strychnine. A lack of association between acetylcholinesterase inhibition and antagonism of GABA-induced depression by bicuculline methochloride has been demonstrated *in vivo* in the spinal cord (CURTIS *et al.*, 1974). In the medial septal nucleus bicuculline methochloride does appear to potentiate excitation induced by acetylcholine as might be expected of an inhibitor of acetylcholinesterase (MILLER & MCLENNAN, 1974) although the effect of this substance on the action of other excitants was not tested. The increased brain levels and rate of release of acetylcholine induced by systemically administered bicuculline (SVENNEBY & ROBERTS, 1974; GARDNER & WEBSTER, 1973) are presumably indirect effects since they can be mimicked by other substances (picrotoxin and leptazol respectively) which are not inhibitors of acetylcholinesterase. It may be relevant, however, that a number of potent anticholinesterases, including physostigmine, increase brain levels of GABA (DATTA, 1968), and that (+)-tubocurarine, an acetylcholine antagonist at the neuromuscular junction, can act as a GABA antagonist in the brain (HILL *et al.*, 1972). GABA and acetylcholine have a common structural element (N—CH_2—CH_2—*X*—C=O; *X* = CH_2 in GABA and O in acetylcholine) which may mean that there are common structural features in the active sites for GABA and acetylcholine on metabolising enzymes or receptors. This may explain the overlapping effects of various drugs on the GABA and acetylcholine systems.

Department of Pharmacology, Australian National University, Canberra, A.C.T., Australia

ELLEN BREUKER*
G. A. R. JOHNSTON

REFERENCES

ALID G., VALDES L. F. & ORREGO F. J. (1974) *Experientia* **30**, 266–268.

AUGUSTINSSON K.-B. & NACHMANSOHN D. (1949) *J. biol. Chem.* **179**, 543–559.

CLELAND W. W. (1963) *Biochim. biophys. Acta.* **67**, 104–137.

CURTIS D. R. & JOHNSTON G. A. R. (1974) *Ergebn. Physiol.* **69**, 97–188.

CURTIS D. R., JOHNSTON G. A. R., GAME C. J. A. & MCCULLOCH R. M. (1974) *J. Neurochem.* **23**, 605–606.

DATTA C. (1968) *Indian J. exp. Biol.* **6**, 88–89.

ELLMAN G. L., COURTNEY K. D., ANDRES V. & FEATHERSTONE R. M. (1961) *Biochem. Pharmac.* **7**, 88–95.

GARDNER C. R. & WEBSTER R. A. (1973) *Br. J. Pharmac.* **47**, 652P.

HILL R. G., SIMMONDS M. A. & STRAUGHAN D. W. (1972) *Nature, Lond.* **240**, 51–52.

JOHNSTON G. A. R., BEART P. M., CURTIS D. R., GAME C. J. A., MCCULLOCH R. M. & MACLACHLAN R. M. (1972) *Nature, New Biol.* **240**, 219–220.

MILLER J. J. & MCLENNAN H. (1974) *Neuropharmacology* **13**, 785–787.

SVENNEBY G. & ROBERTS E. (1973) *J. Neurochem.* **21**, 1025–1026.

SVENNEBY G. & ROBERTS E. (1974) *J. Neurochem.* **23**, 275–277.

VARELA J. M. (1973) *Experientia* **29**, 1347–1349.

* Present address: Department of Human Physiology and Pharmacology, University of Adelaide, Adelaide, South Australia.

APPENDIX 10

Brain Research, 110 (1976) 413–416

Antagonism of GABA-mediated inhibition in the central nervous system by caprolactam derivatives

D. I. B. KERR, BARBARA J. DENNIS, ELISABETH L. M. BREUKER, R. H. PRAGER, A. D. WARD AND THACH DUONG

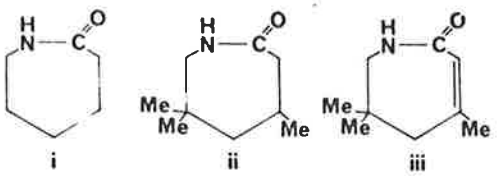
Department of Human Physiology and Pharmacology, and Department of Organic Chemistry, University of Adelaide, Adelaide 5001 (Australia)

(Accepted March 18th, 1976)

Caprolactam enters the CNS relatively rapidly to have a marked convulsant action when given by the intravenous route, although only at rather high dose levels ($\simeq$600 mg/kg)[6]. Lactams obtained from ω-amino acids of a longer chain length have a greater lipid solubility and are even more effective convulsants, as are their corre sponding thio-derivatives[6]. However, neither caprolactam nor the higher GABA homologues such as ω-amino-caprylic acid have been shown to produce any significant activity upon direct application to spinal cord neurones, except for the slight depressant action[1] of ε-amino-caproic acid, from which caprolactam is derived by ring closure. Yet these amino acids, and caprolactam itself, actively block 'hyperpolarising dendritic potentials' and elicit local convulsive discharge upon topical application to the cerebral cortex[10]. In retrospect, it seemed most likely that such actions implicate these compounds in blockade of some form of CNS inhibition, rather than their convulsant properties resulting from direct excitation of CNS neurones. Furthermore, it also seemed most likely that this inhibition would be GABA-mediated, since this predominates over glycinergic inhibition in higher CNS centres[3]. That GABA-mediated inhibition is indeed antagonised by caprolactam and by some of its simple derivatives has now been demonstrated, using the strionigral pathway where GABA is the recognised transmitter readily blocked by the GABA antagonist picrotoxin[9].

Using convulsant activity for preliminary screening, we have synthesised a number of highly active lactams having more nearly optimal log P (partition-coefficient) characteristics[6] than the parent caprolactam (i). The most effective convulsants so far found in this series have alkyl substituents on carbons 4 and 6 of the ring. Thus 4,6,6-trimethyl-caprolactam (ii) and the related Δ3 cyclic amide 4,6,6-trimethyl-Δ3-caprolactam (iii) are potent convulsants with a CD_{50} of 4–6 mg/kg, representing some 100-fold increase in convulsant activity over the parent caprolactam. Thiocaprolactam, where sulphur replaces oxygen in (i), is also an effective convulsant[6].

414



(i) caprolactam: hexahydro-2H-azepin-2-one

(ii) 4,6,6-trimethyl-caprolactam: hexahydro-4,6,6-trimethyl-2H-azepin-2-one

(iii) 4,6,6-trimethyl-Δ3-caprolactam: 1,5,6,7-tetrahydro-4,6,6-trimethyl-2H-azepin-2-one.

Positive field potentials (SN-positivities) evoked in the substantia nigra (SN) by striopallidal (SP) stimulation were blocked following administration of picrotoxin or the lactams (Fig. 1). Conventional monopolar recording from the substantia nigra has been used, responses being evoked with bipolar stimulation of the striopallidum at 3/sec in rabbits under urethane anaesthesia (400 mg/kg). All electrode positions were subsequently checked on Nissl-stained sections cut in the Horsley–Clarke plane. It proved best to average the positive field responses with a Mnemotron CAT 400C, writing out on an X–Y plotter, because convulsant activity generally confused the interpretation of any oscilloscope record during the period of anti-GABA activity. Onset of the blockade of the SN-positivity by intravenous picrotoxin at convulsant doses was often relatively slow (up to 10 min), and the positivity was affected over a period of one or more hours, particularly where cumulative doses had been given. Episodic seizure activity, manifest as muscular twitching, together with large-amplitude waves superimposed on the SN recording, was invariably present whenever the SN-positive response was depressed by picrotoxin. In testing for GABA antagonism, the SN response was averaged only at the onset of each such seizure episode, for each

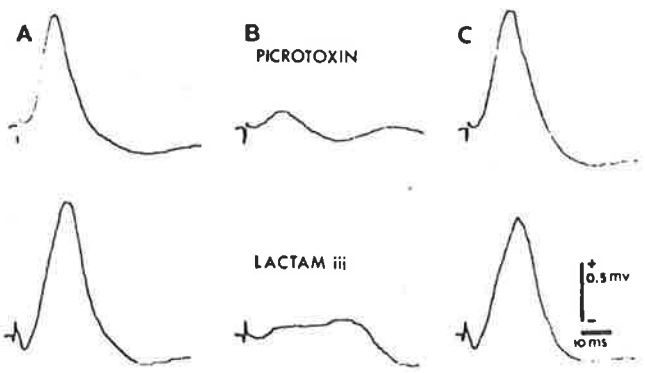


Fig. 1. Picrotoxin and caprolactam derivative (iii) block the positive response evoked in the substantia nigra by striopallidal stimulation in rabbits under urethane anaesthesia. In all records 36 responses were averaged at 3/sec. Upper: A, control; B, during the action of picrotoxin 2 mg kg i.v.; C, recovery 1 h later. Lower: A, control; B, during the action of caprolactam derivative (iii) 6 mg kg i.v.; C, recovery 20 min later. The late positivity, indicating that postictal depression was absent, can be seen in the records taken during the action of the drugs.

episode was followed by a period of postictal depression, during which time no significant potentials could be recorded from SN in response to SP stimulation. These precautions were not mentioned by Precht and Yoshida, although they may be inferred from their similar use of averaged records to demonstrate their blockade effects[9]. It was also sometimes convenient to use records which not only showed the SN-positivity being depressed, but which also showed the development of a later positive component, traceable to field spread from the adjacent reticular formation and thus obviously not obtained during postictal depression.

On the basis of this experience with picrotoxin, the tests were extended to the lactams. Again, demonstration of GABA antagonism was considered reliable only if blockade of the SN-positivity was seen when postictal depression was definitely absent. Under these conditions intravenous doses of the lactams including thiocaprolactam and the 4,6 substituted derivatives (ii) and (iii), at a dose level of 6 mg/kg, also produced significant blockade of the SN-positivity (Fig. 1). A similar GABA antagonism can be inferred for each of the convulsant lactams in the present series and for the higher homologues[4] of larger ring size. In general, these compounds, and more particularly thiocaprolactam (36 mg/kg), precipitated seizures and blockade of the SN-positivity with a surprisingly short delay (little more than one circulation time), and were active against the test potential for relatively short periods (10–15 min on average). Effective repeated dosage was thus possible, and in this regard the compounds are potentially more convenient than picrotoxin[3] for the repeated challenging of GABA in the CNS by the intravenous route, where their relative aqueous solubility at neutral pH is an added advantage. (One ml of a saturated solution of picrotoxin at 25 °C contains 2.8 mg, as against 26 mg for lactam (ii) and 41 mg for lactam (iii).)

Apart from bicuculline methochloride, which remains the most effective water-soluble GABA antagonist for iontophoretic experiments[3], all the presently used GABA antagonists have disadvantages in their use, mainly due to poor aqueous solubility. Tetramine (tetramethylenedisulphotetramine), originally proposed as a GABA antagonist by this laboratory (see ref. 3, p. 113), while an extremely powerful convulsant and effective GABA antagonist[1], is relatively non-specific[2,5] besides being virtually insoluble in water, which greatly limits its use. Bicuculline, too, is difficult to dissolve except at low pH, where it undergoes inactivation more rapidly than is generally supposed, presumably by hydrolytic conversion to bicucine as happens at physiological pH[8]. Picrotoxin is poorly water soluble and cannot be applied reliably by iontophoresis due to lack of any charge on the molecule. Modification to include a charged centre on the lactams would thus be highly desirable, since, unfortunately, the present lactams also carry little effective nett charge and are, apart from having greater solubility, no better than picrotoxin for microiontophoresis.

GABA antagonism by lactams, having resonant NH-CO groups, raises certain questions concerning both the nature of the GABA receptor and the active conformation of the GABA molecule. An 'extended' rather than 'folded' GABA has become favoured as the preferred active conformation[7] on the basis of bicuculline sensitivity. The caprolactams lack any obvious resemblance to 'extended' GABA, and it is suggested that 'folded' GABA receptors may well be concerned in some GABA-mediated in-

hibitions of the higher CNS. The relationship of the GABA antagonists picrotoxinin and tetramine to an 'extended' GABA receptor is also not immediately clear, although Smythies has suggested[11] how these compounds might attach to a GABA receptor.

While the similarity of action of picrotoxin and the lactams in depressing strio-nigral responses has been relied upon for demonstrating GABA antagonism, such depression could well result from transmitter store depletion, as has been suggested for pilocarpine[12], or block of GABA release, for which there is some evidence (Breuker and Johnston, unpublished). Clearly, the lactams should be tested for antagonism of other inhibitory transmitters as well as for antagonist activity on a wider range of systems; particularly with applied GABA to overcome any problem due to a block of GABA release. So far, preliminary results on the isolated toad spinal cord (McNally and Kerr, unpublished) have shown GABA-induced depolarization of dorsal root fibres to be reduced by lactam (ii), further indicating that the caprolactams are indeed GABA antagonists.

A preliminary account of this work has been presented at the 23rd Meeting of the Australian Physiological and Pharmacological Society, May 1975.

We thank Gordon Frith, who synthesized a number of the compounds.

Supported by a Grant-in-Aid No. C72/15060 from the Australian Research Grants Commission.

1 Bowery, N. G., Brown, D. A., and Collins, J. F., Tetramethylenedisulphotetramine: an inhibitor of γ-aminobutyric acid-induced depolarization of the isolated superior cervical ganglion of the rat, *Brit. J. Pharmacol.*, 53 (1975) 422–424.

2 Collins, J. F., Hill, R. G., and Roberts, F., A study of tetramethylenedisulphotetramine (TETS) and related compounds as antagonists of presynaptic inhibition and microiontophoretically applied γ-aminobutyric acid (GABA) and glycine in the rat cuneate nucleus, *Brit. J. Pharmacol.*, 54 (1975) 239P–240P.

3 Curtis, D. R., and Johnston, G. A. R., Amino acid transmitters in the mammalian central nervous system, *Ergebn. Physiol.*, 69 (1974) 97–188.

4 Curtis, D. R., and Watkins, J. C., The excitation and depression of spinal neurones by structurally-related amino acids, *J. Neurochem.*, 6 (1960) 117–141.

5 Dray, A., Tetramethylenedisulphotetramine and amino acid inhibition in the rat brain, *Neuropharmacology*, 14 (1975) 703–705.

6 Elison, C., Lien, E. J., Zinger, A. P., Hussain, M., Tong, G. L., and Golden, M., CNS activities of lactam derivatives, *J. pharm. Sci.*, 60 (1971) 1058–1062.

7 Johnston, G. A. R., Curtis, D. R., Beart, P. M., Game, C. J. A., McCulloch, R. M., and Twitchin, G., *Cis*- and *trans*-4-amino-crotonic acid as GABA analogues of restricted conformation, *J. Neurochem.*, 24 (1975) 157–160.

8 Olsen, R. W., Ban, M., Miller, T., and Johnston, G. A. R., Chemical instability of the GABA antagonist bicuculline under physiological conditions, *Brain Research*, 98 (1975) 383–387.

9 Precht, W., and Yoshida, M., Blockage of caudate-evoked inhibition of neurones in the substantia nigra by picrotoxin, *Brain Research*, 32 (1971) 229–233.

10 Purpura, D. P., Girado, M., Smith, T. G., Callan, D. A., and Grundfest, H., Structure activity determinants of pharmacological effects of amino acids and related compounds on central synapses, *J. Neurochem.*, 3 (1959) 238–268.

11 Smythies, J. R., Relationships between the chemical structure and biological activity of convulsants, *Ann. Rev. Pharmacol.*, 14 (1974) 9–22.

12 Turkanis, S. A., and Esplin, D. W., Evidence for the release and depletion of the postsynaptic inhibitory transmitter by pilocarpine, *Arch. Int. Pharmacodyn.*, 173 (1968) 195–200.

BIBLIOGRAPHY

ALBERT, A. (1975): "The Selectivity of Drugs". Outline Studies in Biology. Chapman and Hall, London.

BOWERY, N.G. and BROWN, D.A. (1974): Depolarizing actions of α-aminobutyric acid and related compounds on rat superior cervical ganglia *in vitro*. *Br. J. Pharmac.*, 50, 205-218.

BRODIE, B.B. and HOGBEN, C.A.M. (1957): Some physico-chemical factors in drug action. *J. Pharm. Pharmac.*, 9, 345-380.

CURTIS, D.R. and JOHNSTON, G.A.R. (1974): Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, 69, 97-188.

CURTIS, D.R. and WATKINS, J.C. (1960): The excitation and depression of spinal neurones by structurally related amino acids. *J. Neurochem.*, 6, 117-141.

ELISON, C., LIEN, E.J., ZINGER, A.P., HUSSAIN, M., TONG, G.L. and GOLDEN, M. (1971): CNS activities of lactam derivatives. *J. Pharmaceut. Sci.*, 60, 1058-1062.

ENNA, S.J. and SNYDER, S.H. (1975): Properties of α-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. *Brain Res.*, 100, 81-97.

GOLDBLATT, M.W., FARQUHARSON, M.E., BENNETT, G. and ASKEW, B.M. (1954): ε-caprolactam. *Br. J. Industr. Med.*, 11, 1-10.

HANSCH, C. and ANDERSON, S.M. (1967): The structure-activity relationship in barbiturates and its similarity to that in other narcotics. *J. Med. Chem.*, 10, 745-753.

HANSCH, C., STEWARD, A.R., ANDERSON, S.M. and BENTLEY, D. (1967): The parabolic dependence of drug action upon lipophilic character as revealed by a study of hypnotics. *J. Med. Chem.*, 11, 1-11.

JAMES, K.C. (1974): "Linear Free Energy Relationships and Biological Action." IN *"Progress in Med. Chem."* 10, p 205, ed. Ellis, G.P. and Bluet, G.

KERR, D.I.B., DENNIS, B.J., BREUKER, E.L.M., PRAGER, R.H., WARD, A.D. and DUONG, T. (1976): Antagonism of GABA-mediated inhibition in the central nervous system by caprolactam derivatives. *Brain Res.*, 110, 413-416.

LIEN, E.J. and KUMLER, W.D. (1967): Dipole moments and pharmacological activity of cyclic ureas, cyclic thioureas, and the N,N'-dimethylated compounds. *J. Med. Chem.*, 11, 214-219.

LIEN, E.J., LIEN, L.L. and TONG, G.L. (1971): Central nervous system activities of thiolactams and lactams. Structure-activity correlations. *J. Med. Chem.*, 14, 846-848.

LITCHFIELD, J.T. (Jr.) and WILCOXON, F. (1949): A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, 96, 99-113.

LOCKETT, M.F. and DAVIS, M.M. (1958): The analgesic action of normorphine administered intracisternally to mice. *J. Pharm. Pharmac.*, 10, 80-85.

OLSEN, R.W., BAYLESS, J.D. and BAN, M. (1975): Potency of inhibitors of α-aminobutyric acid uptake by mouse brain subcellular particles at 0°C. *Mol. Pharmac.*, 11, 558-565.

OLSEN, R.W. and GREENLEE, D. (1976): Binding of gamma-aminobutyric acid to rat brain, synaptic plasma membranes. *Fed. Proc.*, 35, 1647.

PURCELL, W.P., BASS, G.E. and CLAYTON, J.M. (1973): "Strategy of Drug Design: A Guide to Biological Activity". J. Wiley & Sons, New York.

PURPURA, D.P., GIRADO, M., SMITH, T.G., CALLAN, D.A. and GRUNDFEST, H. (1959): Structure-activity determinants of pharmacological effects of amino acids and related compounds in central synapses. *J. Neurochem.*, 3, 238-268.

SCHILD, H.O. (1947): The use of drug antagonists for the identification and classification of drugs. *Br. J. Pharmac.*, 2, 251-258.

SHULMAN, A. and LAYCOCK, G.M. (1967): Action of central nervous system stimulant and depressant drugs in the intact animal. Part 1. Titration of analeptics by pentobarbitone sodium and hydroxydione. *E. J. Pharmac.*, 1, 282-294.

SHULMAN, A. and LAYCOCK, G.M. (1967): Action of central nervous system stimulant and depressant drugs in the intact animal. Part 2. Titration of analeptics by amylobarbitone sodium. *E. J. Pharmac.*, 1, 295-309.

SMYTHIES, J.R. (1974): Relationships between the chemical structure and biological activity of convulsants. *Ann. Rev. Pharmac.*, 4, 9-22.

TAFT, R.W. (1956): "Separation of Polar, Steric, and Resonance Effects in Reactivity." IN *"Steric Effects in Organic Chemistry."* ed. Newman, M.S., J. Wiley & Sons, New York.

TICKU, M.K. and OLSEN, R.W. (1977): α-aminobutyric acid-stimulated chloride permeability in crayfish muscle. *Biochem. Biophys. Acta*, in press.

VAN ROSSUM, J.M. (1963): The relation between chemical structure and biological activity. *J. Pharm. Pharmac.*, 15, 285-316.

ZUKIN, S.R., YOUNG, A.B. and SNYDER, S.H. (1974): Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. nat. Acad. Sci. (Wash.)*, 71, 4802-4807.