Amphibian Neuropeptides:
Isolation, Sequence Determination and Bioactivity

A Thesis submitted
for the
Degree of Doctor of Philosophy
in the
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by

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Chapter 4- Antimalarial Activity of Anuran Peptides

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Chapter 6- Eugenin- a Marsupial Neuropeptide

Abstract

The skin extracts from amphibians have been investigated for over fifty years and have been found to contain numerous components with therapeutic and medicinal uses. Host-defence compounds are secreted onto the dorsal surface of the animal from specialised granular glands in response to a variety of stimuli, such as stress induced by a predator. Isolated peptides can exhibit either pharmacological properties or antibiotic activity.

Previous studies isolated a potent hypotensive neuropeptide, crinia angiotensin II, within skin secretions of the Australian frog *Crinia georgiana*. This prompted further investigations into the isolation and sequence determination of host-defence compounds from other species in this genus- *C. signifera*, *C. riparia* and *C. deserticola*. Fifteen novel peptides were identified. The major peptide components were potent disulfide containing neuropeptides of a type not observed in other Australian anurans that have been previously investigated. The remaining peptides demonstrate either antibiotic activity or inhibit the enzyme neuronal nitric oxide synthase.

The skin components from anurans of the *Litoria* genus have been extensively studied, with a number of peptides exhibiting both antibacterial and pharmacological activity. The skin secretion of *Litoria dentata* has been investigated, with five novel peptides identified. The neuropeptide tryptophyllin L 1.3 was previously isolated from the related frog *L. rubella*. Other components that are unique in structure have not yet been tested for biological activity.

The parasitic disease malaria is responsible for over one million deaths per year. The increase in resistance of current antimalarial compounds has led to the development of new treatments from various animal-derived peptide antimicrobials. A number of amphibian peptides and their derivatives were investigated as potential antiplasmodial agents against the malaria parasite *Plasmodium falciparum*. Results indicate that these compounds inhibit parasite growth with minimal haemolytic activity, making them promising tools for malaria research.

The defence chemistry of amphibian neuropeptides has been extensively studied and is important in understanding both the ecology and physiology of the vertebrate. Neuropeptides are classified into groups with similar structural characteristics. Biological activity occurs via interaction with a G protein-coupled receptor. The most studied of all amphibian neuropeptides is caerulein, which has a similar spectrum of activity to the mammalian peptide cholecystokinin. This includes smooth muscle contraction that occurs via interaction with cholecystokinin receptors.

The pharmacological activity of Australian anuran neuropeptides from various genera was investigated. Two biological assays were conducted- a smooth muscle contraction test and a lymphocyte proliferation assay. A range of neuropeptides contracted smooth muscle at nanomolar concentrations, while others only proliferated lymphocytes. Some peptides were inactive in both assays.

Young marsupials are born at an immature stage of development and rely on immune protection provided by the mother. Eugenin is a host-defence compound isolated from pouch secretions of the Tammar wallaby. The immunomodulator activates CCK_2 receptors, resulting in lymphocyte proliferation. Therefore, eugenin stimulates immune cells in the pouch providing vital immune protection for pouch young.

Statement of Originality

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

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

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Vita Marie Maselli Date

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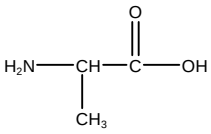
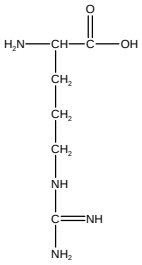
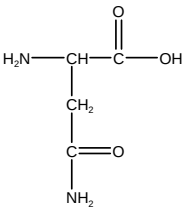
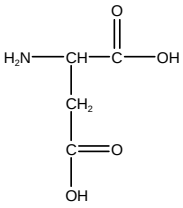
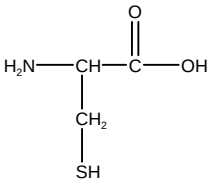
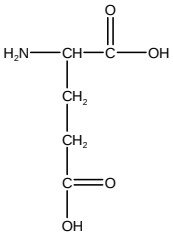
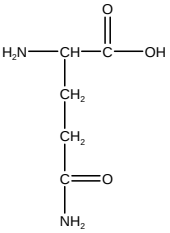
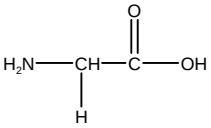
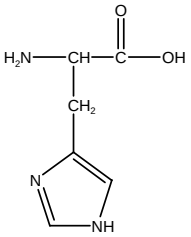
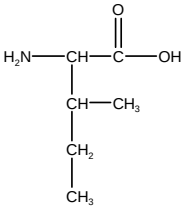
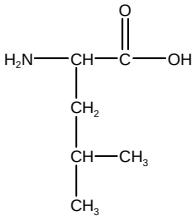
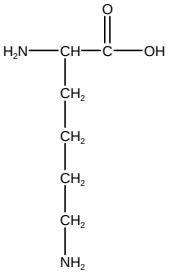
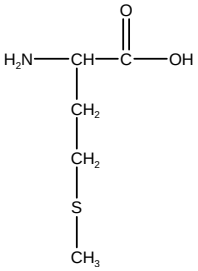
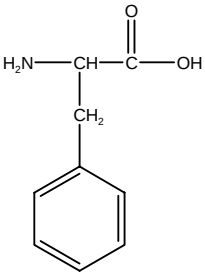
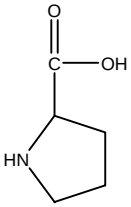
The 20 Common Amino Acids

Alanine Ala (A)	**Arginine** Arg (R)	**Asparagine** Asn (N)	**Aspartate** Asp (D)	**Cysteine** Cys (C)
				
Glutamate Glu (E)	**Glutamine** Gln (Q)	**Glycine** Gly (G)	**Histidine** His (H)	**Isoleucine** Ile (I)
				
Leucine Leu (L)	**Lysine** Lys (K)	**Methionine** Met (M)	**Phenylalanine** Phe (F)	**Proline** Pro (P)
				
Serine Ser (S)	**Threonine** Thr (T)	**Tryptophan** Trp (W)	**Tyrosine** Tyr (Y)	**Valine** Val (V)