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Reviews

Peptides related to vasopressin in invertebrates[1]

by M. Mühlethaler[2], M. Raggenbass and J.J. Dreifuss[3]

Department of Physiology, University Medical Center, CH–1211 Geneva 4 (Switzerland)

Summary. Compounds chemically related to the vertebrate neurohypophysial peptides, vasopressin and neurophysin, have been detected in recent years in the brain and in ganglia of invertebrates. Most data acquired so far have been obtained in insects and in molluscs. Evidence suggesting that these compounds might exert neurohormonal and neurotransmitter functions in these species is reviewed.

1. Introduction

The amino acid sequence of the neurohypophysial hormones of mammals and many other vertebrates was established many years ago. These compounds are cyclic nonapeptides differing only by amino acid substitutions in position 2, 3, 4 and 8. The cyclic structure is due to formation of a disulfide bridge between cysteine residues 1 and 6. There are at present 11 known types of vasopressin-like and oxytocin-like peptides (table). In most vertebrate species oxytocin-like hormones play a role in reproduction whereas vasopressin-like hormones are involved with hydromineral balance. The primary structure of the contemporary neurohypophysial hormones suggests that they have evolved from a single common ancestor[51].

It has recently become apparent that oxytocin and vasopressin, in addition to being secreted into the general circulation, are also carried to various areas within the central nervous system of vertebrates, where they might act as neurotransmitters/neuromodulators[25, 50]. Similar peptides are also present in the nervous system of invertebrates (for review, see Greenberg and Prince[33]). Two complementary approaches have led to the conception that these peptides are of importance to invertebrates: a) neuropeptides have been isolated and characterized in extracts from invertebrate ganglia and b) it has been shown that antibodies directed against neuropeptides found in vertebrates recognize compounds manufactured by invertebrate neurones[58]. Although the study of vasopressin-like peptides in invertebrates is still at an early stage, recent data support the view that such molecules could be present throughout the invertebrate phyla. Studies have been mainly undertaken so far in molluscs and arthropods.

Naturally-occurring neurohypophysial hormones

Hormone	Cys-Www-Xxx-Yyy-Asn-Cys-Pro-Zzz-GlyNH$_2$								
	1	2	3	4	5	6	7	8	9
Arg-vasopressin		Tyr	Phe	Gln				Arg	
Lys-vasopressin		Tyr	Phe	Gln				Lys	
Phenypressin		Phe	Phe	Gln				Arg	
Oxytocin		Tyr	Ile	Gln				Leu	
Arg-vasotocin		Tyr	Ile	Gln				Arg	
Lys-vasotocin		Tyr	Ile	Gln				Lys	
Mesotocin		Tyr	Ile	Gln				Ile	
Valitocin		Tyr	Ile	Gln				Val	
Ichthyotocin		Tyr	Ile	Ser				Ile	
Glumitocin		Tyr	Ile	Ser				Gln	
Aspargtocin		Tyr	Ile	Asn				Leu	

2. Vasopressin-like peptides in insects

Studies on the identification and roles in insects of peptides related to vasopressin have been undertaken in several species of orthoptera, in particular in cockroaches, silkworms and locusts. In these species neuroendocrine cells implicated in water balance are located in the brain and in ganglia of the ventral chain. The link between these two regions has been clarified and recently the existence of an endogenous compound involved in water regulation and structurally related to vasopressin has been demonstrated.

Cerebral centers controlling water-balance

In the early 1950's, Stutinsky[69–71] demonstrated the presence in the pars intercerebralis of several insects of a cluster of Gomori-positive endocrine neurones emitting an axon running towards the corpora cardiaca, and it was postulated that they might serve as a storage site for neurosecretory products (fig. 1). The morphological similarities of this structure to the hypothalamo-neurohypophysial system in vertebrates led Stutinsky to propose that it could participate in osmoregulation in insects, a suggestion also made concomitantly by Hanström[36]. It should be added that neurones of the pars intercerebralis not only project towards the corpora cardiaca, but also to another neurohaemal organ located on the medial ventral surface of the brain (fig. 1, NA). In the orthopter, *Locusta migratoria,* Cazal and Girardie[15] demonstrated in 1968 that electrocoagulation of a region containing the pars intercerebralis induces water

retention. In later work, Girardie[31] found that in *Locusta migratoria* and *Schistocerca gregaria* water retention was in fact caused by destruction of the median sub-ocellar centre which is situated ventrally to the pars intercerebralis and which contains 4–6 large neurosecretory neurones. A similar group of neurosecretory cells is also present in other orthoptera[13, 19, 24, 30].

Presence of vasopressin-like peptides in the ventral chain of insects

For many years neurosecretion related to water balance in insects was only studied at the level of the brain. However, neurosecretory cells are also found in the ventral chain[16–18, 23, 29, 58]. Using specific antibodies raised against vasopressin, vasotocin or neurophysins, Rémy and coworkers[14, 32, 60, 61] discovered 2 large immunoreactive neurones in the sub-oesophagial ganglion of silkworms and locusts (fig. 2). Similar results were reported by Strambi and coworkers in *Acheta, Periplaneta, Blaberus, Gryllus* and *Polistes*[67, 68]. A vasopressin-like compound was also detected in the haemolymph of *Acheta domestica*[67]. The vasopressin-like, diuretic neuropeptide found in *Locusta migratoria* differs from vasopressin in its larger molecular weight and more acidic isoelectric point; at its carboxyl terminal it possesses, however, the same tetrapeptide as arginine-vasopressin[22]. Some authors claim that the vasopressin-related peptides are manufactured only within 2 neurones of the sub-oesophagial ganglion and are absent from neurosecretory neurones of the pars intercerebralis and the median sub-ocellar centre, but this is still controversial. Friedal et al.[26] showed the presence of a neurophysin-like peptide in the A-cells of the pars intercerebralis and in the axons leading from these nerve cells to the corpora cardiaca, from where it is released[27]. Amino acid analysis pointed to a similarity in size and composition with pituitary neurophysin of vertebrates.

The link between the brain and the neurosecretory neurones of the ventral chain

In early studies using electrocoagulation, the respective roles in water balance of the pars intercerebralis and of the median sub-ocellar neurosecretory cells were not differentiated. More recently, the pars intercerebralis was selectively destroyed in migratory locusts and an increase in water elimination ensued which was accompanied by an increased level of the vasopressin-like compound in the hemolymph; in contrast, destruction of the median sub-ocellar region caused water retention and the vasopressin-like compound became undetectable in the hemolymph[52, 53]. These data led to the conjecture that the peptide synthesized in the sub-oesophagial ganglion exerts a diuretic action and that its release is under positive control from the median sub-ocellar region and under negative control from the pars intercerebralis[54]. This suggestion is also supported by stimulation experiments; electrical stimulation of the sub-ocellar region yielded an increase in the level of the vasopressin-like compound in the hemolymph, whereas stimulation of the pars intercerebralis caused a decrease[53]. In addition, Proux and coworkers[55] showed that extracts of the sub-oesophagial ganglion exert a direct diuretic action whereas extracts from the corpora cardiaca apparently act indirectly. Since nervous connections which link cerebral areas to the sub-oesophagial neurosecretory neurones have not been unambiguously demonstrated it has been suggested that secretion of the vasopressin-like diuretic compound may be under hormonal control, but this remains an open question.

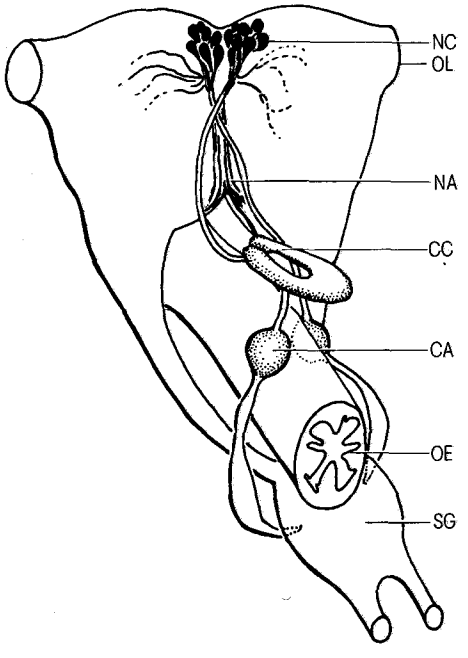

Figure 1 Stereogramm of the nervous system of *Acheta domesticus* showing location of the neurosecretory cells (NC) of the pars intercerebralis, their fiber tracts and the principal areas they innervate: CA, corpus allatum; CC, corpus cardiacum; NA, cerebral neurohaemal area. Other abbreviations: OE, oesophagus; OL, optic lobe; SG, sub-oesophagial ganglion. Another group of neurosecretory cells is located in the median sub-ocellar centre, i.e. between NC and NA. Adapted from Geldiay and Edwards[30].

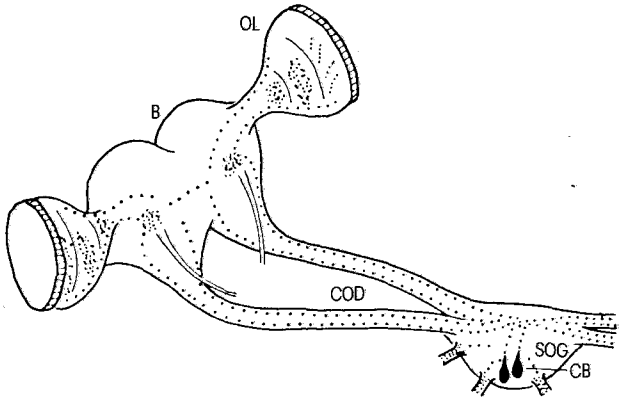

Figure 2. Stereogram of brain and sub-oesophagial ganglion (SOG) of *Locusta migratoria* showing the location of the 2 cell bodies (CB) containing vasopressin/neurophysin-like immunorectivity and their axons coursing along the circum-oesophagial connective (COD) towards the brain (B) and optic lobes (OL). Adapted from Rémy and Girardie[59].

3. *Vasopressin-like peptides in molluscs*

The existence of large nerve cells exhibiting neurosecretory properties was already reported in the 1930's in arthropods, molluscs and worms[63]. Neurosecretory cells were observed for example in the sea hare, *Aplysia,* an observation later confirmed by several groups[20,21,28,62].

Water regulation in Aplysia

Using intracellular recording, a study was made of the response of large neurones located in the abdominal ganglion of *Aplysia* to a change in osmolarity of the fluid bathing the osphradium. This sensory organ is located at the base of the siphon; it is connected to the osphradial ganglion, which is in turn linked through the branchial nerve to the abdominal ganglion. Stinnakre and Tauc[65] showed that a decrease in the osmolarity of the fluid bathing the osphradium leads to a membrane hyperpolarization, a decrease in spontaneous action potential activity and an increase in membrane conductance in nerve cells of the abdominal ganglion. In a later study it was found that this effect was restricted to only one neurone in the ganglion, cell R15[66]. Similar results were also reported by Jahan-Parwar and coworkers[38] and recently confirmed once more by Bablanian and Treistman[4].

These data are of interest in view of reports showing that cell R15 does synthesize, process and transport peptides of low molecular weight[44,45]. Some of these peptides might play a role in water regulation; for example, when Kupfermann and Weiss[39] injected *Aplysias* with an extract obtained from cell R15, they observed a 3–10% weight gain due to water retention. However, they were unable to determine the molecular weight of the active peptides and could not exclude the possibility that several active compounds were present in the extract.

Physiological actions of vertebrate neurohypophysial peptides in ganglia of molluscs

The actions of vertebrate neurohypophysial peptides have been mainly assessed on 2 large molluscan neurones: cell R15 in *Aplysia californica* and cell 11 in the land snail, *Otala lactea.* Both neurones display at times intermittent bursts of action potentials triggered by slow membrane oscillations.

In the dormant *Otala,* cell 11 is either silent or displays spontaneous activity consisting of a beating pattern of spikes occurring at low frequency. Vasopressin at 10^{-9} M–10^{-6} M initiates bursting in a slowly reversible and dose-dependent way[7]. The whole sequence of the vasopressin molecule is needed, since neither the cyclic part alone nor the linear part alone nor vasopressin deprived of its glycinamide terminal were active. On the other hand, oxytocin and arginine-vasotocin were found to be as potent as vasopressin[8]. Iontophoretic studies indicated that responsiveness to the peptide was confined to the axon and its hillock. Similar excitatory effects were found by the same authors on cell R15 in *Aplysia*[8] and by others in the land snail, *Helix pomatia*[41-43]. In the giant African snail, *Achatina fulica,* Takeuchi and coworkers[72,73] described excitatory effects induced by an oxytocin analogue and by a vasotocin analogue. A structural analogue of arginine-vasopressin, deamino-dicarba-vasopressin, which is a rather selective antidiuretic agonist in mammals, was inactive even at high concentrations, suggesting that the receptor located on snail neurones differs from the vasopressin receptor present on kidney cells in mammals.

Further investigating the electrophysiological behavior of cell 11 in *Otala* under voltage clamp, Barker and Smith[9] found that a voltage step produced a non-spike current which had the following properties: a) it was directed inwardly and was linearly proportional to voltages imposed in the hyperpolarizing direction; b) in the depolarizing direction, there was a negligibly small outward current when the membrane potential was moderately shifted; with stronger depolarization, a voltage-dependent, outward current became apparent. In the presence of vasopressin, the small depolarizing step caused an inward current, which showed a marked voltage-dependency and whose magnitude was determined by the extracellular sodium concentration. The authors concluded that vasopressin induces bursting activity by enhancing a voltage-dependent sodium current, although they could not exclude an additional effect mediated by potassium. These membrane effects of vertebrate neurohypophysial peptides on neurones in molluscs have been extensively reviewed[5,6,10,11].

Levitan and coworkers[42] addressed the question of the nature of the second messengers mediating these effects. They found that vasopressin and oxytocin, as well as ganglionic extracts, were able to increase the level of cyclic nucleotides in *Aplysia* and *Helix* neurones. They also observed that bursting pacemaker potentials could be elicited in cell R15 of *Aplysia* and in cell F-1 of *Helix* by substances known to increase the intracellular levels of cAMP and cGMP (fig. 3). Neither cAMP alone, nor cGMP alone, was able to induce oscillatory activity. Therefore they proposed that the vasopressin-like com-

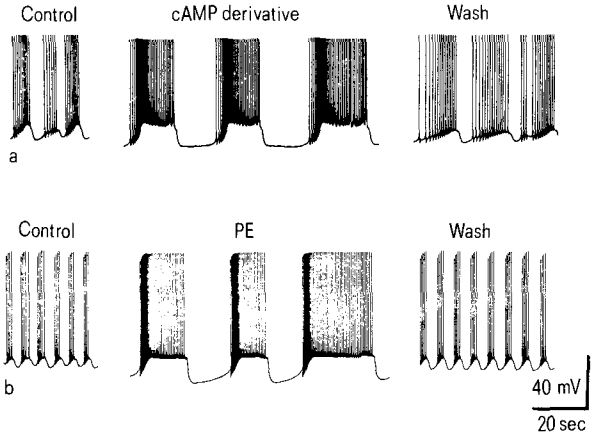

Figure 3. Similarity of responses of a snail neurone to the application a) of an 8-substituted derivative of cyclic AMP and b) of a peptide-containing extract (PE) obtained from the nervous system of *Helix* and *Aplysia.* Note that both reversibly increase the duration of on- and off-phases of firing. Firing pattern in normal medium is shown before (control) and 60–90 min after (wash) cAMP and PE treatment. From Levitan[41].

pound present in invertebrate ganglia increases the intracellular level of both cyclic nucleotides.

Identification of vasotocin in Aplysia

The chemical nature of the endogenous vasopressin-like compound was first investigated by Ifshin and coworkers[37] after finding that extracts of *Otala* and *Aplysia* circum-oesophagial ganglia can induce bursting activity in cell 11 of *Otala*. They showed that the active compound present in the extract was a peptide, since the extract became ineffective after proteolytic pretreatment. However, the peptide differed from oxytocin or vasopressin, since its activity was not destroyed by chymotrypsin. Levitan and coworkers[42,43] showed that an extract obtained from the circum-oesophagial ganglia of *Helix pomatia* was as potent as vasopressin in enhancing burst duration in cell F-1 (fig. 3); in addition, chromatography on neurophysin-sepharose affinity columns indicated the presence in the extract of a compound closely related to vasopressin and oxytocin. Using radioimmunoassay and reverse phase high pressure liquid chromatography, Moore and coworkers[48,49] established recently that the endogenous compound present in *Aplysia* which possesses vasopressin-like effects is arginine-vasotocin (fig. 4). Vasotocin is present in large amounts in the left pleural ganglion (one of the circum-oesophagial ganglia), whereas only traces are found in the abdominal ganglion. Vasotocin-containing neurones may project from the left pleural ganglion towards neurones located in the abdominal ganglion, actually Berry and Geinisman[12] had previously demonstrated the transport of low molecular weight peptides along this pathway.

When perifused at concentrations ranging from 10^{-6} to 10^{-12} M, vasotocin increased the bursting activity of cell R15, while shortening the bursts in cells L3–L6[49]. At similar concentrations, vasotocin also reduced the amplitude of the reflex gill withdrawal in response to stimulation of the siphon, increased the rate of habituation of this withdrawal reflex and reduced the neural activity in gill motoneurones[46,75].

Immunocytochemical studies in other molluscs

While no immunocytochemical data have been reported so far concerning *Aplysia californica,* immunocytochemical data are available for other molluscs. In the pond snail, *Lymnae stagnalis,* Schot and coworkers[64] demonstrated the presence of neurones reacting with antibodies directed against vasopressin, vasotocin, oxytocin and neurophysins. Immunoreactive cell bodies were found in several ganglia and immunoreactive fibers throughout the whole nervous system. The authors believe that the endogenous peptide(s) might act as neurotransmitter(s) because immunoreactive fibers were seen to establish close contacts with neurones.

A class of neurones in the brain of cephalopods send their axon towards the vena cava. These neurones are deemed to be neurosecretory since they contain dense-cored granules within terminals which establish a close contact with blood vessels. Using immunocytochemical techniques in *Octopus vulgaris,* Martin and coworkers[47] showed recently vasopressin-neurophysin-like immunoreactivity in these terminals, the function(s) of which is not yet known.

4. Conclusion

The presence of vasotocin is firmly established in *Aplysia californica.* In 2 other molluscs and in various insects the presence of neurohypophysial peptides is based on radioimmunoassay and immunocytochemical data. The fact that neurones are found which react with vasopressin antibodies as well as with neurophysin antibodies is of interest. It suggests that the endogenous peptides are not only structurally, but also biosynthetically related to the neurohypophysial hormones, since in vertebrates, vasopressin and neurophysin derive from a common precursor[40].

Recently, Grimmelikhuizen and coworkers[34,35] found that neurones, previously characterized as neurosecretory in the coelenterate *Hydra attenuata,* could be stained with antibodies raised against oxytocin, vasopressin or mesotocin. Although it is recognized by oxy-

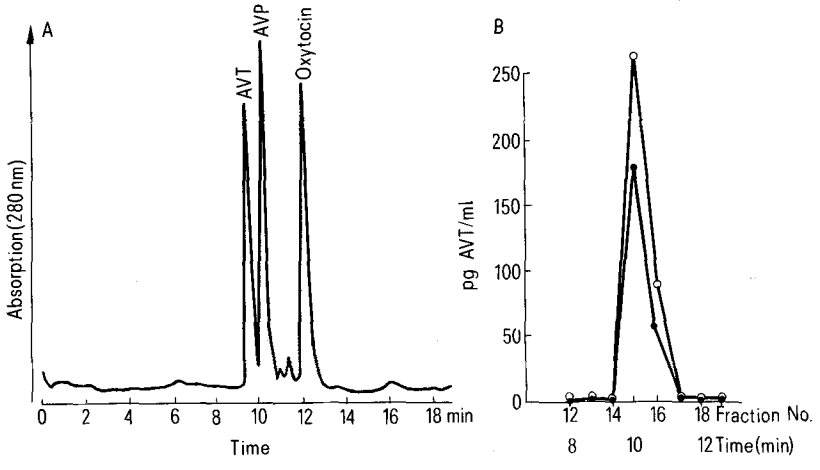

Figure 4. Presence of arginine-vasotocin in *Aplysia* nervous system. *A* HPLC of 50 µg of arginine-vasotocin (AVT), arginine vasopressin (AVP) and oxytocin. *B* Fractions of the column effluent were collected at 40-sec intervals from the moment of injection of 6 µg AVT (○) or extract of Aplysia anterior ganglia (●). The AVT content of each fraction was determined by radioimmunoassay. The elution time in B differs from that in A due to the additional time required for the effluent to pass from the cell of the spectrophotometer to the fraction collector. From Moore et al.[49].

tocin and vasopressin antibodies, the compound present in *Hydra* apparently differs from both, according to evidence from radioimmunoassay. It is nevertheless remarkable that neuropeptides which appear to be structurally related to vertebrate neurohypophysial hormones are already found in such extremely primitive invertebrates.

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2 Present address: Department of Physiology and Biophysics, New York University, New York 10016, USA.

3 Address correspondence and reprint requests to: Prof. J.J. Dreifuss, Département de Physiologie, Centre Médical Universitaire, 1 rue Michel-Servet, CH-1211 Genève 4.

4 Bablanian, G.M., and Treistman, S.N., Seawater osmolarity influences bursting pacemaker activity in intact Aplysia californica. Brain Res. *271* (1983) 342–345.

5 Barker, J.L., Peptides: role in neuronal excitability. Physiol. Rev. *56* (1976) 435–452.

6 Barker, J.L., Physiological roles of peptides in the nervous system, in: Peptides in Neurobiology, pp. 295–343. Ed. H. Gainer. Plenum Press, New York, 1977.

7 Barker, J.L., and Gainer, H., Peptide regulation of bursting pacemaker activity in a molluscan neurosecretory cell. Science *184* (1974) 1371–1373.

8 Barker, J.L., Ifshin, M.S., and Gainer, H., Studies on bursting pacemaker potential activity in molluscan neurones. III. Effects of hormones. Brain Res. *84* (1975) 501–513.

9 Barker, J.L., and Smith, T.G. Jr, Peptide regulation of neuronal membrane properties. Brain Res. *103* (1976) 167–170.

10 Barker, J.L., and Smith, T.G., Electrophysiological studies of molluscan neurons generating bursting pacemaker potential activity, in: Abnormal Neuronal Discharges, pp. 359–387. Eds N. Chalazonitis and M. Boisson. Raven Press, New York, 1978.

11 Barker, J.L., and Smith, T.G., Bursting pacemaker activity in a peptidergic and peptide-sensitive neuron, in: The Role of Peptides in Neuronal Function, pp. 189–228. Eds J.L. Barker and T.G. Smith. Dekker, New York, 1980.

12 Berry, R., and Geinisman, Y., Interganglionic axonal transport of neural peptides within the nervous system of Aplysia. J. Neurobiol. *10* (1979) 489–498.

13 Bessé, N. de, Mise en évidence de cellules neurosécrétrices protocérébrales impliquées dans la régulation de l'équilibre hydrique et de la sécrétion salivaire chez les adultes de Leucophaea maderae, C.r. Acad. Sci. Paris D*286* (1978) 1695–1698.

14 Camier, M., Girardie, J., Rémy, C., Girardie, A., and Cohen, P., Identification of immunoreactive neurophysin-like proteins in the central nervous system of an insect; Locusta migratoria. Biochem. biophys. Res. Commun. *93* (1980) 792–796.

15 Cazal, M., and Girardie, A., Contrôle humoral de l'équilibre hydrique chez Locusta migratoria migratorioides. J. Insect Physiol. *14* (1968) 655–668.

16 Chalaye, D., Recherches histochimiques et histophysiologiques sur la neurosécrétion de la chaîne nerveuse ventrale du criquet migrateur, Locusta migratoria. C.r. Acad. Sci. Paris D*260* (1965) 7010–7013.

17 Chalaye, D., Recherche sur la destination des produits de neurosécrétion de la chaîne nerveuse ventrale du criquet migrateur, Locusta migratoria. C.r. Acad. Sci. Paris D *262* (1966) 161–164.

18 Chalaye, D., Neurosécrétion au niveau de la chaîne nerveuse ventrale de Locusta migratoria migratorioides R. et F. (orthoptère acridien). Bull. Soc. Zool. Fr. *92* (1967) 87–108.

19 Charlet, M., Mise en évidence d'un centre neurosécréteur protocérébral intervenant dans l'équilibre hydrique de la larve d'Aeshna cyanea Müll. (Insecte, Odonate). C.r. Acad. Sci. Paris D *279* (1974) 835–838.

20 Coggeshall, R.E., A light and electron microscope study of the abdominal ganglion of Aplysia californica. J. Neurophysiol. *30* (1967) 1263–1287.

21 Coggeshall, R.E., Kandel, E.R., Kupferman, I., and Waziri, R., A morphological and functional study on a cluster of identifiable neurosecretory cells in the abdominal ganglion of Aplysia californica. J. Cell Biol. *31* (1966) 363–368.

22 Cupo, A., and Proux, J., Biochemical characterization of a vasopressin-like neuropeptide in Locusta migratoria. Evidence of high molecular weight protein encoding vasopressin sequence. Neuropeptides *3* (1983) 309–318.

23 Delphin, F., The histology and possible functions of neurosecretory cells in the ventral ganglia of Schistocerca gregaria Forskal (Orthoptera:Acrididae). Trans. R. ent. Soc. Lond. *117* (1965) 167–214.

24 Dogra, G.S., and Ewen, A.B., Histology of the neurosecretory system and the retrocerebral endocrine glands of the adult migratory grasshopper, Melanoplus sanguinipes (Fab.) (Orthoptera: Acrididae). J. Morph. *130* (1970) 451–466.

25 Dreifuss, J.J., Mühlethaler, M., and Gähwiler, B.H., Electrophysiology of vasopressin in normal rats and in Brattleboro rats. Ann. N.Y. Acad. Sci. *394* (1982) 689–702.

26 Friedel, T., Orchard, I., and Loughton, B.G., Release of neurosecretory protein from insect neurohaemal tissue following electrical stimulation. Brain Res. *208* (1981) 451–455.

27 Friedel, T., Loughton, B.G., and Andrew, R.D., A neurosecretory protein from Locusta migratoria. Gen. comp. Endocr. *41* (1980) 487–498.

28 Frazier, W.T., Kandel, E.R., Kupfermann, I., Waziri, F., and Coggeshall, R.E., Morphological and functional properties of identified neurons in the abdominal ganglion of Aplysia californica. J. Neurophysiol. *30* (1967) 1288–1351.

29 Freon, G., Recherches histophysiologiques sur la neurosécrétion dans la chaîne nerveuse ventrale du criquet migrateur, Locusta migratoria. C.r. Acad. Sci. Paris D *259* (1964) 1565–1568.

30 Geldiay, S., and Edwards, J.S., The protocerebral neurosecretory system and associated cerebral neurohemal area of Acheta domesticus. Z. Zellforsch. *45* (1973) 1–22.

31 Girardie, A., Mise en évidence dans le protocérébron de Locusta migratoria migratorioides et de Schistocerca gregaria, de nouvelles cellules neurosécrétrices contrôlant le métabolisme hydrique. C.r. Acad. Sci. Paris D *721* (1970) 504–507.

32 Girardie, J., and Rémy, C., Particularités histo-cytologiques des prolongement distaux des 2 cellules à 'vasopressin-neurophysine-like' du criquet migrateur. J. Physiol., Paris *76* (1980) 265–271.

33 Greenberg, M.J., and Prince, D.A., Invertebrate neuropeptides: native and naturalized. A. Rev. Physiol. *45* (1983) 271–288.

34 Grimmelikhuizen, C.J.P., Coexistence of neuropeptides in Hydra. Neuroscience *9* (1983) 837–845.

35 Grimmelikhuizen, C.J.P., Dierickx, K., and Boer, G.J., Oxytocin/vasopressin-like immunoreactivity is present in the nervous system of Hydra. Neuroscience *7* (1982) 3191–3199.

36 Hanström, B., Neurosecretory pathways in the head of crustaceans, insects and vertebrates. Nature *171* (1953) 72–73.

37 Ifshin, M., Gainer, H., and Barker, J.L., Peptide factor extracted from molluscan ganglia that modulates bursting pacemaker activity. Nature *254* (1975) 72–74.

38 Jahan-Parwar, B., Smith, M., and von Baumgarten, R., Activation of neurosecretory cells in Aplysia by osphradial stimulation. Am. J. Physiol. *216* (1967) 1246–1257.

39 Kupfermann, L., and Weiss, R., Water regulation by a presumptive hormone contained in identified neurosecretory cell R15 of Aplysia. J. gen. Physiol. *67* (1976) 113–123.

40 Land, H., Schütz, G., Schmale, H., and Richter, D., Nucleotide sequence of cloned cDNA encoding bovine arginine-vasopressin-neurophysin II precursor. Nature *295* (1982) 299–303.

41 Levitan, I.B., Modulation of neuronal activity by peptides and neurotransmitters: possible role of cyclic nucleotides. J. Physiol., Paris *74* (1978) 521–526.

42 Levitan, I.B., Harmar, A.J., and Adams, W.B., Synaptic and hormonal modulation of a neuronal oscillator: a search for molecular mechanisms. J. exp. Biol. *81* (1979) 131–151.

43 Levitan, I.B., and Treistman, S.N., Modulation of electrical activity and cyclic nucleotide metabolism in molluscan nervous system by a peptide-containing nervous system extract. Brain Res. *136* (1977) 307–317.

44 Loh, Y.P., and Gainer, H., Low molecular weight specific proteins in identified molluscan neurons. I. Synthesis and storage. Brain Res. *92* (1975) 181–192.

45 Loh, Y.P., and Gainer, H., Low molecular weight specific proteins in identified molluscan neurons. II. Processing, turnover and transport. Brain Res. *92* (1975) 193–205.

46 Lukowiak, K., Thornhill, J.A., Cooper, K.E., and Veale, W.L., Vasopressin increases the central nervous system suppressive control over gill reflex behaviours and associated neural activity in Aplysia. Can. J. Physiol. Pharmac. *58* (1980) 583–587.

47 Martin, R., Frosch, D., and Voigt, K.H., Immunocytochemical evidence for melanotropin- and vasopressin – like material in a cephalopod neurohormonal organ. Gen. comp. Endocr. *42* (1980) 235–243.
48 Moore, J.G., Reversed phase high pressure liquid chromatography for the identification and purification of neuropeptides. Life Sci. *30* (1982) 995–1002.
49 Moore, G.J., Thornhill, J.A., Gill, V., Lederis, K., and Lukowiak, K., An arginine vasotocin-like neuropeptide is present in the nervous system of the marine mollusc Aplysia californica. Brain Res. *206* (1981) 213–218.
50 Mühlethaler, M., Sawyer, W.H., Manning, M.M., and Dreifuss, J.J., Characterization of a uterine-type oxytocin receptor in the rat hippocampus. Proc. natl Acad. Sci. USA *80* (1983) 6713–6717.
51 Pliška, V., Phylogeny of neurohypophysial hormones: parsimonial phylogenetic trees and evolution of some biological activities, in: Neuroendocrinology of Vasopressin, Corticoliberin and Opiomelanocortins, pp. 177–189. Eds A.J. Baertschi and J.J. Dreifuss. Academic Press, London 1982.
52 Proux, J., Influence de l'électrocoagulation de la pars intercerebralis et des cellules neurosécrétrices sous-ocellaires médianes sur le comportement pondéral, hydrique et alimentaire du criquet migrateur. Bull. Soc. Zool. Fr. *104* (1979) 89–103.
53 Proux, J., and Girardie, A., Neurosecretory regulation of the haemolymphatic titre of a vasopressin-like substance in the migratory locust. Neurosci. Lett. *33* (1982) 73–77.
54 Proux, J., and Rougon-Rapuzzi, G., Evidence of vasopressin-like molecule in migratory locust. Radioimmunological measurements in different tissues: correlation with various states of hydratation. Gen. comp. Endocr. *42* (1980) 378–383.
55 Proux, J., Rougon-Rapuzzi, G., and Cupo, A., Enhancement of excretion across locust malpighian tubules by a diuretic vasopressin-like hormone, Gen. comp. Endocr. *47* (1982) 449–457.
56 Proux, J., Rougon-Rapuzzi, G., and Rémy, C., Influence de la pars intercerebralis et des cellules neurosécrétrices sousocellaires médianes sur la teneur en substance apparentée à la vasopressine dans la chaîne nerveuse ventrale et l'hémolymphe du criquet migrateur. Etude radio-immunologique et immunohistologique. J. Physiol., Paris *76* (1980) 277–282.
57 Raabe, M., Etude des phénomènes de neurosécrétion au niveau de la chaîne nerveuse des phasmidés. Bull. Soc. Zool. Fr. *90* (1965) 631–654.
58 Rémy, C., Parentés immunochimiques entre produits de neurosécrétion d'invertébrés et neuropeptides de vertébrés. J. Physiol., Paris *78* (1982) 514–522.
59 Rémy, C., and Girardie, J., Anatomical organization of two vasopressin-neurophysin-like neurosecretory cells throughout the central nervous system of the migratory locust, Gen. comp. Endocr. *40* (1980) 27–35.
60 Rémy, C., Girardie, J., and Dubois, M.P., Exploration immunocytologique des ganglions cérébroïdes et sous-oesophagien du phasme Clitumnus extradentatus: Evidence d'une neurosécrétion apparentée à la vasopressine-neurophysine, C.R. Acad. Sci. Paris D *285* (1977) 1495–1497.
61 Rémy, C., Girardie, J., and Dubois, M.P., Vertebrate neuropeptide-like substances in the suboesophageal ganglion of two insects: Locusta migratoria R. and F. (Orthoptera) and Bombyx mori L. (Leptidoptera). Immunocytological investigation. Gen. comp. Endocr. *37* (1979) 93-100.
62 Rosenbluth, J., The visceral ganglion of Aplysia californica. Z. Zellforsch. Mikrosk. Anat. *60* (1963) 213–236.
63 Scharrer, B., Über sekretorisch tätige Nervenzellen bei wirbellosen Tieren. Naturwissenschaften *25* (1935) 131–142.
64 Schot, L.P.C., Boer, H.H., Swaab, D.F., and van Norden, S., Immunocytochemichal demonstration of peptidergic neurons in the central nervous system of the pond snail Lymnea stagnalis with antisera raised to biologically active peptides of vertebrates. Cell Tissue Res. *16* (1981) 273–291.
65 Stinnakre, J., and Tauc, L., Effets de l'activation osmotique de l'osphradium sur les neurones du système nerveux central de l'Aplysie. J. Physiol., Paris *58* (1966) 266–267.
66 Stinnakre, J., and Tauc, L., Central neuronal response to the activation of osmoreceptors in the osphradium of Aplysia. J. exp. Biol. *51* (1969) 347–361.
67 Strambi, C., Rougon-Rapuzzi, G., Cupo, A., Martin, A., and Strambi, A., Mise en évidence immunocytologique d'un composé apparenté à la vasopressine dans le système nerveux du grillon Acheta domesticus. C.r. Acad. Sci. Paris D *288* (1979) 131–133.
68 Strambi, C., Strambi A., Cupo, A., Rougon-Rapuzzi, G., and Martin, N., Etude des taux d'une substance apparentée à la vasopressine dans le système nerveux des grillons soumis à différentes conditions hygrométriques. C.r. Acad. Sci. Paris D *287* (1978) 1227–1233.
69 Stutinsky, F., Etude de l'innervation du complexe rétrocérébral chez Periplaneta americana à l'aide de l'hématoxyline de Gomori. Bull. Soc. Zool. Fr. *76* (1951) 307–308.
70 Stutinsky, F., Etude du complexe rétro-cérébral de quelques insectes avec l'hématoxyline chromique. Bull. Soc. Zool. Fr. *77* (1952) 61–67.
71 Stutinsky, F., Mise en évidence d'une substance antidiurétique dans le cerveau et le complexe rétrocérébral d'une blatte. Bull. Soc. Zool. Fr. *78* (1953) 202–204.
72 Takeuchi, H., Matsumoto, M., and Mori, A., Modification of effects of biologically active peptides, caused by enzyme treatment on the excitability of identifiable giant neurones of an African giant snail (Achatina fulica Ferussac). Experientia *33* (1977) 249–251.
73 Takeuchi, H., Sokai, A., and Mori, A. Effects of three synthetic peptides analogous to neurohypophysial hormones on the excitability of giant neurones of Achatina fulica Ferrussac. Experientia *32* (1976) 1554–1566.
74 Thornhill, J.A., Lukowiak, K., Cooper, K.E., and Veale, W.L., Arginine vasotocin, an endogenous neuropeptide of Aplysia, suppresses the gill withdrawl reflex and reduces the evoked synaptic input to central gill motor neurons. J. Neurobiol. *12* (1981) 533–544.

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Molecular aspects of the imipramine 'receptor'

by A. Davis[1,2]

Departments of Pharmacology and Psychiatry, University of Toronto, 250 College Street, Toronto (Ontario, Canada)

1. *Introduction*

The imipramine binding site has been characterized for only 4–5 years[99], and yet in that short time, it has emerged as a potentially powerful tool for investigation of depression. Because its possible usefulness was recognized from the start imipramine was used in clinical studies almost immediately[7,14]. As traditional paths of investigation were by-passed in the case of imipramine, a real gap in basic knowledge about the imipramine binding site exists. During the last year, studies in this and other laboratories have attempted to fill part of this gap with the intention of providing a better rationale for the use of this drug in the clinical situation.