J. JOOSSE

What is special about peptides as neuronal messengers?

Nerve cells use various chemical messengers, including acetylcholine, monoamines, amino acids and neuropeptides. In the past 10 years it has become clear that there is a great molecular diversity of such messengers particularly amongst the amino acids. However, the molecular diversity of neuropeptides exceeds by far that of the amino acids. There is now evidence that in one animal species as many as 100-200 different peptide molecules may serve as neuronal messengers (Kandel 1983; Nieuwenhuys 1985; Joosse 1986).

Neuropeptides occur in nervous systems of all animals, even in the most simple such as coelenterates (Schaller *et al.*, 1984). For many years neuronal peptides were thought to have only a neuroendocrine role. The present view however is that, in addition to their role as neurohormones, they may act as typical synaptic neurotransmitters and as paracrine or neurocrine regulators at non-synaptic sites (Buma & Roubos 1985; Nieuwenhuys 1985).

Immunocytochemical and biochemical studies provide substantial evidence that structurally related peptides are found in many different phyla. Well-known examples are insulin-like peptides and FMRFamide. An important consequence of these findings is that some of the peptides found so far may have arisen from a smaller number of ancestral molecules, possibly at the prokaryote stage (Joosse 1987).

A large part of the present volume concerns the molecular diversity and functions of neuropeptides in invertebrates. In view of the great diversity in structure and function of neuropeptides and their presence throughout the animal kingdom, the question arises as to the adaptive value of peptide variety.

This question is discussed in the literature only occasionally, and emphasis is placed on the fact that, at least until now, unlike classical transmitters no special enzymatic machinery has been identified which effects the rapid breakdown of peptides at the sites of their release. Therefore, their actions last longer than those of classical transmitters. In the nervous system, peptides may diffuse to more widely distributed receptors (Kandel 1983) and this is likely to be important, for example, when peptides are co-released with classical transmitters. Here, peptides may recruit numerous neurones in a nucleus (Jan & Jan 1983). Others have stressed that some neuropeptides are able to bind to several different receptors, which means that these molecules contain more information than classical transmitters (Schwyzer 1980).

Previously, little attention has been given to the eco-physiological aspects of neuronal messengers. For example, what are the energy costs involved in the production of various messengers? In this respect, studies should be made of the energy budget of peptide transport from the site of synthesis (cell body), to the release point. Classical transmitters are often synthesized near their site of release, which reduces transport costs. Recycling of materials is also important. Little is known about reprocessing of peptides and their amino acid components. (Probably the single amino acid transmitters are the most economic signal molecules.) Acetylcholine reabsorption from the synaptic cleft is a well-known recycling phenomenon: after exocytosis, the membranes of the elementary granules are reprocessed locally. In peptidergic neurones, the multilamellar bodies which contain similar membrane material need to be transported retrogressively to the cell bodies at considerable energy cost (see also Golding & Pow, Chapter 1).

Any consideration of the selective value of neurotransmitter variety must include their genetics. Classical neurotransmitters are synthesized by one or a series of enzymes, from precursors in the blood or in cells, as components of typical metabolic pathways. Thus, it is not the structures of the neurotransmitters, but that of the synthesizing enzymes that are encoded by the genome. On the other hand, with neuropeptides the primary molecular structures of the signal molecules themselves are encoded by the genome; triplets of nucleotides code for each amino acid of the peptide molecules. Here we perceive a basic difference between categories of neuronal messenger (cf. Joosse 1987). Classical transmitters have an indirect relationship with the genome, whereas for peptides this relationship is direct. The mechanisms which operate in changing the genome are not restricted to point mutations, but show great diversity. All such changes may affect the nucleotide sequence in genes coding for peptides, and consequently the composition and sequence of the amino acids comprising the peptide messengers. Clearly, genomic changes will also affect genes coding for enzymes involved in the synthesis of classical transmitters. The effects of the latter changes are difficult to predict, but it may be that the quantity of messenger produced changes, or that a particular enzyme in the synthetic pathway of the messenger is unable to function. However, the chances are extremely low that the structure of the messenger concerned is changed. Moreover, classical transmitters are small molecules, and a change in their structure will often be lethal, since their functioning is not only dependent on receptor binding, but also on subsequent enzymatic breakdown and/or reabsorption. On the other hand, changes in the amino acid composition of peptides have the advantage of being gradual: a change from one hydrophobic amino acid to another may alter the characteristics of the complete molecule only slightly, whereas a change from any amino acid into proline could alter the shape of the entire molecule with severe consequences for receptor binding. This graduality of structural alterations makes peptide messengers suitable candidates for a crucial role in the evolutionary development of regulatory systems. Small changes in

molecular structure may alter the rate or duration of the process controlled and therefore may be more easily successful when exposed to selection pressure. Another positive aspect of the direct genomic coding of neuropeptide structure is the occurrence of families of genes coding for the same neuropeptide precursor molecule, but with slightly different molecular structures for the final product (Hakanson & Thorell 1985). From the viewpoint of population genetics this is a highly advantageous situation for the successful control of adaptive changes.

In conclusion it appears that peptides are a special category of messenger. These molecules are suitable candidates for the control of the great number of processes related to animal adaption. This may explain why peptides are involved in the control of great diversity of physiological processes and why these molecules have persisted as messengers throughout animal evolution, despite their apparently higher energy budget compared with classical transmitters.

References

Buma, P. & Roubos, E.W. (1985). Ultrastructural demonstration of nonsynaptic release sites in the central nervous system of the snail Lymnaea stagnalis, the insect Periplaneta americana, and the rat. *Neuroscience* **17**, 867-79.

Hakanson, R. & Thorell, J. (eds.) (1985). Biogenetics of Neurohormonal Peptides. Academic Press, pp. 303.

Jan, Y.N. & Jan, L.Y. (1983). A LHRH-like peptidergic neurotransmitter capable of 'action at a distance' in autonomic ganglia. TINS 6, 320-25.

Joosse, J. (1986). Neuropeptides: peripheral and central messengers of the brain. In: Comparative endocrinology, developments and directions, ed. C.L. Ralph, pp. 12-32. *Progr. Clin. biol. Res.*, Vol. 205. New York: Allan Liss.

Joosse, J. (1987). Functional and evolutionary perspectives of neuropeptides and their precursors. *Proc. Firstt Internat. Congress Neuroend.* (In press).

Kandel, E.R. (1983). Neurobiology and molecular biology: the second encounter, *Cold Spring Harb. Symp. quant. Biol.* **48**, 891-908.

Nieuwenhuys, R. (1985). *Chemoarchitecture of the brain,* pp.1-246. Berlin: Springer.

Schaller, H.C., Hoffmeister, S. & Bodenmuller, H. (1984). Hormonal control of regeneration in Hydra. In: *Biosynthesis, metabolism and mode of action of invertebrate hormones*, ed. J.A. Hoffman & M. Porchet, pp. 5-9. Berlin: Springer.

Schwyzer, R. (1980). Organization and transduction of peptide information. *Trends in Pharmacol. Sc.* **3**, 327-31.

PART I

Immunocytochemistry and Ultrastructure

D. W. GOLDING & D. V. POW

The new neurobiology – ultrastructural aspects of peptide release as revealed by studies of invertebrate nervous systems

Ultrastructure of invertebrate nervous systems

Neurosecretory and other neurones

Examination of invertebrate nervous systems reveals that many are richly endowed with neurones resembling classical neurosecretory cells in cytology and ultrastructure. Such cells are clearly specialized for peptide secretion. They contain an abundance of rough endoplasmic reticulum (RER), and secretory granules (variously known as elementary granules, large dense-cored vesicles, etc.) generated by Golgi bodies, accumulate in large numbers within the perikarya. Although many are doubtless endocrine cells, others (Figs. 1–3) have axons which extend not to blood cavities, but into the central neuropile where the secretory material is discharged.

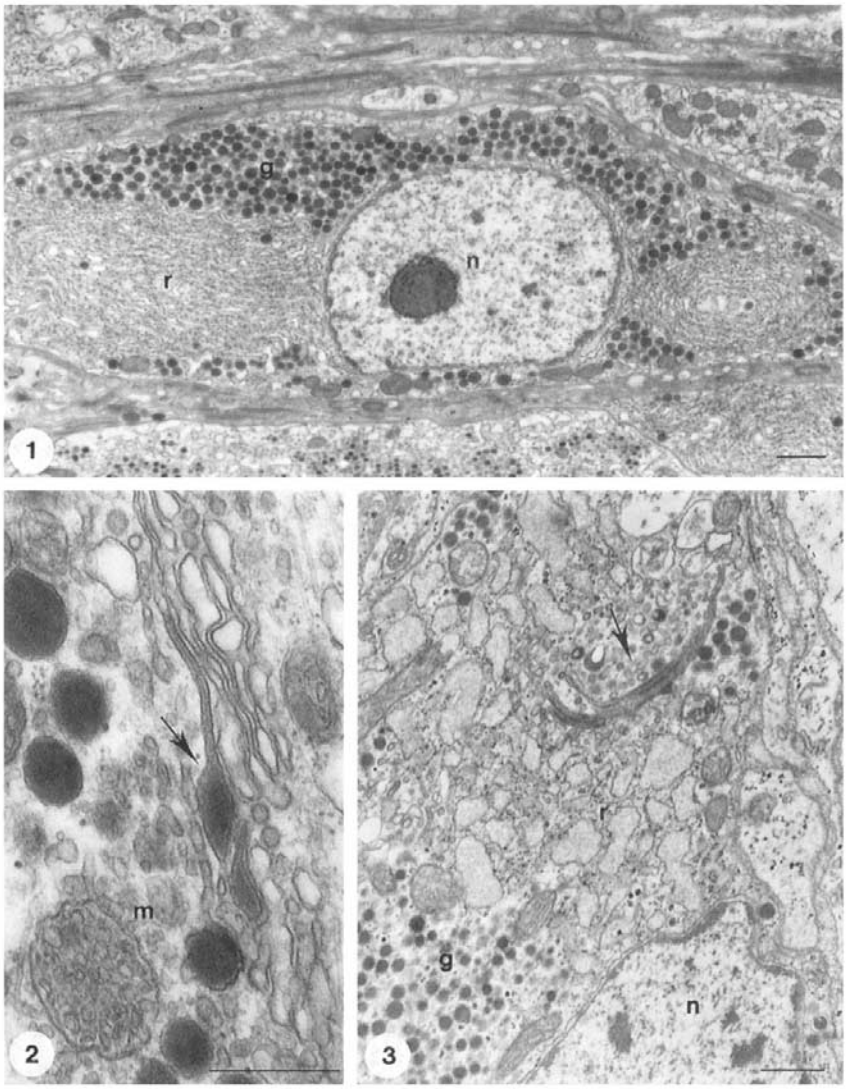
Furthermore, some secretory granules are evident in virtually all neurones (Golding & Whittle 1977), and this is consistent with the finding that many and perhaps all neurones, including those with conventional transmitters, also secrete peptides (review by Hokfelt, Johansson & Goldstein 1984).

Nerve terminals: vesicles and granules

In most nerve terminals, whether in the central or peripheral nervous systems, large numbers of synaptic vesicles are encountered (Figs. 4 & 5). Measuring 20–50nm in diameter, the vesicles in all but a small minority of terminals (Fig. 6) have lucent contents, except following exposure to a mixture of Zinc iodide and Osmium tetroxide (the ZIO reagent) (Fig. 7) which deposits extremely electron-dense material within them (May & Golding 1982). The vesicles cluster densely adjacent to sites of specialized contact with other cells. Pre- and postsynaptic thickenings are present and the synaptic clefts are wider and often more regular in form than other intercellular spaces, and contain moderately dense material.

Secretory granules also accumulate in synaptic terminals (Figs. 4–7). In contrast to synaptic vesicles, granules show great variety in size (80–200nm in diameter), form, electron density of the core, presence of a clear halo, etc. (Fig. 8). The majority 'stand back' from the synaptic junction and occupy more peripheral regions of the terminal. Unlike synaptic vesicles, they show little or no affinity for the ZIO reacent.

Figs. 1–3. Non-endocrine peptidergic cells. g. secretory granules; m, multivesicular body; n, nucleus; r, rough endoplasmic reticulum; arrows, Golgi apparatus giving rise to granules. Fig. 1. *Lumbricus terrestris* (Annelida), cerebral ganglion; bar 1000 nm. Fig. 2. *L. terrestris*, cerebral ganglion; bar 500 nm. Fig. 3. *Dendrocoelum lacteum* (Platyhelminthes), cerebral ganglion; bar 500 nm.



Immunocytochemical and other studies have established that neuropeptides are stored in the granules and not within synaptic vesicles (review by Hokfelt *et al.* 1984). Their apparent presence in the cytoplasm outside the granules is generally regarded as an artefact. Comparable findings relate to endocrine peptides in neurosecretory cells (review by Morris, Nordmann & Dyball 1978).

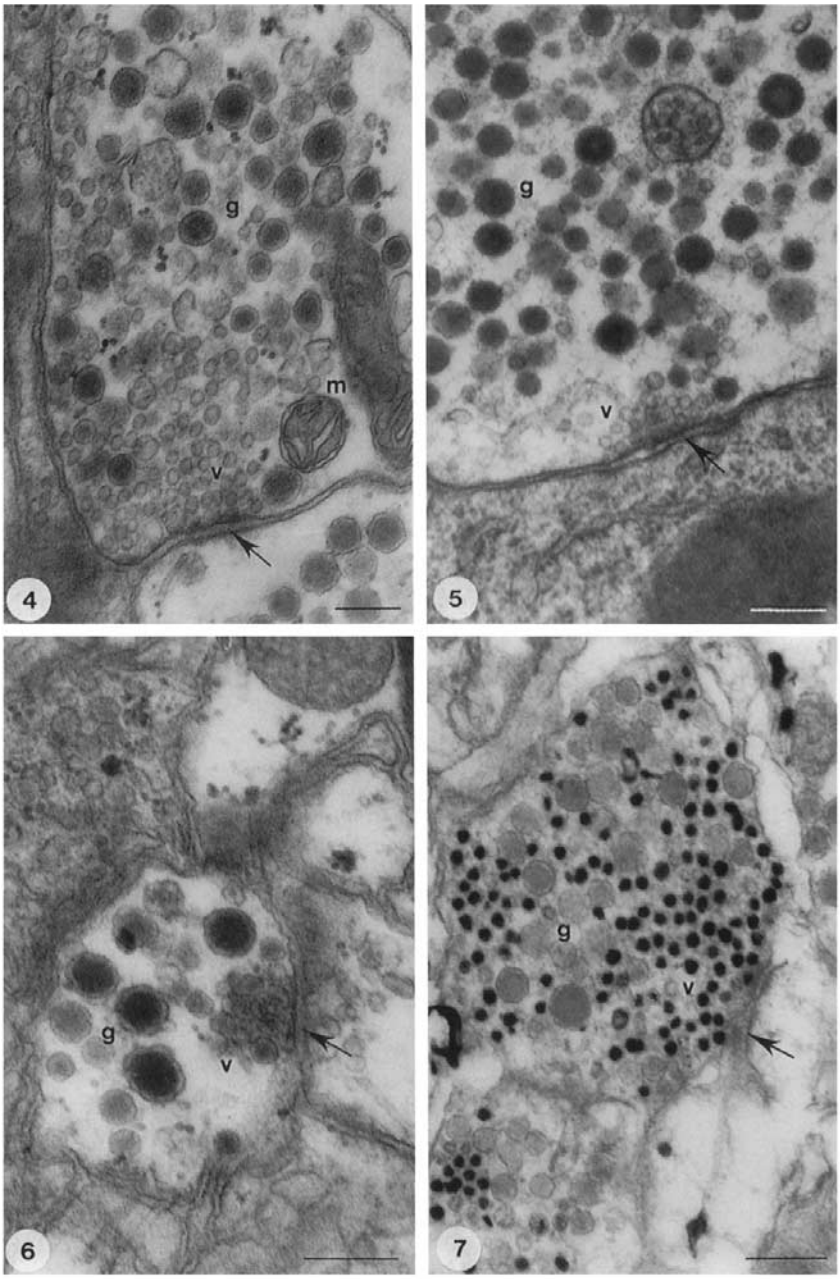
Secretory discharge by exocytosis

Synaptic vesicles engaged in exocytosis are encountered in extremely rare cases, the bounding membrane of the vesicle having fused with the presynaptic membrane, presumably allowing discharge into the synaptic cleft.

In contrast, exocytosis of secretory granules occurs mainly from within apparently unspecialized regions of the phasmalemma of non-endocrine nerve terminals. Demonstrated clearly during studies of annelid central nervous systems (Golding & May, 1982), the process is now recognized as a phenomenon of fundamental neurobiological mportance and has been reported with respect to a wide range of invertebrates (Figs. 8–11) (Shkolnik & Schwartz, 1980; Golding & Bayraktaroglu, 1984; Buma, Roubos & Buijs, 1984). Similar findings relate to vertebrates (Buma & Roubos, 1986), including the classic cholinergic synapses innervating the adrenal medulla and its homologues (Golding & Pow, 1987). Several lines of evidence indicate that the phenomenon is not a fixation artefact, but a correlate of secretory release resulting from neural activity. Sites of discharge are marked by the formation of omega profiles, with material of varying density being present within the indentations, and several sites of discharge may be present in a single fibre profile. Furthermore, compound exocytosis, in which one or more granules have apparently fused with another already engaged in discharge, is sometimes encountered (Fig. 9). Dissolution of the material does not always keep pace with release and pools of material then accumulate in the extracellular space.

Although granule exocytosis is associated mainly with areas of undifferentiated membrane, discharge into synaptic clefts does occur, albeit more rarely. It seems to be encountered with unusual frequency within regions of membrane immediately adjacent to areas occupied by membrane thickenings (Fig. 12). Exocytosis also occurs from within areas of the terminal in contact with glia as well as those adjacent to neuronal elements. Indeed, in the corpus cardiacum of the locust, quantitative studies show that exocytosis is not targeted towards the postsynaptic gland cells but is equally likely to be associated with regions of the plasmalemma adjacent to other fibres, glia, etc. (Pow & Golding, 1987). Last, granule discharge in the corpus cardiacum is associated with varicosities and does not apparently occur from within the narrow regions of fibres which connect them (Pow & Golding, 1987).

In most cases, sites of granule exocytosis and synaptic contact, respectively, are not sharply segregated from each other within separate varicosities. However, in



some types of neurone in annelids, in which granule exocytosis is abundant, synapses and synaptic vesicles are at most extremely rare, and similar findings apparently apply to *Lymnaea* (Buma & Roubos 1986).

Exoycytotic profiles captured by tannic acid

The study of glandular discharge has been greatly facilitated by the use of tannic acid (Fig.10), a complex mixture of glucosides whose orthophenol radicles are negatively charged at neutral pH. Infused into living ganglia maintained in saline solutions *in vitro* (the TARI method, Buma, Roubos & Buijs, 1984), or injected *in vivo* (the TAIV method, Pow & Golding, 1987), tannic acid prevents the dissolution of granule cores as they are discharged and enhances their eventual electron density. Exocytotic profiles, 'frozen' *in situ*, are thus progressively accumulated as secretion continues (Fig. 11). However, the two techniques should be applied with caution, since in at least some organs, cytolysis and aberrant granule discharge results from exposure to tannic acid for extended periods of time (Pow, unpublished).

Membrane retrieval

Omega profiles resulting from granule exocytosis are sometimes etched by one or more coated pits (Fig. 13), apparently giving rise to vesicles 30–50 nm in diameter (Golding & May 1982). In other cases, omega profiles may be coated *in toto* (Golding & Bayraktaroglu 1984; Buma & Roubos 1985) probably indicating that granule membrane is being retrieved by endocytosis of 'vacuoles' similar in size to the granules.

A dual vesicle hypothesis

Correlation of ultrastructural observations with information drawn more widely, led Golding & May (1982) to conclude that neurones typically possess two, dichotomous, secretory mechanisms for the elaboration and discharge of neurochemical mediators (Fig. 14).

Origins

Secretory inclusions may well differ, first, in their respective origins within the neurone (Fig. 14a). Granules are undoubtedly generated by the Golgi apparatus in

Figs. 4–7. Synaptic terminals containing secretory granules (g), and synaptic vesicles (v), focussed on membrane thickenings with differentiated clefts (arrows). Fig. 4. *L. terrestris; m*, mitochondria; bar 200 nm. Fig. 5. *Schistocerca gregaria* (Insecta); bar 200 nm. Fig. 6. *Nereis diversicolor* (Annelida), showing dense-cored synaptic vesicles; bar 200 nm. Fig. 7. *N. diversicolor*, showing synaptic vesicles 'stained' with ZIO; bar 200 nm. Courtesy, Dr. Barbara A. May, University of Newcastle upon Tyne.