Peptidergic synaptic transmission in sympathetic ganglia[1]

TERRENCE J. SEJNOWSKI[2]

Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT

Biologically active peptides have been localized in neuronal cell bodies, axons, and synaptic boutons of sympathetic ganglia; some of these peptides may be neurotransmitters. For example, substances immunologically similar to substance P and luteinizing hormone-releasing hormone appear to be released from nerve terminals in sympathetic ganglia. In each case, the postsynaptic action of the peptide lasts for several minutes and is accompanied by a combination of decreases and increases in the membrane conductance that are voltage dependent. These peripheral peptidergic synapses may be models for peptidergic transmission in the central nervous system where detailed analysis is more difficult.—**Sejnowski, T. J.** Peptidergic synaptic transmission in sympathetic ganglia. *Federation Proc.* 41: 2923–2928; 1982.

Until recently, peptides in brain tissue were thought to serve a neuroendocrine function. The discovery that a variety of peptides are contained in neurons throughout the nervous system has raised the possibility, however, that they are classical transmitters at synapses (16, 40, 44, 47, 50). Peripheral autonomic ganglia are suitable for studying this possibility: ganglia are easily isolated for intracellular recording, the neurons are relatively homogeneous, and the input and output are often segregated.

In sympathetic ganglia immunoreactivity has been reported for peptide-like substance P (15), Met- and Leu-enkephalin (48), vasoactive intestinal peptide (14), somatostatin (13), cholecystokinin (34), bombesin (16), avian pancreatic polypeptide (35), and luteinizing hormone-releasing hormone (LHRH) (20, 21). The reactive substances have not been purified from the ganglia and sequenced, but their structure is sufficiently similar to the known peptides to cross-react with specific antiserums. The anatomical localization of peptides in sympathetic ganglia has far outpaced physiological and biochemical studies: in only a few cases is there substantial evidence that the peptide is a neurotransmitter.

In this article peptidergic transmission is examined in two preparations: the inferior mesenteric ganglion of the guinea pig where a substance P-like peptide may be a transmitter, and the paravertebral ganglia of the frog where a peptide similar to LHRH may mediate synaptic transmission. In both of these sympathetic ganglia, peptidergic transmission lasts much longer than the familiar fast cholinergic transmission and is produced by different mechanisms.

SUBSTANCE P

The first peptide to be proposed as a neurotransmitter was substance P for primary afferents in the spinal cord (43). Substance P is present in the spinal cord in dense networks of fibers (17) and is released when the spinal cord is bathed in high [K^+] solution (2). Although substance P has an excitatory effect on spinal motor neurons when applied iontophoretically or in the bathing solution, it has not been possible to measure directly synaptic potentials mediated by substance P because of the anatomical complexity of the spinal cord (45).

In the inferior mesenteric ganglion of the guinea pig the principal cells are surrounded with baskets of varicosities and fibers that are positive for substance P immunoreactivity (15). The source of these fibers appears to be a population of small cells in dorsal root ganglia, as suggested by the retrograde transport of horseradish peroxidase and ligation experiments (3, 9, 30, 36); the fibers may be collaterals from sensory nerve fibers passing through the ganglion from the gastrointestinal tract to the dorsal root ganglia (Fig. 1).

In 1978 Neild (39) reported a slow depolarization in neurons of the guinea pig inferior mesenteric ganglion after repetitive stimulation of the hypogastric nerves, a response that was not affected by cholinergic blocking agents. Shortly thereafter it was found that a similar depolarization could be produced in these cells by externally applied substance P (6, 30); this depolarization was not affected by a reduction of [Ca^{2+}] or by an increase of [Mg^{2+}] in the bathing solution, which indicates that substance P acts directly on ganglion cells. The possibility that substance P is a transmitter in the inferior mesenteric ganglion was strengthened by the demonstration that substance P-like immunoreactive material is released from the ganglion in a bathing

[1] From the Neurobiology and Behavior Thematic Symposium *Peptidergic Neurons: Physiology and Biochemistry* programed by the American Society for Pharmacology and Experimental Therapeutics and The American Physiological Society at the 65th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlanta, Georgia, April 13, 1981.

[2] Present address: Department of Biophysics, The Johns Hopkins University, Baltimore, MD 21218.

0014-9446/82/0041-2923/$01.25. © FASEB

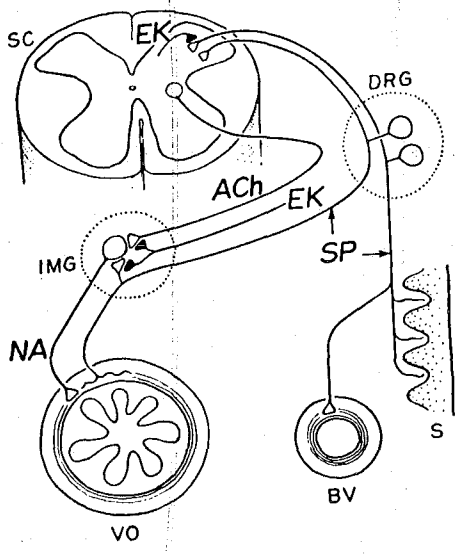


Figure 1. Diagram illustrating innervation of the inferior mesenteric ganglion of the guinea pig. Some nerve fibers in the ganglion contain peptide immunoreactivity in addition to those shown, including vasoactive intestinal polypeptide and cholecystokinin (14, 16). In addition to norepinephrine, many of the cell bodies in the ganglion also contain a somatostatinlike peptide (13). ACh: acetylcholine; NA: norepinephrine; EK: enkephalin; SP: substance P; BV: blood vessel; DRG: dorsal root ganglion; IMG: inferior mesenteric ganglion; S: skin; SC: spinal cord; VO: visceral organ. From ref 31, through the courtesy of Dr. Masanori Otsuka.

solution containing high $[K^+]$, but only if Ca^{2+} is present (30).

The nerve-evoked depolarization and the depolarization induced by authentic substance P are usually associated with a decrease in the membrane conductance (5, 7, 30). In some cells, however, conductance increases have also been reported (5, 7, 38), and several mechanisms may therefore contribute to this peptidergic response. It is difficult to space clamp all the synapses on a mammalian ganglion cell to the same potential because many synapses are made on dendritic processes; consequently the voltage dependence of the peptidergic response is still uncertain.

Enkephalinlike immunoreactivity is also found in nerve fibers and terminals in the inferior mesenteric ganglion of the guinea pig (48) and in the spinal cord where it is believed to inhibit synaptic transmission. In the mesenteric ganglion the bath application of an analog of enkephalin inhibits the release of substance P-like immunoreactivity in a high $[K^+]$ bathing solution, and also inhibits the nerve-evoked peptidergic response in ganglion cells without affecting the depolarizing response to externally applied substance P. This suggests that enkephalin acts presynaptically to reduce the release of substance P-like transmitter from nerve terminals (29, 31). The inhibitory effect of enkephalin on the release of substance P and on the nerve-evoked depolarization is reversed by naloxone, an opiate antagonist. Enkephalin inhibits its fast cholinergic transmission in an apparently similar manner (29).

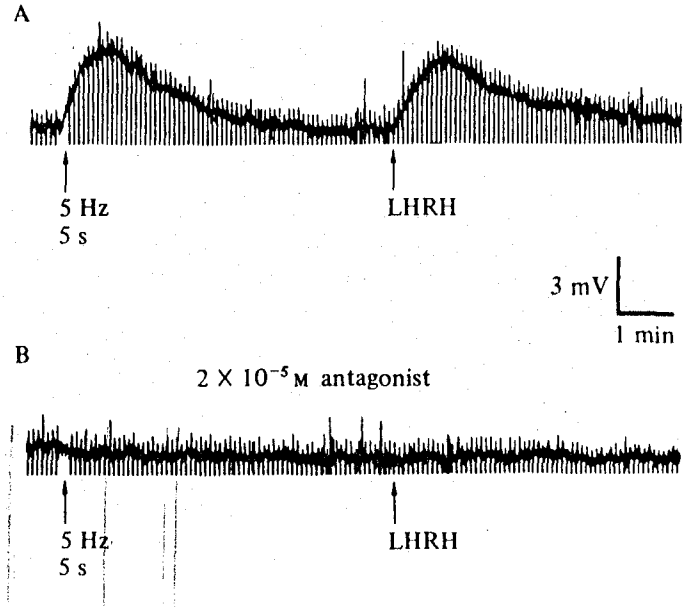
LHRH

In 1968 Nishi and Koketsu (42) reported that prolonged stimulation of the slowly conducting preganglionic fibers of bullfrog paravertebral sympathetic ganglia produces a long-lasting depolarization of lumbar paravertebral sympathetic ganglion cells. The response, which is insensitive to cholinergic blocking agents, rises to a peak about 1 min after stimulation and can continue for over 10 min (Fig. 2). Recently, Jan et al. (20, 21) reported evidence that the transmitter for this slow synaptic potential is a peptide resembling LHRH:

1) In nerves whose stimulation leads to the noncholinergic response, a protease-sensitive substance with a molecular weight of about 1000 can be detected by radioimmunoassay for LHRH. *2)* LHRH-like immunoreactivity is present within synaptic boutons surrounding ganglion cells (18, 19). *3)* Five days after cutting the appropriate nerves, about 95% of the substance disappears from the ganglia. At the same time the content of the substance central to the cut is increased, which suggests that it is concentrated in axons and transported to the periphery from the spinal cord. *4)* The substance is released in solutions containing high $[K^+]$, but only if Ca^{2+} is present. *5)* Application of the authentic LHRH mimics the action of the nerve-released transmitter in a specific manner (Fig. 2). Both substances cause similar changes in the postsynaptic membrane conductance and in the excitability of neurons (33). *6)* An analog of LHRH, which in mammals blocks the release of gonadotropins, blocks the depolarizing effect of nerve-released transmitter and of applied LHRH in ganglion cells (Fig. 2).

The minimum concentration of LHRH that produces a depolarization in ganglion cells is about 1 μM. Several analogs of LHRH that are 10–100 times more potent in releasing gonadotropins from the anterior pituitary of rats are also more effective in depolarizing sympathetic ganglion cells of the frog. For example, [D-Ala^6]LHRH is about 100 times more potent in mammals (51) and acts at concentrations of about 10^{-8} M in sympathetic ganglia

Figure 2. *A)* Comparison of nerve-evoked and LHRH-induced peptidergic intracellular responses in sympathetic ganglion cells. *B)* Bath application of an LHRH antagonist, [D-pGlu^1, D-Phe^2, D-Trp^{3,6}]LHRH, which in rats inhibits the release of gonadotropins from the pituitary, blocked both responses without affecting the other cholinergic responses in the same neuron. The vertical lines in these recordings are test pulses whose peaks are not shown. From ref 21.



(20, 21). The LHRH-like substance in sympathetic ganglia of the frog has chromatographic characteristics different from hypothalamic LHRH in the frog and probably has a different sequence (8). Although the sequence of LHRH varies slightly among vertebrates, the hypothalamic LHRH appears to have the same structure in mammals and amphibians (28).

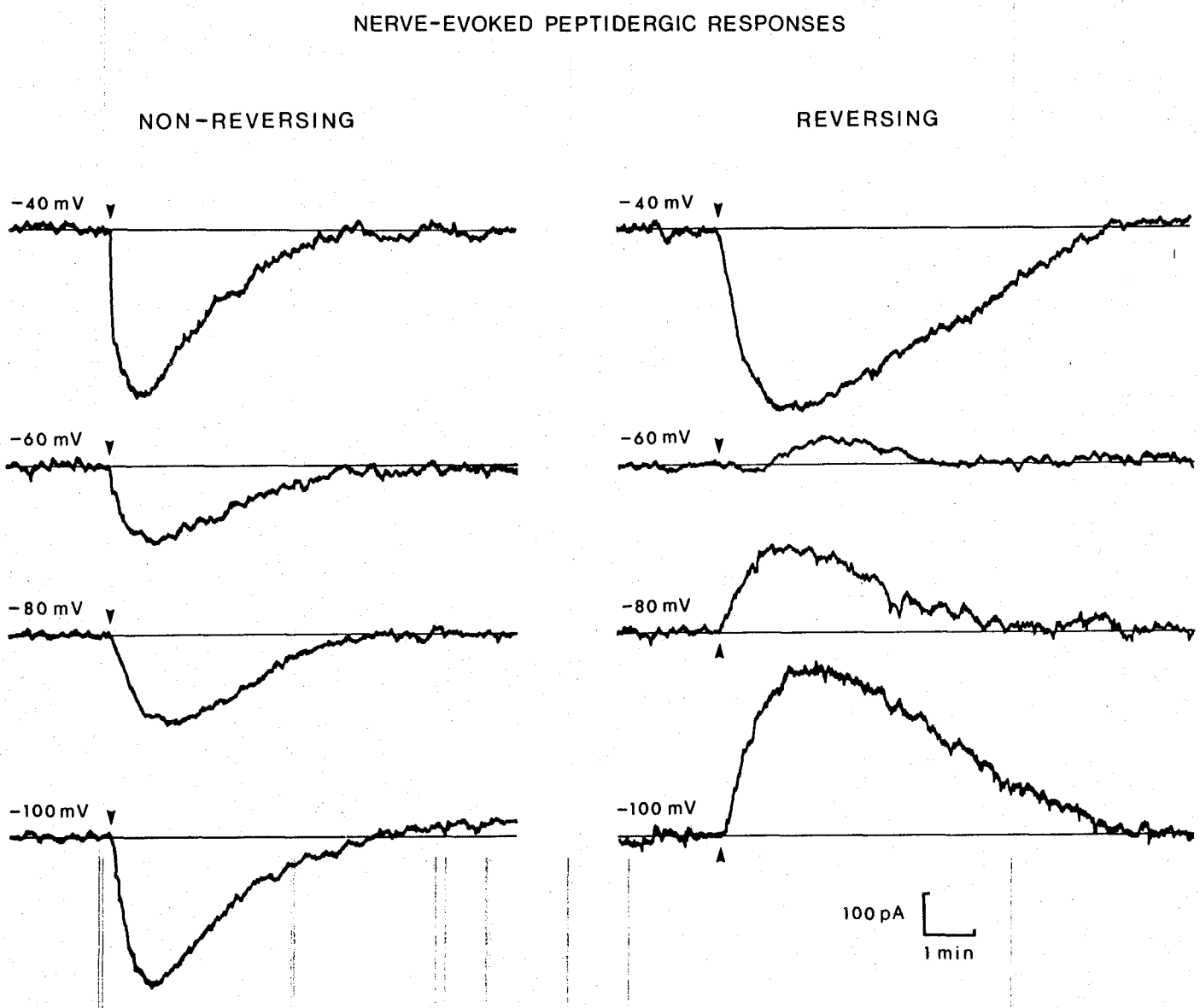
Taken together, these lines of evidence strongly favor the possibility that an LHRH-like peptide is a transmitter in these ganglia.

IONIC MECHANISMS ACTIVATED BY LHRH

Neurons in bullfrog sympathetic ganglia lack dendrites and are well suited for voltage clamp analysis. The preganglionic cholinergic axons cover a substantial fraction of the axon hillock and cell body with synaptic boutons. In the large B neurons of the ninth and tenth ganglia, the peptidergic inputs can be stimulated independently from the cholinergic inputs. A train of stimuli to the seventh and eighth spinal nerves carrying the peptidergic fibers produces an inward current in all ganglion cells when they are voltage clamped at their resting potentials (Fig. 3).

The voltage dependence of the peptidergic current varied among ganglion cells, as shown for two representative cells in Fig. 3. In about half of the ganglion cells the synaptic current increased when the cell was clamped at membrane potentials that were either depolarized or hyperpolarized from resting potential. In contrast, for a few

Figure 3. Nerve-evoked peptidergic responses in two representative B neurons in sympathetic ganglia of the bullfrog. Cells from the ninth or tenth ganglia in an isolated chain of ganglia were voltage clamped at the membrane potential indicated to the left of each current recording and the seventh and eighth spinal nerves carrying peptidergic fibers were stimulated at 20 Hz for 5 s at the position of the arrow. The cell on the left responded with an inward current at all membrane potentials tested. In the cell on the right the current reversed polarity; however, the early part of the response reversed at a somewhat different membrane potential than the later part. At −60 mV, for example, the response is nearly null for the first minute after stimulation, but the later portion of the response has already reversed in polarity. The resting potentials of these cells were around −50 mV and the external $[K^+]$ was 2 mM. A single-electrode voltage clamp was used; the electrodes had resistances of about 20 MΩ and were filled with 3 M KCl. From S. W. Kuffler and T. J. Sejnowski (unpublished).



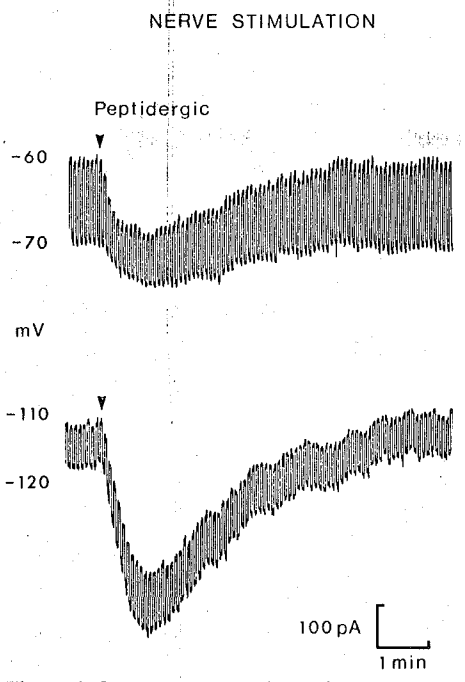


Figure 4. Current responses in a voltage-clamped sympathetic ganglion cell after stimulation of the peptidergic nerves at 20 Hz for 5 s. The membrane potential was alternately clamped every 5 s between the two voltages indicated to the left of each current recording. After a voltage jump between two clamped potentials, a fast jump in the holding current occurs that relaxes to a new steady-state equilibrium in less than 1 s (4); the envelope of the current steps simultaneously provides the slow membrane currents during the response at two clamped voltages. In addition, the change in the amount of current required to step between the two potentials during the response is a measure of the change in steady-state conductance of the membrane. At the peak of the response 40% less steady-state current was required to step between −60 and −70 mV compared to the current required to step the same 10 mV before the response. At −60 mV both the conductance and voltage sensitivity of the conductance are altered during the response because the instantaneous current jump after a voltage jump from −60 to −70 mV decreased by 32% at the peak of the response and the relaxing component of the current jump decreased by 46%. In contrast, 35% more current was required at the peak of the response when stepping between −110 and −120 mV. At these hyperpolarized potentials the current jump had no relaxing component. From S. W. Kuffler and T. J. Sejnowski (unpublished).

neurons the polarity of the current changed from an inward to an outward current at hyperpolarized potentials. In addition, neurons with intermediate types of responses were occasionally found in which it was possible to reverse the polarity of the first minute of the response (as for the reversing type), but the later portion of the response could not be reversed at any clamped potential between −30 and −120 mV (as for the nonreversing type). This diversity of voltage dependence of peptidergic responses was seen among ganglion cells that could not otherwise be distinguished on morphological grounds in the living preparation or on their electrical properties such as their resting potentials and action potentials. Cells that have been dissociated from ganglia have no axons, but their responses to externally applied LHRH exhibit a similar diversity; thus, the soma of a ganglion cell is itself capable of generating peptidergic responses and the diversity cannot be attributed to the axon.

Conductance measurements were made during peptidergic responses using a voltage jump technique, as illustrated in Fig. 4. A decrease in the membrane conductance occurs in most cells of both the reversing and nonreversing types when clamped near the resting potential during peptidergic responses to nerve stimulation or to the external application of LHRH. Examples of conductance decreases in response to a chemical transmitter have been reported at several other synapses, including a slow muscarinic synapse in sympathetic ganglion cells of the frog (53), a molluscan serotonergic synapse (10), and at synapses in the carp retina (22).

Ganglion cells in the frog have a voltage-sensitive K^+ conductance, called the M current, which decreases when LHRH is applied to the bathing solution (1). Although a decrease in the K^+ conductance is probably involved in the nerve-evoked peptidergic response, it cannot by itself explain the variety of responses that have been observed. First, the M current is inactivated at membrane potentials more negative than −60 mV; however, a synaptic current is measured in nearly all cells at membrane potentials hyperpolarized beyond −60 mV, whether or not the polarity has reversed (Fig. 3). Second, in cells for which the polarity of the peptidergic response did reverse, it was generally not possible to find a potential at which the response was null for the duration of the response, as might be expected for a single mechanism (Fig. 3). Third, in some ganglion cells a conductance increase occurs when the cell is clamped at hyperpolarized potentials (Fig. 4).

Nishi and Katayama (23, 24, 41) have suggested that two mechanisms may contribute to the peptidergic response: a conductance decrease to K^+ that dominates near the resting potential in nearly all cells, and a conductance increase, perhaps to Na^+ and K^+, that is most apparent at hyperpolarized potentials. The diversity of the observed responses could be explained if the relative proportion of these two mechanisms were to vary from cell to cell. This hypothesis can be tested directly by recording from single channels with patch recording techniques.

COMPARISON WITH CHOLINERGIC TRANSMISSION

Acetylcholine is known to be a preganglionic transmitter in sympathetic ganglia and only recently has there been evidence for other transmitters. Fast cholinergic transmission in sympathetic ganglia is similar to the well-studied fast cholinergic transmission between vertebrate motor neurons and skeletal muscle: acetylcholine is released from nerve terminals, binds to receptors in the postsynaptic membrane, and opens ionic channels, a sequence of events that takes only a few milliseconds (27). Two putative peptide transmitters in sympathetic ganglia, substance P and LHRH, share some features with acetylcholine and other classical transmitters, but have actions that are different. Like fast cholinergic transmission, the peptides are released from nerve terminals on depolarization in the presence of Ca^{2+}; however, the peptidergic response lasts for several minutes.

A part of the long duration of the peptidergic responses in sympathetic ganglia could be due to slow delivery of the transmitter. In lumbar paravertebral ganglia of the frog, synaptic boutons with LHRH-like immunoreactivity are found mainly around a population of small ganglion cells, which may be C cells; inasmuch as most large B cells respond to stimulation of the peptidergic inputs, the transmitter may diffuse to them from neighboring cells (18, 19). Many other factors could also contribute to the long duration of the peptidergic responses, including an intracellular second messenger, as proposed for other slow synaptic responses in sympathetic ganglia (11) and reviewed elsewhere (12, 37).

Cyclic nucleotides do not appear to be second messengers for the response to LHRH in sympathetic ganglia of the frog because the application of dibu-

tyryl cyclic AMP (dibutyryl cAMP) or dibutyryl cyclic GMP (dibutyryl cGMP) to the bathing solution or the intracellular injection of cAMP or cGMP did not mimic the nerve-evoked response (41). However, cAMP may *modulate* peptidergic transmission: the peptidergic response of ganglion cells to nerve stimulation is augmented by bath-applied catecholamines acting on β-adrenergic receptors, and this augmentation is mimicked by bath-applied dibutyryl cAMP, intracellularly injected cAMP, or bath-applied isobutyl methylxanthine, a phosphodiesterase inhibitor, but not by bath-applied dibutyryl cGMP or intracellularly injected cGMP (41). Interestingly, catecholamines and dibutyryl cAMP also potentiate the fast cholinergic response in the same ganglion cells, apparently by increasing the release of acetylcholine from presynaptic terminals (32). Although frog sympathetic ganglia have a sparse population of small intensely fluorescent cells containing catecholamines, no physiological role has yet been established for them (52).

During fast cholinergic transmission, the conductance of the membrane briefly increases as ionic channels are opened. The principal action of LHRH on neurons in the paravertebral sympathetic ganglia of the frog and of substance P on the inferior mesenteric ganglia of the guinea pig is to decrease the conductance of the membrane. Under some conditions conductance increases also occurred in neurons from both preparations; thus, several mechanisms may contribute to the peptidergic responses in these two ganglia and similar mechanisms may be found at other peptidergic synapses. The application of substance P to neurons in the myenteric plexus of the guinea pig small intestine also causes a slow depolarization that is accompanied by a conductance decrease (25, 26). It will be of interest to examine this peptidergic response at hyperpolarized potentials to see if a conductance increase can be detected as well.

CONCLUSION

Sympathetic ganglia were once thought to be simple excitatory relay stations but some are now believed to have integrative functions because individual ganglion cells receive converging inputs from spinal nerves and generate several types of postsynaptic responses with a wide range of time scales. For example, the interaction of three different synaptic signals can be studied in single paravertebral ganglion cells of the frog that differ in their duration by factors of up to 10,000 (33), and interactions in the inferior mesenteric ganglion of the guinea pig may be even more complicated (31).

The functional role of peptides in the central nervous system (CNS) is uncertain. In frog sympathetic ganglia the peptidergic signal increases the excitability of neurons for many minutes, both by bringing the membrane potential of the cell closer to threshold and by increasing the membrane resistance, thereby enhancing the effectiveness of fast cholinergic signals (46). Unexpectedly, however, the size of a third excitatory postsynaptic potential, a slow atropine-sensitive cholinergic response, is reduced during the peptidergic response (21). It appears that the activation of distinct muscarinic and peptidergic receptors control shared ionic mechanisms in single ganglion cells (18, 49, and S. W. Kuffler, T. J. Sejnowski, unpublished).

The study of peptidergic transmission in the autonomic nervous system has barely begun. Although we have fragmentary knowledge of peptidergic mechanisms in a few ganglia, the function of these responses in the animal is not yet known and their general applicability to the CNS remains to be established. FP

I am indebted to the late Stephen Kuffler with whom I have collaborated on some of the experiments reported here and to my colleagues in the Department of Neurobiology for their comments on the manuscript.

REFERENCES

1. **Adams, P. R.; Brown, D. A.** Luteinizing hormone-releasing factor and muscarinic agonists act on the same voltage-sensitive K^+-current in bullfrog sympathetic neurones. *Br. J. Pharmacol.* 68: 353–355; 1980.
2. **Akagi, H.; Otsuka, M.; Yanagisawa, M.** Identification by high-performance liquid chromatography of immunoreactive substance P released from isolated rat spinal cord. *Neurosci. Lett.* 20: 259–263; 1980.
3. **Baker, S. C.; Cuello, A. C.; Matthews, M. R.** Substance P-containing synapses in a sympathetic ganglion, and their possible origin as collaterals from sensory nerve fibers. *J. Physiol. (London)* 308: 76P–77P; 1980.
4. **Brown, D. A.; Adams, P. R.** Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neuron. *Nature (London)* 283: 673–676; 1980.
5. **Dun, N. J.; Jiang, Z. G.** Non-cholinergic excitatory transmission in inferior mesenteric ganglia of the guinea-pig: possible mediation by substance P. *J. Physiol. (London)* 325: 145–159; 1982.
6. **Dun, N. J.; Karczmar, A. G.** Actions of substance P on sympathetic neurons. *Neuropharmacology* 18: 215–218; 1979.
7. **Dun, N. J.; Minota, S.** Effects of substance P on neurons of the inferior mesenteric ganglia of the guinea-pig. *J. Physiol. (London)* 321: 259–271; 1981.
8. **Eiden, L. E.; Eskay, R. L.** Characterization of LRF-like immunoreactivity in the frog sympathetic ganglia: non-identity with LRF decapeptide. *Neuropeptides* 1: 29–38; 1980.
9. **Elvin, L.-G.; Dalsgaard, C. J.** Retrograde axonal transport of horseradish peroxidase in afferent fibers of the inferior mesenteric ganglion of the guinea pig. Identification of the cells of origin in dorsal root ganglia. *Brain Res.* 126: 149–153; 1977.
10. **Gerschenfeld, H. M.; Paupardin-Tritsch, D.** Ionic mechanisms and receptor properties underlying the responses of molluscan neurons to 5-hydroxytryptamine. *J. Physiol. (London)* 243: 427–456; 1974.
11. **Greengard, P.** Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. *Nature (London)* 260: 101–108; 1976.
12. **Hartzell, H. C.** Mechanisms of slow postsynaptic potentials. *Nature (London)* 291: 539–544; 1981.
13. **Hökfelt, T.; Elfvin, L.-G.; Elde, R.; Schultzberg, M.; Goldstein, M.; Luft, R.** Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. *Proc. Natl. Acad. Sci. USA* 74: 3587–3591; 1977.
14. **Hökfelt, T.; Elfvin, L.-G.; Schultzberg, M.; Fuxe, K.; Said, S. I.; Mutt, V.; Goldstein, M.** Immunohistochemical evidence of vasoactive intestinal polypeptide-containing neurons and nerve fibers in sympathetic

ganglia. *Neuroscience* 2: 885–896; 1977.

15. **Hökfelt, T.; Elfvin, L.-G.; Schultzberg, M.; Goldstein, M.; Nilsson, G.** On the occurrence of substance P-containing fibers in sympathetic ganglia: immunohistochemical evidence. *Brain Res.* 132: 29–41; 1977.
16. **Hökfelt, T.; Johansson, O.; Ljungdahl, A.; Lundberg, J. M.; Schultzberg, M.** Peptidergic neurones. *Nature (London)* 284: 515–521; 1980.
17. **Hökfelt, T.; Kellerth, J.-O.; Nilsson, G.; Pernow, B.** Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. *Brain Res.* 100: 235–252; 1975.
18. **Jan, L. Y.; Jan, Y. N.** Peptidergic transmission in sympathetic ganglia of the frog. *J. Physiol. (London)* 327: 219–246; 1982.
19. **Jan, L. Y.; Jan, Y. N.; Brownfield, M. S.** Peptidergic transmitters in synaptic boutons of sympathetic ganglia. *Nature (London)* 288: 380–382; 1980.
20. **Jan, Y. N.; Jan, L. Y.; Kuffler, S. W.** A peptide as a possible transmitter in sympathetic ganglia of the frog. *Proc. Natl. Acad. Sci. USA* 76: 1501–1505; 1979.
21. **Jan, Y. N.; Jan, L. Y.; Kuffler, S. W.** Further evidence for peptidergic transmission in sympathetic ganglia. *Proc. Natl. Acad. Sci. USA* 77: 5008–5012; 1980.
22. **Kaneko, A.; Shimazaki, H.** Synaptic transmission from photoreceptors to bipolar and horizontal cells in the carp retina. *Cold Spring Harbor Symp. Quant. Biol.* 40: 537–546; 1976.
23. **Katayama, Y.; Inokuchi, H.; Nishi, S.** Voltage clamp study of the late slow excitatory postsynaptic current in bullfrog sympathetic ganglion cells. *Neurosci. Lett. Suppl.* 6: S63; 1981.
24. **Katayama, Y.; Nishi, S.** The ionic mechanism of the late slow EPSP in amphibian sympathetic ganglion cells. *Proc. Int. Union Physiol. Sci.* 13: 371; 1977.
25. **Katayama, Y.; North, R. A.** Does substance P mediate slow synaptic excitation within the myenteric plexus? *Nature (London)* 274: 387–388; 1978.
26. **Katayama, Y.; North, R. A.; Williams, J. T.** The action of substance P on neurons of the myenteric plexus of the guinea-pig small intestine. *Proc. R. Soc. London* B206: 191–208; 1979.
27. **Katz, B.** *The release of neural transmitter substances.* Springfield, OH: Charles C Thomas; 1969.
28. **King, J. A.; Millar, R. P.** Heterogeneity of vertebrate luteinizing hormone-releasing hormone. *Science* 206: 67–69; 1979.
29. **Konishi, S.; Tsunoo, A.; Otsuka, M.** Enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia. *Nature (London)* 282: 515–516; 1979.
30. **Konishi, S.; Tsunoo, A.; Otsuka, M.** Substance P and noncholinergic excitatory synaptic transmission in guinea-pig sympathetic ganglia. *Proc. Jpn. Acad.* 55B: 525–530; 1979.
31. **Konishi, S.; Tsunoo, A.; Yanaihara, N.; Otsuka, M.** Peptidergic excitatory and inhibitory synapses in mammalian sympathetic ganglia: roles of substance P and enkephalin. *Biomed. Res.* 1: 528–536; 1980.
32. **Kuba, K.; Kato, E.; Kumamoto, E.; Koketsu, K.; Hirai, K.** Sustained potentiation of transmitter by adrenaline and dibutyryl cyclic AMP in sympathetic ganglia. *Nature (London)* 291: 654–656; 1981.
33. **Kuffler, S. W.** Slow synaptic responses in autonomic ganglia and the pursuit of a peptidergic transmitter. *J. Exp. Biol.* 89: 257–286; 1980.
34. **Larsson, L. I.; Rehfeld, J. F.** Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. *Brain Res.* 165: 201–218; 1979.
35. **Lundberg, J. M.; Hökfelt, T.; Anggard, A.; Kimmel, J.; Goldstein, M.; Markey, K.** Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons. *Acta Physiol. Scand.* 110: 107–109; 1980.
36. **Matthews, M. R.; Cuello, A. C.** Substance P-immunoreactive peripheral branches of sensory neurons innervate guinea pig sympathetic neurons. *Proc. Natl. Acad. Sci. USA* 79: 1668–1672; 1982.
37. **McAfee, D. A.; Henon, B. K.; Whiting, G. J.; Horn, J. P.; Yarowsky, P. J.; Turner, D. K.** The action of cAMP and catecholamines in mammalian sympathetic ganglia. *Federation Proc.* 39: 2997–3002; 1980.
38. **Minota, S.; Dun, N. J.; Karczmar, A. G.** Substance P depolarization of sympathetic neurons: not simple K^+ inactivation. *Brain Res.* 216: 224–228; 1981.
39. **Neild, T. O.** Slowly-developing depolarization of neurones in the guinea-pig inferior mesenteric ganglion following repetitive stimulation of the preganglionic nerves. *Brain Res.* 140: 231–239; 1978.
40. **Nicoll, R. A.; Schenker, C.; Leeman, S. E.** Substance P as a transmitter candidate. *Annu. Rev. Neurosci.* 3: 227–268; 1980.
41. **Nishi, S.; Katayama, Y.** The non-cholinergic excitatory transmission in sympathetic ganglia. Salanki, J., ed. *Advances in Physiological Science, Vol. 4, Physiology of excitable membranes.* New York: Pergamon; 1981: 322–327.
42. **Nishi, S.; Koketsu, K.** Early and late afterdischarges of amphibian sympathetic ganglion cells. *J. Neurophysiol.* 31: 109–118; 1968.
43. **Otsuka, M.; Konishi, S.** Substance P and excitatory transmitter of primary sensory neurons. *Cold Spring Harbor Symp. Quant. Biol.* 40: 135–143; 1976.
44. **Otsuka, M.; Takahashi, T.** Putative peptide neurotransmitters. *Annu. Rev. Pharmacol. Toxicol.* 17: 425–439; 1977.
45. **Otsuka, M.; Yanagisuwa, M.** The effects of substance P and baclofen on motoneurons of isolated spinal cord of the newborn rat. *J. Exp. Biol.* 89: 201–214; 1980.
46. **Schulman, J. A.; Weight, F. F.** Synaptic transmission: long-lasting potentiation by postsynaptic mechanism. *Science* 194: 1437–1439; 1976.
47. **Schultzberg, M.; Hökfelt, T.; Lundberg, J. M.** Peptide neurons in the autonomic nervous system. Eranko, O.; Soinila, S.; Paivarinto, H., eds. *Histochemistry and cell biology of autonomic neurons, SIF cells, and paraneurons.* New York: Raven; 1980: 341–348.
48. **Schultzberg, M.; Hökfelt, T.; Terenius, L.; Elfvin, L. G.; Lundberg, J. M.; Brandt, J.; Elde, R. P.** Enkephalin immunoreactive nerve fibres and cell bodies in sympathetic ganglia of the guinea-pig and rat. *Neuroscience* 4: 249–270; 1979.
49. **Sejnowski, T. J.; Kuffler, S. W.** Integration of peptidergic and muscarinic synaptic transmission in amphibian sympathetic ganglion neurons. *Soc. Neurosci. Abst.* 7: 726; 1981.
50. **Snyder, S.** Brain peptides as neurotransmitters. *Science* 209: 976–983; 1980.
51. **Vale, W.; Rivier, C.; Brown, M.; Rivier, J.** Pharmacology of thyrotropin releasing factor (TRF) and luteinizing hormone releasing factor (LRF) and somatostatin. Porter, J. C., ed. *Hypothalamic peptide hormones and pituitary regulation.* New York: Plenum; 1977: 123–156.
52. **Weight, F. F.; Smith P. A.** Small intensely fluorescent cells and the generation of the slow postsynaptic inhibition in sympathetic ganglia. Eranko, O.; Soinila, S.; Paivarinto, H., eds. *Histochemistry and cell biology of autonomic neurons, SIF cells, and paraneurons.* New York: Raven; 1980: 159–171.
53. **Weight, F. F.; Votava, J.** Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science* 170: 755–758; 1970.