PUTATIVE PEPTIDE NEUROTRANSMITTERS

♣6689

Masanori Otsuka and Tomoyuki Takahashi
Department of Pharmacology, Faculty of Medicine, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, Japan

The purpose of this review is to examine the present status of peptides as putative neurotransmitters. Special emphasis is placed on the evaluation of evidence for or against the transmitter role of peptides, particularly in mammalian CNS, according to the well-established criteria for transmitter identification (1, 2). The field of peptide transmitters is a new and rapidly developing one, and therefore, we can derive from the available results only tentative views as to whether or not certain peptides are likely to be transmitters. Although a number of peptides are known to influence various neural activities such as behavior and sleep (3–5), this review deals only with the peptides of known structures for which there is certain or definite neurochemical as well as electrophysiological evidence implicating their transmitter functions.

The notion that certain nerve cells secrete peptides from their axon terminals has been well known in the field of endocrinology since the early 1950s (6). Bargmann et al (7) proposed the term *peptidergic neuron* for hypothalamic neurosecretory cells, and suggested that these neurons not only release peptide hormones into the blood stream but also form peptidergic synapses on endocrine epithelial cells. Recent discovery of the powerful effects of physalaemin (8) and vasopressin on central neuronal activities (9) in 1971 sparked interest in the possibility that certain peptides may serve as neurotransmitters in mammalian CNS (10). This was timely because the recent progress in peptide chemistry was ready to promote the subsequent development of the field of peptide neuropharmacology. A considerable amount of data has accumulated that suggests the transmitter role of certain peptides. Particularly good evidence has been obtained for two peptides, substance P and proctolin, which are described below in detail.

SUBSTANCE P

In 1931, Euler & Gaddum (11) detected a smooth muscle–stimulating and vasodilating agent in extracts of equine brain and intestine. This agent was called substance P (12, 13) and was shown to be of peptide nature (14). Studies of substance P

distribution in the early 1950s (15–19) revealed that mammalian spinal dorsal root contains a larger amount of substance P than the ventral root. Based on these results, Lembeck proposed a hypothesis that substance P may be an excitatory transmitter of primary sensory neurons (16). The studies of substance P, however, had long been hampered by the ambiguity of its chemical nature. In fact, no pure substance P preparation was available, and for many years Lembeck's hypothesis could not be properly tested. In the early study of Galindo et al (20) with the crude preparation, no direct action of substance P on cat central neurons could be detected.

A breakthrough in this field was made by the recent studies of Leeman and her colleagues, who succeeded in purifying substance P from bovine hypothalamus, determining its structure as an undecapeptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2), and synthesizing the peptide (21–23). Before the discovery of the structure of the undecapeptide substance P, the definition of substance P was based on its pharmacological activities (24). Thus so-called substance P was postulated to comprise multiple peptides (25–27). However, it is now generally agreed that substance P denotes the peptide characterized by Chang et al (22).

Evidence for a Transmitter of Primary Sensory Neurons

PRESENCE OF SUBSTANCE P IN PRIMARY SENSORY NEURONS Preferential distribution of substance P in spinal dorsal root as opposed to ventral root (15–19) was confirmed by recent studies using radioimmunoassay as well as bioassay combined with column chromatography (28–30). Thus it was revealed that the concentration of substance P in bovine and feline dorsal root is 9–27 times higher than that in the ventral root. Furthermore, the chemical, pharmacological, and immunological properties of substance P extracted from bovine dorsal root were shown (28, 29) to be identical with those of substance P extracted from hypothalamus and characterized by Chang et al (22). In order to examine further the relation between substance P and primary sensory neurons, Takahashi & Otsuka (30, 31), using bioassay, examined the distribution of substance P in cat spinal cord. It was thus shown that substance P is particularly concentrated in dorsal horn where a large part of the primary afferent fibers terminates and forms synapses (32). The highest level of substance P was found in the dorsal part of dorsal horn. After sectioning the incoming dorsal roots, the level of the peptide in the dorsal horn was markedly reduced. Furthermore, when the dorsal root was ligated, a large amount of substance P accumulated on the ganglion side of the ligature, which suggests that substance P is synthesized in spinal ganglia and transported through the dorsal root to their nerve terminals in the cord (30, 31). All these findings by bioassay were recently confirmed by immunohistochemical studies of Hökfelt and his colleagues, who showed that substance P-like immunoreactivity is selectively localized in nerve fibers of laminae I–III of spinal cord of the rat and cat (33, 34). The latter finding agrees with the results of subcellular fractionation studies using bioassay (35–37) as well as radioimmunoassay (38, 39), which indicate that substance P is most concentrated in synaptosomal fraction.

ACTION OF SUBSTANCE P ON SPINAL NEURONS Application of synthetic substance P in quite low concentration induced a depolarization of motoneurons in

isolated frog spinal cord (28, 40, 41). The depolarizing potency of substance P was, on a molar basis, about 200 times higher than that of L-glutamate, another excitatory transmitter candidate (42). Substance P-induced depolarization of frog spinal motoneurons was accompanied by the increase of membrane conductance (41). In order to examine the possibility that substance P may depolarize the motoneurons by a transsynaptic mechanism, substance P was applied after the synaptic transmission in the spinal cord was blocked by reducing Ca concentration in the medium or by adding tetrodotoxin (40, 41). Substance P still induced similar depolarizing responses indicating that the peptide acts directly on the motoneurons.

Recently the effect of substance P on mammalian spinal cord was studied. For this purpose, an isolated spinal cord preparation of the newborn rat was developed (43). Substance P was applied into the perfusion bath in known concentrations and the effects were recorded either extracellularly from the ventral root or intracellularly from the motoneurons. Substance P again induced a depolarization accompanied by high frequency spike discharges of the motoneurons (44–46). The depolarizing potency of substance P was 1000–9000 times higher than that of L-glutamate. When the preparation was soaked in a low Ca (0.2 mM) and high Mg (5 mM) medium, the spinal reflexes were completely blocked. Repetitive stimulation of the dorsal root did not produce any detectable synaptic potentials when recorded from the ventral root. Under such conditions, substance P still produced a similar depolarizing response although the dose-response curve was slightly displaced to the right. These results suggest that substance P causes the depolarization of rat motoneurons by both direct effect on the motoneurons and indirect effect activating the excitatory interneurons synapsing with the motoneurons (44–46; S. Konishi and M. Otsuka, unpublished). There is also evidence that substance P activates the inhibitory interneurons in the spinal cord (44). Electrophoretic application of substance P also produced an excitant effect on dorsal horn neurons and cuneate neurons of the cat (47, 48).

ANTAGONISTS OF SUBSTANCE P Several substances, e.g. AMP, cystine-di-β-naphthylamide, and trimethaphan camphorsulfonate (Arfonad®), were reported to antagonize the action of substance P on guinea pig ileum (49). It may be interesting to see whether or not these compounds antagonize the excitant action of substance P on central neurons. It was recently reported that baclofen [β-(4-chlorophenyl)-γ-aminobutyric acid, Lioresal®] antagonizes the depolarizing action of substance P on rat spinal motoneurons (45, 46). The depolarizing action of L-glutamate was also reduced, but to a smaller extent (45, 46; cf 50). Since baclofen readily blocked the monosynaptic and polysnaptic reflexes as well as the dorsal root potential (45, 46, 50–52), it was proposed that baclofen blocks the primary afferent transmission by antagonizing the transmitter action of substance P (45, 46). The antagonism between baclofen and substance P was also observed by electrophoretic application on neurons of cat spinal cord and brain, although the excitant effects of L-glutamate and acetylcholine were also depressed by baclofen in some but not all cells (53–55). Further studies are needed to clarify the mechanism of action of baclofen.

STRUCTURE-ACTIVITY RELATIONSHIP From the studies on frog and rat spinal motoneurons, it has become evident that the C-terminal amino acid sequence is essential for the motoneuron-depolarizing activity of substance P (40, 46, 56, 57). Omission of one to five amino acids at the N-terminus did not cause any serious loss of the motoneuron-depolarizing activity. Two C-terminal analogues of substance P, i.e. hepta- and hexapeptides, were considerably more active than the undecapeptide substance P. By contrast, when the C-terminal methionine was omitted, the depolarizing activity was completely lost (46, 57). Hypotensive and gut-contracting activities of the substance P analogues were roughly parallel with their motoneuron-depolarizing activities (58).

RELEASE OF SUBSTANCE P Early studies of Angelucci (59) and Ramwell et al (60) showed the presence of substance P-like activity in the perfusate of frog spinal cord. Recently, the isolated spinal cord of newborn rat was perfused, and the perfusates were analyzed by radioimmunoassay for substance P (61). Repetitive stimulation of dorsal roots caused a marked increase of substance P–like immunoreactivity in the perfusate. By contrast, when the preparation was perfused with a low Ca and high Mg medium where the synaptic transmission was completely blocked, the same stimulation of dorsal roots produced no change of the immunoreactivity. Perfusion of the spinal cord with high K (55 mM) solution caused a large increase in release of substance P–like immunoreactivity. Potassium-evoked release of substance P–like immunoreactivy was also observed from rat hypothalamic slices, and this release was completely abolished in the low Ca and high Mg medium (62).

INACTIVATION OF SUBSTANCE P There are two kinds of mechanisms known to inactivate transmitter substances, i.e. enzymatic breakdown and reuptake. When slices of rat spinal cord, substantia nigra, and hypothalamus were incubated with ^{125}I-labeled substance P, no accumulation of the peptide into the tissues could be detected (33, 62, 63). On the other hand, the presence of substance P-inactivating enzyme system in various regions of CNS, e.g. basal ganglia, hypothalamus, and spinal cord, was reported by many workers (64–70). The inactivating enzyme was extracted and partially purified from mammalian brain (69, 70). Studies of detailed distribution of this enzyme in nervous system will help to elucidate its physiological role. Furthermore, it will be interesting to find specific inhibitors of the substance P–inactivating enzyme and to examine their effect on primary afferent transmission in the spinal cord. In this connection, Krivoy reported that lysergic acid diethylamide (LSD-25) inhibits the enzymatic inactivation of substance P by neural tissue extracts, and also enhances the fourth wave of the dorsal root potential in the cat (67, 71).

STATUS OF SUBSTANCE P AS A SENSORY TRANSMITTER Among the criteria for transmitter identification, the presence in presynaptic neurons and the release in response to presynaptic stimulation have been fairly well established for substance P as a transmitter of primary afferent fibers. Further studies are needed to clarify

the details of substance P action, e.g. the reversal potential and ionic mechanism, its inactivation process at synaptic site, and its synthesis. However, judging from the data available at the present time, substance P seems likely to be an excitatory transmitter of spinal dorsal root fibers.

The question arises whether substance P is a transmitter of all dorsal root fibers or of only a part of them. Immunohistochemical studies of Hökfelt et al (34, 72) showed that only 10–20% of neurons in the rat spinal ganglia were substance P–positive. However, the possibility cannot be excluded that many more spinal ganglion cells contain substance P, because in the cat spinal ganglia the substance P–positive fluorescence could be demonstrated in the cell bodies only after preventing the axonal transport of the peptide (33). Henry (54, 73) showed that electrophoretically applied substance P caused excitation of cat spinal neurons which were activated by noxious stimuli applied to the skin and suggested that substance P is specifically related to nociception.

An important question is whether or not substance P is an excitatory transmitter of Group Ia fibers that monosynaptically excites spinal motoneurons. Some observations are consistent with the transmitter role of the peptide in this place. Namely, substance P exerts a direct depolarizing action on spinal motoneurons (40, 41, 44, 46); spinal monosynaptic reflex is blocked by baclofen which antagonizes the depolarizing action of substance P (45, 46); and the level of substance P in the ventral horn is slightly reduced after the section of dorsal roots (30). To settle this question it may be crucial to find the drugs that correspond to curare or eserine in cholinergic synapse, and then to examine the effect of these drugs on monosynaptic excitatory postsynaptic potential (EPSP) recorded in the motoneurons.

When substance P was applied to the isolated rat spinal cord, the time course of the depolarization of motoneurons was slightly slower than that of glutamate-induced depolarization (44-46). This may be at least partly due to the diffusion of the peptide through the cord tissue. Krnjević and his colleagues (47, 48), on the other hand, reported that the excitant effect of electrophoretically applied substance P on cuneate and spinal neurons appeared with a delay of 10–30 sec and persisted for 1–2 min after the end of application. In the experiment of Walker et al (74) in brain stem neurons, however, the effect of electrophoretically applied substance P appeared within 4 sec and subsided with a similarly fast time course. It would be desirable to examine the effect of substance P by iontophoretic application on synaptic sites of central neurons under visual control (75), possibly by using thin tissue slices or neurons in cell culture.

Henry et al (48) showed that substance P potentiated the excitant effect of glutamate on cat spinal neurons, and proposed that the function of substance P is a form of sensitization or modulation. However, the simultaneous application of substance P and L-glutamate on the motoneurons in isolated rat or frog spinal cord produced a simply additive effect (41, 46).

Other Possible Transmitter Functions of Substance P

Substance P is widely and selectively distributed in mammalian CNS (17–19, 76–78). Particularly high levels of substance P were found in trigeminal nerve nucleus,

hypothalamus, substantia nigra, etc. Immunohistochemical studies of Nilsson et al (79) suggested that substance P is concentrated in nerve endings in hypothalamus and other regions of rat CNS. Electrophoretic application of substance P on cerebral cortical as well as substantia nigral neurons caused excitatory responses (55, 74, 80). Duffy & Powell (81) reported that substance P stimulates the brain adenylate cyclase activity.

An interesting possibility is that substance P may be released from the peripheral nerve terminals of primary sensory neurons and may serve as a transmitter of axon reflex vasodilatation. Dale in 1935 already suggested that the transmitter of axon reflex vasodilatation is closely related to the transmitter released by sensory neurons at the central synapse (82). In this connection, Hökfelt et al (33) found substance P–positive fibers around blood vessels in the cat skin.

Substance P exerts a powerful stimulant action on various smooth muscles (11, 23, 29, 83). Immunohistochemical studies of Nilsson et al (84) showed the occurrence of substance P–positive fibers in mammalian gastrointestinal tract. Substance P extracted from equine intestine was shown by Studer et al (85) to be identical with substance P isolated from hypothalamus and characterized by Chang et al (22).

The above results suggest that substance P may serve as a transmitter in many places in mammalian central and peripheral nervous system.

PROCTOLIN

Brown (86) found in the extract of cockroach gut a substance that causes a contraction of longitudinal muscle of the gut. This factor was first called *gut factor.* The concentration of the gut factor in the nerves innervating intestinal muscle was up to 150 times greater than that in the thoracic peripheral nerves which innervate somatic muscle. This gut factor is present in considerable amount in the rectum, and it is depleted from the rectum after surgical section of the innervating nerves, which suggests that the gut factor is of neural origin. In the homogenate of rectum, the gut factor is associated with subcellular particles which may correspond to synaptic vesicles. Based on these findings, Brown proposed that the gut factor functions as an excitatory transmitter in insect intestinal muscle.

Recently the gut factor was purified and its structure determined as a pentapeptide (Arg-Tyr-Leu-Pro-Thr), which was named proctolin (87, 88). Synthetic proctolin in quite a low concentration (10^{-9} M) produced a contraction of the rectum, and the peptide was fully active on tetrodotoxin-treated or surgically denervated muscle, indicating that proctolin acts directly on muscle membrane. Tyramine (10^{-6} M) suppressed the responses evoked both by proctolin and by nerve stimulation. Furthermore, when a nerve-rectum preparation was perfused, an active substance with similar pharmacological activity as proctolin was released during repetitive nerve stimulation. Although further experiments are needed for elucidating, for example, the reversal potential of proctolin action and the identification of the active substance released from the nerve-rectum preparation, the above results provide impressive evidence for proctolin as a transmitter in cockroach gut. Starratt & Brown (88) found that a substance chemically indistinguishable from proctolin is present

in eight other species of insects and proposed that proctolin is a universal constituent of the Insecta.

CARNOSINE

The cell bodies of primary olfactory neurons lie in the nasal olfactory epithelium, and send their axons to the olfactory bulb where these axons form synapses with mitral and periglomerular cells. Margolis (89, 90) and Neidle & Kandera (91) found that carnosine (β-alanyl-L-histidine) is highly concentrated in the olfactory bulb of mouse, rat, and guinea pig. The concentration of carnosine in the olfactory bulb of the mouse was about 20 times higher than that in cerebral hemisphere (91). Carnosine occurs also in olfactory epithelium in uniquely high concentration (89, 90). Degeneration studies showed that both carnosine and carnosine synthetase are highly localized in the primary olfactory pathway (89, 90, 92). Based on these findings, Margolis proposed a hypothesis that carnosine is a neurotransmitter of the primary olfactory pathway (89, 90, 92). A crucial test of this hypothesis would be to see the effect of carnosine on mitral and periglomerular cells, but this has not yet been performed.

OTHER PEPTIDES

Several peptides occur in mammalian CNS and exert either excitant or depressant action on central neurons when applied by microiontophoresis. Although these peptides were proposed as central neurotransmitters, evidence is still far from convincing. It would be desirable to examine the possible transmitter role of each of these peptides at anatomically defined synapses according to the criteria of transmitter identification.

Enkephalin

The presence of the opiate receptor, which specifically binds to morphine agonists and its antagonist, was recently shown in mammalian brain and intestine (93–95). These findings prompted several workers to search for an endogenous substance which acts on this receptor and has pharmacological properties similar to morphine (96–98). Consequently, a peptide factor, termed *enkephalin* (98), was found from brain extract. Enkephalin inhibits competitively the binding of dihydromorphine and naloxone, a morphine antagonist, to opiate receptor (96, 99, 100). Like morphine, enkephalin inhibits neurally evoked contractions of vas deferens, and this effect is antagonized by naloxone (97, 98). Enkephalin is unevenly distributed in mammalian CNS and its distribution parallels that of the opiate receptor (97, 99, 101). Recently enkephalin was shown to be localized in the synaptosomal fraction (99, 102).

Hughes et al (103) identified enkephalin as comprising two pentapeptides, methionine enkephalin (Tyr-Gly-Gly-Phe-Met) and leucine enkephalin (Tyr-Gly-Gly-Phe-Leu). Although the hypothesis was proposed that enkephalin might serve as a neurotransmitter (100, 104), the hypothesis depends largely on the assumption

that morphine acts at certain synapses as an agonist of the transmitter, and this remains to be seen. Effects of enkephalin on neural activities were studied. Intracerebroventricular administration of enkephalin to mice resulted in an analgesic effect (105). Iontophoretic application of methionine enkephalin produced a depressant effect on brain stem neurons of the rat and cat, and this effect was blocked by naloxone in the rat (106) but not in the cat (107).

Hypothalamic Releasing Factors

So far three hypothalamic releasing factors were structurally identified, i.e. thyrotropin- releasing hormone (TRH) (108, 109), luteinizing hormone–releasing hormone (LHRH) (110, 111), and somatostatin (growth hormone release inhibiting factor) (112). TRH and somatostatin are widely distributed in mammalian hypothalamic and extrahypothalamic brain tissues (113–116) as well as in the spinal cord (72, 117, 118). With immunofluorescence and immunoperoxidase techniques, evidence was obtained that these releasing factors are localized in certain nerve terminals (72, 117–120). Hökfelt et al (72, 117) observed that somatostatin-like immunoreactivity is present in certain neuronal cell bodies in spinal ganglia and in fibers in the dorsal horn of the spinal cord, and suggested that somatostatin may play a role as a transmitter of primary sensory neurons. When administered by microiontophoresis, TRH, LHRH, and somatostatin produced a depressant or excitant effect on neurons of different regions of CNS (121–124).

Neurohypophysial Peptides

Vasopressin is synthesized primarily in supraoptic neurosecretory cells and released into the circulation from their axon terminals which are located in the neurohypophysis. Nicoll & Barker (5, 9) found that vasopressin administered by microiontophoresis decreased the firing rate of supraoptic neurosecretory cells. Based on this and other findings they proposed a hypothesis that this posterior pituitary hormone might also be released from recurrent collaterals of the supraoptic neurons and serve as the transmitter of recurrent inhibition. This hypothesis was supported by the observation of Vincent & Arnauld (125) that recurrent inhibition of supraoptic neurosecretory cells in the monkey disappeared after 5 days of water deprivation when a total depletion of vasopressin is expected to occur. However, a doubt as to the hypothesis has recently been raised by Dreifuss et al (126) who observed the recurrent inhibition of supraoptic neurosecretory cells in the Brattleboro strain of rats, which do not synthesize vasopressin.

Moss et al (127) observed that iontophoretically applied oxytocin excited selectively the paraventricular neurosecretory cells that produce oxytocin. The physiological meaning of this observation is unknown.

Angiotensin II

Mammalian brain contains angiotensin I and II (128, 129) as well as angiotensin-converting enzyme (130). Electrophoretic application of angiotensin II on supraoptic neurosecretory cells as well as on neurons of subfornical organ produced an excitant effect (131–134). The question remains whether this effect of angiotensin

reflects the transmitter action on these neurons or the response to the circulating angiotensin.

CONCLUSIONS

It now seems likely that a new class of substances, peptides, must be added to the list of neurotransmitters. Considerable evidence is available that substance P and proctolin are excitatory transmitters in mammalian primary sensory neurons and in insect peripheral nerves respectively. In view of the relatively small molecular weights of the established neurotransmitters, it may appear rather unexpected that a peptide of more than 1000 daltons serves a transmitter function. However, if we consider the many common characteristics shared by neurotransmission and neurosecretion (135), together with the fact that neurosecretory cells secrete various peptides, then the idea of peptide neurotransmitters may not be novel. In fact, it has been suggested by many authors that certain neurosecretory cells release peptides as hormones or releasing factors from some nerve endings into the circulation and the same peptides as neurotransmitters from other nerve endings at synapses (9, 121, 123, 125). This may be parallel with the case of primary sensory neurons which probably release substance P as excitatory transmitter from their intraspinal nerve endings and the same peptide as transmitter of axon reflex vasodilatation from the peripheral nerve endings contacting the blood vessels.

A particular advantage for the study of peptide neurotransmitters is the availability of immunological techniques. With a highly specific radioimmunoassay, it is possible to determine as little as 10^{-15} moles of substance P (38, 58, 136). Furthermore, immunohistochemical techniques enable one to locate specific peptide immunoreactivities at the light microscopic as well as electron microscopic level (33, 72, 117–120). Such techniques will provide a useful means for mapping specific neurons in CNS, and may also be useful in chemical pathology for revealing abnormalities in diseases.

Another promising field which may possibly be introduced by peptide transmitters is its pharmacology. Little is known about the possible pharmacological manipulation of peptidergic neurotransmission. With modern techniques of peptide chemistry it is probably not difficult to synthesize various peptides which act as agonists or antagonists of peptide transmitters. If we can synthesize peptides that penetrate into the CNS, a group of new drugs acting on the CNS may be introduced.

The question remains why the nervous system needs relatively large and possibly expensive molecules such as undecapeptide to transmit either an excitatory or inhibitory effect if precise neuronal connections are assured by anatomical contacts at synapses. For example, do primary afferent fibers need diverse peptides for mediating different modalities of sensation? Future studies will clarify whether or not the functions of peptide transmitters are explicable in terms of traditional concepts of chemical transmission.

Literature Cited

1. Eccles, J. C. 1964. *The Physiology of Synapses.* Berlin: Springer. 316 pp.
2. Werman, R. 1966. Criteria for identification of a central nervous system transmitter. *Comp. Biochem. Physiol.* 18:745–66
3. de Wied, D., Witter, A., Greven, H. M. 1975. Behaviorally active ACTH analogues. *Biochem. Pharmacol.* 24: 1463–68
4. Pappenheimer, J. R., Koski, G., Fencl, V., Karnovsky, M. L., Krueger, J. 1975. Extraction of sleep-promoting factor S from cerebrospinal fluid and from brains of sleep-deprived animals. *J. Neurophysiol.* 38:1299–1311
5. Barker, J. L. 1976. Peptides: Roles in neuronal excitability. *Physiol. Rev.* 56:435–52
6. Scharrer, E., Scharrer, B. 1954. Hormones produced by neurosecretory cells. *Recent Prog. Horm. Res.* 10:183–240
7. Bargmann, W., Lindner, E., Andres, K. H. 1967. Über Synapsen an endokrinen Epithelzellen und die Definition sekretorischer Neurone. *Z. Zellforsch. Mikrosk. Anat.* 77:282–98
8. Konishi, S., Otsuka, M. 1971. Actions of certain polypeptides on frog spinal neurons. *Jpn. J. Pharmacol.* 21:685–87
9. Nicoll, R. A., Barker, J. L. 1971. The pharmacology of recurrent inhibition in the supraoptic neurosecretory system. *Brain Res.* 35:501–11
10. Bloom, F. E. 1972. Amino acids and polypeptides in neuronal function. *Neurosci. Res. Program Bull.* 10:127–251
11. Euler, U.S.v., Gaddum, J. H. 1931. An unidentified depressor substance in certain tissue extracts. *J. Physiol. London* 72:74–87
12. Chang, H. C., Gaddum, J. H. 1933. Cholinc esters in tissue extracts. *J. Physiol. London* 79:255–85
13. Gaddum, J. H., Schild, H. 1934. Depressor substances in extracts of intestine. *J. Physiol. London* 83:1–14
14. Euler, U.S.v. 1936. Untersuchungen über Substanz P, die atropinfeste, darmerregende und gefäßerweiternde Substanz aus Darm und Hirn. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 181:181–97
15. Hellauer, H. 1953. Zur Charakterisierung der Erregungssubstanz sensibler Nerven. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 219: 234–41
16. Lembeck, F. 1953. Zur Frage der zentralen Übertragung afferenter Impulse. III. Mitteilung. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückenmarks. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 219:197–213
17. Pernow, B. 1953. Studies on substance P—purficiation, occurrence, and biological actions. *Acta Physiol. Scand.* 29:Suppl. 105, pp. 1–90
18. Kopera, H., Lazarini, W. 1953. Zur Frage der zentralen Übertragung afferenter Impulse. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 219: 214–22
19. Amin, A. H., Crawford, T. B. B., Gaddum, J. H. 1954. The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol. London* 126:596–618
20. Galindo, A., Krnjević, K., Schwartz, S. 1967. Micro-iontophoretic studies on neurones in the cuneate nucleus. *J. Physiol. London* 192:359–77
21. Chang, M. M., Leeman, S. E. 1970. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J. Biol. Chem.* 245:4784–90
22. Chang, M. M., Leeman, S. E., Niall, H. D. 1971. Amino-acid sequence of substance P. *Nature New Biol.* 232: 86–87
23. Tregear, G. W., Niall, H. D., Potts, J. T. Jr., Leeman, S. E., Chang, M. M. 1971. Synthesis of substance P. *Nature New Biol.* 232:87–89
24. Lembeck, F., Zetler, G. 1971. Substance P. In *International Encyclopedia of Pharmacology and Therapeutics,* ed. J. M. Walker, Sect. 72. 1:29–71. Oxford:Pergamon. 305 pp.
25. Zetler, G. 1961. Zwei neue pharmakologisch aktive Polypeptide in einem Substanz P-haltigen Hirnextrakt. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 242:330–52
26. Meinardi, H., Craig, L. C. 1966. Studies of substance P. In *Hypotensive Peptides,* ed. E. G. Erdös, N. Back, F. Sicuteri, A. F. Wilde, 594–607. Berlin: Springer. 660 pp.
27. Iven, H., Zetler, G. 1973. Comparison of crude bovine subcortical substance P with synthetic substance P. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 276:123–31

28. Otsuka, M., Konishi, S., Takahashi, T. 1972. A further study of the montoneuron-depolarizing peptide extracted from dorsal roots of bovine spinal nerves. *Proc. Jpn. Acad.* 48:747–52
29. Takahashi, T., Konishi, S., Powell, D., Leeman, S. E., Otsuka, M. 1974. Identification of the motoneuron-depolarizing peptide in bovine dorsal root as hypothalamic substance P. *Brain Res.* 73:59–69
30. Takahashi, T., Otsuka, M. 1975. Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. *Brain Res.* 87:1–11
31. Takahashi, T., Otsuka, M. 1974. Distribution of substance P in cat spinal cord and the alteration following unilateral dorsal root section. *Jpn. J. Pharmacol.* 24: Suppl., p. 105
32. Sprague, J. M., Ha, H. 1964. The terminal fields of dorsal root fibers in the lumbosacral spinal cord of the cat, and the dendritic organization of the motor nuclei. In *Organization of the Spinal Cord, Progr. Brain Res.*, ed. J. C. Eccles, J. P. Schadé, 11:120–54. Amsterdam: Elsevier. 285 pp.
33. Hökfelt, T., Kellerth, J. O., Nilsson, G., Pernow, B. 1975. Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. *Brain Res.* 100:235–52
34. Hökfelt, T., Kellerth, J. O., Nilsson, G., Pernow, B. 1975. Substance P: Localization in the central nervous system and in some primary sensory neurons. *Science* 190:889–90
35. Inouye, A., Kataoka, K. 1962. Subcellular distribution of the substance P in the nervous tissues. *Nature* 193: 585
36. Cleugh, J., Gaddum, J. H., Mitchell, A. A., Smith, M. W., Whittaker, V. P. 1964. Substance P in brain extracts. *J. Physiol. London* 170:69–85
37. Ryall, R. W. 1962. Sub-cellular distribution of pharmacologically active substances in guinea pig brain. *Nature* 196:680–81
38. Powell, D., Leeman, S. E., Tregear, G. W., Niall, H. D., Potts, J. T. Jr. 1973. Radioimmunoassay for substance P. *Nature New Biol.* 241:252–54
39. Duffy, M. J., Mulhall, D., Powell, D. 1975. Subcellular distribution of substance P in bovine hypothalamus and substantia nigra. *J. Neurochem.* 25: 305–7
40. Konishi, S., Otsuka, M. 1974. The effects of substance P and other peptides on spinal neurons of the frog. *Brain Res.* 65:397–410
41. Nicoll, R. A. 1976. Promising peptides. *Neurosci. Symp.* 1:99–122
42. Graham, L. T. Jr., Shank, R. P., Werman, R., Aprison, M. H. 1967. Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine, and glutamine. *J. Neurochem.* 14:465–72
43. Otsuka, M., Konishi, S. 1974. Electrophysiology of mammalian spinal cord in vitro. *Nature* 252:733–34
44. Konishi, S., Otsuka, M. 1974. Excitatory action of hypothalamic substance P on spinal motoneurones of newborn rats. *Nature* 252:734–35
45. Saito, K., Konishi, S., Otsuka, M. 1975. Antagonism between Lioresal and substance P in rat spinal cord. *Brain Res.* 97:177–80
46. Otsuka, M., Konishi, S. 1976. Substance P and excitatory transmitter of primary sensory neurons. *Cold Spring Harbor Symp. Quant. Biol.* 40:135–43
47. Krnjević, K., Morris, M. E. 1974. An excitatory action of substance P on cuneate neurones. *Can. J. Physiol. Pharmacol.* 52:736–44
48. Henry, J. L., Krnjević, K., Morris, M. E. 1975. Substance P and spinal neurones. *Can. J. Physiol. Pharmacol.* 53:423–32
49. Stern, P., Huković, S. 1961. Specific antagonists of substance P. In *Symposium on Substance P*, ed. P. Stern, 83–88. Sarajevo: Sci. Soc. Bosnia Herzegovina. 143 pp.
50. Davidoff, R. A., Sears, E. S, 1974. The effects of Lioresal on synaptic activity in the isolated spinal cord. *Neurology* 24:957–63
51. Pedersen, E., Arlien-Søborg, P., Grynderup, V., Henriksen, O. 1970. GABA derivative in spasticity (β-(4-chlorophenyl)-γ-aminobutyric acid, Ciba, 34.647-Ba). *Acta Neurol. Scand.* 46:257–66
52. Pierau, F. K., Zimmermann, P. 1973. Action of a GABA-derivative on postsynaptic potentials and membrane properties of cats' spinal motoneurones. *Brain Res.* 54:376–80
53. Phillis, J. W. 1976. Is β-(4-chlorophenyl)-GABA a specific antagonist of substance P on cerebral cortical neurons? *Experientia* 32:593–94

54. Ben-Ari, Y., Henry, J. L. 1976. Effects of the para-chlorophenyl derivative of GABA on spinal neurones in the cat. *J. Physiol. London* 259:46–47P
55. Davies, J., Dray, A. 1976. Substance P in the substantia nigra. *Brain Res.* 107:623–27
56. Otsuka, M., Konishi, S., Takahashi, T. 1972. The presence of a motoneuron-depolarizing peptide in bovine dorsal roots of spinal nerves. *Proc. Jpn. Acad.* 48:342–46
57. Otsuka, M., Konishi, S. 1977. Electrophysiological and neurochemical evidence for substance P as a transmitter of primary sensory neurons. In *Substance P*, ed. U.S.v. Euler, B. Pernow. New York: Raven. In press
58. Yanaihara, N., Yanaihara, C., Hirohashi, M., Sato, H., Hashimoto, T., Sakagami, M., Iizuka, Y. 1976. Substance P analogues: Synthesis and biological and immunological properties. See Ref. 57
59. Angelucci, L. 1956. Experiments with perfused frog's spinal cord. *Br. J. Pharmacol.* 11:161–70
60. Ramwell, P. W., Shaw, J. E., Jessup, R. 1966. Spontaneous and evoked release of prostaglandins from frog spinal cord. *Am. J. Physiol.* 211:998–1004
61. Otsuka, M., Konishi, S. 1976. Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. *Nature* 264:83–84
62. Iversen, L. L., Jessell, T., Kanazawa, I. 1976. Release and metabolism of substance P in rat hypothalamus. *Nature* 264:81–83
63. Segawa, T., Nakata, Y. 1976. Substance P in rabbit brain and spinal cord: Regional and subcellular distribution and evidence for lack of high-affinity uptake system. *Jpn. J. Pharmacol.* 26: Suppl., 100 P.
64. Gullbring, B. 1943. Inactivation of substance P by tissue extracts. *Acta Physiol. Scand.* 6:246–55
65. Umrath, K. 1953. Über die fermentative Verwandlung von Substanz P aus sensiblen Neuronen in die Erregungssubstanz der sensiblen Nerven. *Pflügers Arch.* 258:230–42
66. Eber, O., Lembeck, F. 1956. Über den enzymatischen Abbau der Substanz P. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 229:139–47
67. Krivoy, W. A. 1957. The preservation of substance P by lysergic acid diethylamide. *Br. J. Pharmacol.* 12:361–64
68. Hooper, K. C. 1962. The catabolism of some physiologically active polypeptides by homogenate of dog hypothalamus. *Biochem. J.* 83:511–17
69. Claybrook, D. L., Pfiffner, J. J. 1968. Purification and properties of a substance P-inactivating enzyme from bovine brain. *Biochem. Pharmacol.* 17: 281–93
70. Benuck, M., Marks, N. 1975. Enzymatic inactivation of substance P by a partially purified enzyme from rat brain. *Biochem. Biophys. Res. Commun.* 65:153–60
71. Krivoy, W. A. 1961. Potentiation of substance P by lysergic acid diethylamide in vivo. *Br. J. Pharmacol.* 16:253–56
72. Hökfelt, T., Elde, R., Johansson, O., Luft, R., Nilsson, G., Arimura, A. 1976. Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. *Neuroscience* 1:131–36
73. Henry, J. L. 1976. Excitation of spinal nociceptive neurons by substance P. *Nobel Symp. 37, Substance P, Stockholm,* pp. 29–31 (Abstr.)
74. Walker, R. J., Kemp, J. A., Yajima, H., Kitagawa, K., Woodruff, G. N. 1976. The action of substance P on mesencephalic reticular and substantia nigral neurones of the rat. *Experientia* 32:214–15
75. Dennis, M. J., Harris, A. J., Kuffler, S. W. 1971. Synaptic transmission and its duplication by focally applied acetylcholine in parasympathetic neurons in the heart of the frog. *Proc. R. Soc. London Ser. B.* 177:509–39
76. Zetler, G., Schlosser, L. 1955. Über die Verteilung von Substanz P und Cholinacetylase im Gehirn. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 224:159–75
77. Paasonen, M. K., Vogt, M. 1956. The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain. *J. Physiol. London* 131:617–26
78. Kanazawa, I., Jessell, T. 1976. Postmortem changes and regional distribution of substance P in the rat and mouse nervous system. *Brain Res.* 117:362–67
79. Nilsson, G., Hökfelt, T., Pernow, B. 1974. Distribution of substance P-like immunoreactivity in the rat central nervous system as revealed by immunohistochemistry. *Med. Biol.* 52:424–27

80. Phillis, J. W., Limacher, J. J. 1974. Substance P excitation of cerebral cortical Betz cells. *Brain Res.* 69:158–63
81. Duffy, M. J., Powell, D. 1975. Stimulation of brain adenylate cyclase activity by the undecapeptide substance P and its modulation by the calcium ion. *Biochim. Biophys. Acta* 385:275–80
82. Dale, H. 1935. Pharmacology and nerve-endings. *Proc. R. Soc. Med.* 28:319–32
83. Euler, U.S.v., Hedqvist, P. 1974. Effects of substance P on the response of guinea pig vas deferens to transmural nerve stimulation. *Acta Physiol. Scand.* 90: 651–53
84. Nilsson, G., Larsson, L.-I., Håkanson, R., Brodin, E., Pernow, B., Sundler, F. 1975. Localization of substance P-like immunoreactivity in mouse gut. *Histochemistry* 43:97–99
85. Studer, R. O., Trzeciak, A., Lergier, W. 1973. Isolierung und Aminosaüresequenz von Substanz P aus Pferdedarm. *Helv. Chim. Acta* 56:860–66
86. Brown, B. E. 1967. Neuromuscular transmitter substance in insect visceral muscle. *Science* 155:595–97
87. Brown, B. E. 1975. Proctolin: A peptide transmitter candidate in insects. *Life Sci.* 17:1241–52
88. Starratt, A. N., Brown, B. E. 1975. Structure of the pentapeptide proctolin, a proposed neurotransmitter in insects. *Life Sci.* 17:1253–56
89. Margolis, F. L. 1974. Carnosine in the primary olfactory pathway. *Science* 184:909–11
90. Margolis, F. L., Roberts, N., Ferriero, D., Feldman, J. 1974. Denervation in the primary olfactory pathway of mice: Biochemical and morphological effects. *Brain Res.* 81:469–83
91. Neidle, A., Kandera, J. 1974. Carnosine —an olfactory bulb peptide. *Brain Res.* 80:359–64
92. Margolis, F. L., Ferriero, D., Herding, J. 1975. Carnosine (β-alanyl-L-histidine) in the olfactory nerve: A putative transmitter candidate. *Proc. Int. Congr. Pharmacol., 6th, Helsinki* 2:61–69
93. Terenius, L. 1973. Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. *Acta Pharmacol. Toxicol.* 33:377–84
94. Pert, C. B., Snyder, S. H. 1973. Opiate receptor: Demonstration in nervous tissue. *Science* 179:1011–14
95. Simon, E. J., Hiller, J. M., Edelman, I. 1973. Stereospecific binding of the potent narcotic analgesic [^{3}H]etorphine to rat-brain homogenate. *Proc. Natl. Acad. Sci. USA* 70:1947–49
96. Terenius, L., Wahlström, A. 1975. Search for an endogenous ligand for the opiate receptor. *Acta Physiol. Scand.* 94:74–81
97. Hughes, J. 1975. Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* 88:295–308
98. Hughes, J., Smith, T., Morgan, B., Fothergill, L. 1975. Purification and properties of enkephalin—the possible endogenous ligand for the morphine receptor. *Life Sci.* 16:1753–58
99. Pasternak, G. W., Goodman, R., Snyder, S. H. 1975. An endogenous morphine-like factor in mammalian brain. *Life Sci.* 16:1765–69
100. Simantov, R., Snyder, S. H. 1976. Isolation and structure identification of a morphine-like peptide "enkephalin" in bovine brain. *Life Sci.* 18:781–88
101. Simantov, R., Kuhar, M. J., Pasternak, G. W., Snyder, S. H. 1976. The regional distribution of a morphine-like factor enkephalin in monkey brain. *Brain Res.* 106:189–97
102. Simantov, R., Snowman, A. M., Snyder, S. H. 1976. A morphine-like factor "enkephalin" in rat brain: Subcellular localization. *Brain Res.* 107:650–57
103. Hughes, J., Smith, T., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., Morris, H. R. 1975. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258:577–79
104. Kosterlitz, H. W., Hughes, J. 1975. Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci.* 17:91–96
105. Büscher, H. H., Hill, R. C., Römer, D., Cardinaux, F., Closse, A., Hauser, D., Pless, J. 1976. Evidence for analgesic activity of enkephalin in the mouse. *Nature* 261:423–25
106. Bradley, P. B., Briggs, I., Gayton, R. J., Lambert, L. A. 1976. Effects of microiontophoretically applied methionine-enkephalin on single neurones in rat brainstem. *Nature* 261:425–26
107. Gent, J. P., Wolstencroft, J. H. 1976. Effects of methionine-enkephalin and leucine-enkephalin compared with those of morphine on brainstem neurones in cat. *Nature* 261:426–27
108. Burgus, R., Dunn, T. F., Desiderio, D., Ward, D. N., Vale, W., Guillemin, R. 1970. Characterization of ovine hypo-

thalamic hypophysiotropic TSH-releasing factor. *Nature* 226:321–25
109. Nair, R. M. G., Barrett, J. F., Bowers, C. Y., Schally, A. V. 1970. Structure of porcine thyrotropin releasing hormone. *Biochemistry* 9:1103–6
110. Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T. W., Debeljuk, L., White, W. F. 1971. Isolation and properties of the FSH and LH-releasing hormone. *Biochem. Biophys. Res. Commun.* 43:393–99
111. Amoss, M., Burgus, R., Blackwell, R., Vale, W., Fellows, R., Guillemin, R. 1971. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. *Biochem. Biophys. Res. Commun.* 44:205–10
112. Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., Guillemin, R. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 179:77–79
113. Jackson, I. M. D., Reichlin, S. 1974. Thyrotropin-releasing hormone (TRH): Distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. *Endocrinology* 95:854–62
114. Oliver, C., Eskay, R. L., Ben-Jonathan, N., Porter, J. C. 1974. Distribution and concentration of TRH in the rat brain. *Endocrinology* 96:540–46
115. Winokur, A., Utiger, R. D. 1974. Thyrotropin-releasing hormone: Regional distribution in rat brain. *Science* 185:265–67
116. Brownstein, M., Arimura, A., Sato, H., Schally, A. V., Kizer, J. S. 1975. The regional distribution of somatostatin in the rat brain. *Endocrinology* 96: 1456–61
117. Hökfelt, T., Elde, R., Johansson, O., Luft, R., Arimura, A. 1975. Immunohistochemical evidence for the presence of somatostatin, a powerful inhibitory peptide, in some primary sensory neurons. *Neurosci. Lett.* 1: 231–35
118. Hökfelt, T., Fuxe, K., Johansson, O., Jeffcoate, S., White, N. 1975. Thyrotropin releasing hormone (TRH)-containing nerve terminals in certain brain stem nuclei and in the spinal cord. *Neurosci. Lett.* 1:133–39
119. Hökfelt, T., Fuxe, K., Johansson, O., Jeffcoate, S., White, N. 1975. Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. *Eur. J. Pharmacol.* 34:389–92
120. Pelletier, G., Labrie, F., Puviani, R., Arimura, A., Schally, A. V. 1974. Immunohistochemical localization of luteinizing hormone-releasing hormone in the rat median eminence. *Endocrinology* 95:314–17
121. Dyer, R. G., Dyball, R. E. J. 1974. Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus. *Nature* 252: 486–88
122. Renaud, L. P., Martin, J. B. 1975. Thyrotropin releasing hormone (TRH): Depressant action on central neuronal activity. *Brain Res.* 86:150–54
123. Renaud, L. P., Martin, J. B., Brazeau, P. 1975. Depressant action of TRH, LH-RH and somatostatin on activity of central neurones. *Nature* 255: 233–35
124. Kawakami, M., Sakuma, Y. 1974. Responses of hypothalamic neurons to the microiontophoresis of LH-RH, LH and FSH under various levels of circulating ovarian hormones. *Neuroendocrinology* 15:290–307
125. Vincent, J. D., Arnauld, E. 1975. Vasopressin as a neurotransmitter in the central nervous system: Some evidence from the supraoptic neurosecretory system. *Prog. Brain Res.* 42:57–66
126. Dreifuss, J. J., Nordmann, J. J., Vincent, J. D. 1974. Recurrent inhibition of supraoptic neurosecretory cells in homozygous Brattleboro rats. *J. Physiol. London* 237:25 P
127. Moss, R. L., Dyball, R. E. J., Cross, B. A. 1972. Excitation of antidromically identified neurosecretory cells of the paraventricular nucleus by oxytocin applied iontophoretically. *Exp. Neurol.* 34:95–102
128. Ganten, D., Marquez-Julio, A., Granger, P., Hayduk, K., Karsunky, K. P., Boucher, R., Genest, J. 1971. Renin in dog brain. *Am. J. Physiol.* 221: 1733–37
129. Fischer-Ferraro, C., Nahmod, V. E., Goldstein, D. J., Finkielman, S. 1971. Angiotensin and renin in rat and dog brain. *J. Exp. Med.* 133:353–61
130. Yang, H-Y. T., Neff, N. H. 1972. Distribution and properties of angiotensin converting enzyme of rat brain. *J. Neurochem.* 19:2443–50
131. Nicoll, R. A., Barker, J. L. 1971. Excitation of supraoptic neurosecretory cells by angiotensin II. *Nature New Biol.* 233:172–74

132. Sakai, K. K., Marks, B. H., George, J., Koestner, A. 1974. Specific angiotensin II receptors in organ-cultured canine supra-optic nucleus cells. *Life Sci.* 14:1337–44
133. Felix, D., Akert, K. 1974. The effect of angiotensin II on neurones of the cat subfornical organ. *Brain Res.* 76: 350–53
134. Felix, D. 1975. Peptide and acetylcholine action on neurones of the cat subfornical organ. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 292:15–20
135. Bern, H. A., Knowles. F. G. W. 1966. Neurosecretion. In *Neuroendocrinology,* ed. L. Martini, W. F. Ganong, 1:139–86. New York: Academic. 774 pp.
136. Nilsson, G., Pernow, B., Fischer, G. H., Folkers, K. 1976. Radioimmunoassay of substance P. *Nobel Symp. 37, Substance P, Stockholm,* p. 8 (Abstr.)