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SUBSTANCE P AS A TRANSMITTER CANDIDATE[1]

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INTRODUCTION

Substance P is a peptide which was first described in 1931 (von Euler & Gaddum 1931) in extracts of brain and intestine, but which was not purified to homogeneity until 1970 (Chang & Leeman 1970). The isolation of substance P was accomplished subsequent to the discovery of a sialogogic peptide in hypothalamic extracts (Leeman & Hammerschlag 1967), which was shortly thereafter characterized as substance P. The name substance P (for preparation) had been used in the laboratory of origin to designate the active agent in a particular preparation of tissue extracts. This nondescript term entered the literature in 1934 and has persisted (Gaddum & Schild 1934). The amino acid sequence of substance P (SP), H-ARG-PRO-LYS-PRO-GLN-GLN-PHE-PHE-GLY-LEU-MET-NH_2, was established in 1971 (Chang et al 1971) and shortly thereafter synthetic peptide was pre-

[1]Abbreviations used: ACh, acetylcholine; AChE, acetylcholinesterase; CAT, choline acetyltransferase; CNS, central nervous system; DA, dopamine; 5,6-DHT, 5,6-dihydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; EPSP, excitatory postsynaptic potential; GAD, glutamic acid decarboxylase; 5-HT, 5-hydroxytryptamine; mepp, miniature endplate potential; NE, norepinephrine; RIA, radioimmunoassay SP, substance P; SPLI, substance P-like immunoreactivity.

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pared (Tregear et al 1971), permitting the development of precise methods for biochemical, histochemical, physiological, and pharmacological studies of the peptide.

In addition to the hypotensive and smooth muscle-contracting activity that led to its discovery (von Euler & Gaddum 1931), SP has many pharmacological effects (see Skrabanek & Powell 1977) that may be of functional significance. One likely physiological role for SP is that of a neurotransmitter. Indeed, SP is widely but selectively distributed in both the central and peripheral nervous systems, is found in fiber tracts and nerve endings, is released upon depolarization, and can alter the activity of some neurons when applied in their vicinity.

In this review we examine the evidence that SP may be a transmitter in three *substance P systems:* the nociceptive primary afferents, the striatonigral tract, and the habenulo-interpeduncular tract. Other areas where SP may play such a role are only briefly considered. In addition, we discuss several topics which are both sufficiently well documented and interesting enough to merit separate treatment, namely (*a*) the coexistence of SP and 5-HT in some neurons, (*b*) the functional interaction of the postulated SP receptor with the calcium channel in salivary gland cells, (*c*) the interaction of SP with some nicotinic receptors, and (*d*) the association of SP with blood vessels. For convenience we have summarized in Table 1 the distribution of SP in the nervous system of various species.

Our discussion draws primarily on the literature published after 1971. Pernow (1953, 1963) and Lembeck & Zetler (1962, 1971) have reviewed earlier work. For a discussion of general pharmacological, endocrine, cardiovascular, and behavioral effects of SP we refer to the following reviews: Skrabanek & Powell (1977), the most complete source of references, listing and briefly describing virtually all publications dealing with SP that have appeared before the end of 1977; von Euler & Pernow (1977), a collection of research papers presented at the 37th Nobel Symposium and covering a wide range of topics on the chemistry and pharmacology of SP; and Mroz & Leeman (1977), a rather complete general review discussing the chemistry, distribution, and pharmacology of SP as well as assay methods.

A Few Caveats to Consider

Following and evaluating the interdisciplinary literature dealing with peptides in the nervous system presents obvious problems. For the sake of the nonspecialist we have, therefore, selected a few areas in which intrinsic difficulties in interpretation are easily overlooked.

IMMUNOCHEMISTRY Immunological methods have been extremely important in determining the amounts of a particular peptide present in a

tissue sample by the use of radioimmunoassy (RIA) and for localizing the peptide to specific neuronal structures by immunohistochemical techniques. The immunological methods are critically dependent on the species of antisera used which may cross-react with compounds structurally related to the immunogen and hence may give rise to *false positives.* To control for this an antiserum is generally characterized in terms of its cross-reactivity with known peptides and their analogs. Furthermore, partial identity of immunoreactive tissue extracts with synthetic peptide can be checked by independent means (e.g. chromatography, electrophoresis). However, even if these controls are carried out, one cannot exclude cross-reactivity with some unknown compound, particularly in immunohistochemical studies. This problem is further discussed by Mroz & Leeman (1977) and by Ljungdahl et al (1978a).

Immunochemical methods can also give rise to *false negatives.* Antigenic sites may be lost during fixation and antibodies may not penetrate to all antigenic sites. The indirect immunofluorescence technique of Coons (see Coons 1958) is sometimes not sufficiently sensitive to visualize very low concentrations of antigen in areas where immunoreactivity has been demonstrated by RIA. Axons and cell bodies seem to be particularly difficult to detect with some antisera (Ljungdahl et al 1978a), perhaps because they do not cross-react with a possible precursor. The peroxidase-antiperoxidase method of Sternberger (see Sternberger et al 1970) may provide greater sensitivity and has the advantage of producing an electron dense reaction product, thus permitting visualization in the electron microscope.

In the case of SP, oxidation of the methionine residue slowly occurs when the peptide is kept in dilute solution (Floor & Leeman, in press). Since the relative immunoreactivity of SP-sulfoxide, as compared to SP, is different for different antisera, this may introduce errors in measurement of tissue levels of SP. Addition of 2-mercapto-ethanol to the tissue extraction and resuspension medium largely prevents such oxidation. To what extent SP is oxidized during tissue preparation for histochemistry is not known.

In summary, depending on the method and antisera used, immunochemical procedures may give rise both to false positives and false negatives. On the other hand, cross-reactivity with a precursor or metabolite is of obvious usefulness for biosynthetic or metabolic studies. In this review we have used the term *substance P-like immunoreactivity* (SPLI) when referring to values obtained by immunochemical procedures.

ELECTROPHYSIOLOGY/IONTOPHORESIS

Specificty of the effect Changes in membrane potential or in neuronal activity subsequent to iontophoresis of a peptide can be nonspecific. An

observed response could be due to a nonspecific membrane effect by the peptide (as opposed to an action mediated via a specific receptor) or to a simple effect of current or pH. (Peptides are usually ejected as cations at an acidic pH.) Because the transport number of most peptides is quite low, relatively large currents may be required to obtain effects and thus current and pH artifacts become more of a problem. The technique of pressure ejection is, therefore, gaining popularity in studies on the neuronal action of peptides. Blockade of a peptide response with a specific antagonist is probably the most meaningful test of specificity. However, no such antagonist is known for substance P.

Apparent time-course of the response The time course of the response observed upon iontophoresis of a peptide depends on the time it takes for the peptide to reach the target membrane, as well as the time course of the physiological response. The first component is largely determined by the time it takes to eject the peptide from the electrode, which in turn depends on the transport number of the peptide and the amount and duration of retaining current used (which electrophoreses the peptide up the electrode, away from the tip). It is possible to determine the relative contributions of these two components (electrophoretic vs physiological) on the apparent time course of the response by calibrating the micropipettes used. The extent to which a given retaining current delays the ejection of SP from micropipettes was specifically investigated by Guyenet et al (1979). As expected, a larger retaining current led to a greater delay in ejecting SP from the micropipettes, as determined by RIA. Similarly, when tested on locus coeruleus neurons in vivo, iontophoretic ejection of SP, following a large retaining current (13 nA), gave a sluggish increase in firing rate whereas following a weak retaining current (0.4 nA) the SP-induced rise in activity was brisk (5 sec lag) and no different from that induced by ACh. The magnitude of the response was also increased.

Apparent mode of action A given response (excitatory or inhibitory) to an applied substance can be due to a direct effect on the neuron from which the recording is made, or it can be mediated via local interneurons or equivalent structures. If the response is mediated by an inhibitory interneuron, the direct membrane action (excitatory or inhibitory) of the applied substance will result in the opposite (inhibitory or excitatory) overall effect. Such indirect effects have been clearly established for glutamate in the olfactory bulb (Nicoll 1971) and enkephalin in the hippocampus (Zieglgänsberger et al 1979). The use of Co^{2+} or Mg^{2+} to block local transmission can reveal such indirect effects, but this test assumes that the substance is not acting via a Ca^{2+} channel. If one knows the identity of a suspected inter-

vening transmitter, specific antagonists can be used to block the indirect effect.

Comparing the membrane effects of a transmitter candidate with those of natural stimulation presents an additional set of problems. Natural stimulation may be heterogeneous in terms of input neurons and their conductance changes. Selective stimulation of a homogeneous population of input neurons, however, may not be possible. Furthermore, if the neuron has extended dendrites, it may not be possible to accurately record dendritic synaptic conductance changes from the soma.

In short, it is exceedingly difficult to determine the direct action of a peptide (or other compound) in the CNS and to satisfy the criterion of mimicry. On the other hand, electrophysiological studies in the peripheral nervous system and in tissue culture have been particularly rewarding.

ELECTRONMICROGRAPHS Electronmicrographs represent a slice in time of normally dynamic processes. This is particularly relevant to the appearance of synaptic boutons that discharge vesicles during activity. Specifically, differences in vesicle size, number, and immunoreactivity in synaptic boutons may reflect differences in their past histories or intrinsic differences between the neurons to which they belong. It is difficult to distinguish between these two possibilities without an extensive study directly aimed at the problem (e.g. see Basbaum & Heuser 1979).

THE DISTRIBUTION OF SUBSTANCE P

Table 1 summarizes the distribution of SPLI in the CNS of various species, as determined by RIA. Small amounts of SPLI have also been measured in various peripheral organs of several species (Nilsson & Brodin 1977). Immunohistochemical data is difficult to tabulate or describe without doing injustice to the often exquisite pictures. We therefore refer directly to the original papers. The distribution, determined by Hökfelt's group, of immunofluorescent SP in the rat central nervous system is summarized in Ljungdahl et al (1978a,b), which also contains an extensive list of references; that determined by the Cambridge Medical Research Council group is summarized in Cuello & Kanazawa (1978). SP immunofluorescence has been described in the mammalian central nervous system (Nilsson et al 1974, Hökfelt et al 1976b, 1977c, 1978b,c) in primary afferents (Nilsson et al 1974, Hökfelt et al 1975, 1976a, 1977c,d, Schultzberg et al 1978b), and in the periphery (Hökfelt et al 1977b, 1978d, Schultzberg et al 1978a). Hökfelt et al (1978a) compare the relative distributions of various peptides in the nervous system of the rat.

Table 1 The distribution of substance P in the central nervous system[a]

Region	Rat		Human		Pigeon
	pmol/10 mg wet weight[b]	pmol/mg protein[c]	pmol/mg protein[d]	pmol/10 mg wet weight[e]	pmol/10 mg wet weight[f]
Somatosensory system					
Dorsal root ganglia	0.6				
Dorsal horn	9.4				10.1
Trigeminal nucleus	12.1				9.0
Dorsal column	1.1				
Dorsal column nucleus	1.5				
Thalamic nucleus	0.2				
Somatosensory cortex	0.2				
Visual system					
Retina					< 0.1
Optic nerve					< 0.1
Lateral geniculate body	0.7	0.9			
Superior colliculus		1.8			
Visual cortex	0.2				
Basal ganglia					
Striatum		0.9			3.3[g]
Caudate nucleus	2.2		3.7		
Putamen			3.3		
Globus pallidus	2.9		18.0		1.2[h]
Substantia nigra	15.1				7.5[i]
Pars compacta		2.9	47.2	4.1	
Par reticularis		11.38	47.9	7.4	
Pars lateralis		3.0			
Subthalamic nucleus	2.0				
Hypothalamus		2.1	5.2		2.1
Medial	5.5				
Middle	4.5				
Lateral	4.3				
Medial preoptic nucleus		4.4			
Lateral preoptic nucleus		3.3			
Periventricular nucleus		3.3			
Suprachiasmatic nucleus		1.6			
Supraoptic nucleus		1.6			
Anterior hypothalamic nucleus		3.2			
Paraventricular nucleus		3.1			
Arcuate nucleus		2.5			
Ventromedial nucleus		2.5			
Dorsomedial nucleus		3.5			
Perifornical nucleus		2.9			
Ventral premammilary nucleus		3.3			
Dorsal premammilary nucleus		1.7			
Posterior hypothalamic nucleus		2.8			
Medial forebrain bundle, anterior		3.1			
Medial forebrain bundle, posterior		2.3			
Median eminence		1.0			
Posterior pituitary		0.5[j]			

Table 1 *(Continued)*

Region	Rat		Human		Pigeon
	pmol/10 mg wet weight[b]	pmol/mg protein[c]	pmol/mg protein[d]	pmol/10 mg wet weight[e]	pmol/10 mg wet weight[f]
Limbic system					
Olfactory bulb	0.5	0.2			0.7
Olfactory tubercle	2.6				
Olfactory cortex	0.4				
Amygdala	3.3	3.4			
Hippocampus	0.3				
Habenula	3.3				
Interpeduncular nucleus	5.2	5.9			8.5
Septum	3.5	1.2			
Dorsal septal nucleus		2.8			
Lateral septal nucleus		3.6			
Interstitial nucleus of stria terminals		3.3			
Nucleus accumbens	2.4				4.3
Mammilary body	1.8				
Anterior thalamic nucleus	1.9				
Other regions					
Locus coeruleus					5.3
Red nucleus		1.3			
Medial geniculate		0.8			
Inferior colliculus		1.2			
Central gray		2.9			
Cerebellum	< 0.1	< 0.1	0.2		< 0.1
Pineal	< 0.1[j]		0.5		
Ventral horn					1.1
Anterior pituitary	0.2[j]				

[a] As determined by RIA; standard errors have been omitted and values rounded off.

[b] From Kanazawa & Jessel (1976); original values in ng/g wet weight have been converted to pmol/10 mg wet weight using a molecular weight of SP to facilitate comparison with other studies; value/10 mg wet weight is on the same scale as value/mg protein since nervous tissue is about 10% protein.

[c] From Brownstein et al (1976).

[d] From Gale et al (1978).

[e] From Kanazawa et al (1977a); original values in ng/g.

[f] From Reubi & Jessel (1978); original values in pmol/g.

[g] Paleostriatum augmentatum, a possible counterpart of the mammalian striatum.

[h] Paleostriatum primitivum.

[i] Nucleus tegmenti pedunculo-ponti.

[j] M. H. Fernstrom and S. E. Leeman, unpublished.

EVIDENCE FOR SUBSTANCE P AS A TRANSMITTER

Our concepts of chemical transmission, as derived from the study of the neuromuscular junction, may need to be expanded when applied to the central nervous system. On morphological grounds, all combinations of interaction between axons, dendrites, and cell bodies have been postulated (see Sheperd 1974). Furthermore, a released compound may act on several

target cells in its vicinity [Beaudet & Descarries (1978) discuss this possibility for monoamines in the cerebral cortex] or it may act upon its neuron of origin, as suggested for the dendritically released DA in the substantia nigra (Groves et al 1975). In each of these cases the site of action of the released compound will be determined by the location of specific receptors relative to the location and efficiency of some inactivating system. In our discussion of SP as a neurotransmitter, we will include any or all of the above possibilities in addition to a simple presynaptic-to-postsynaptic action. Such diversity of action is in fact suggested by the ultrastructural appearance of boutons with SPLI in the spinal cord (Barber et al 1979), as discussed below.

Since the biosynthesis of SP has not yet been characterized and its mode of degradation has not been described at a site relevant to transmission, the evidence supporting a role of SP in neurotransmission is limited to presence, release, and mimicry.

Substance P in Nociceptive Primary Afferent Fibers

The most convincing evidence for a role of SP in neurotransmission comes from studies on the sensory C fibers (and possibly A delta fibers) which convey noxious information. The search for the sensory transmitter(s) has a long and interesting history. Dale, for instance, maintained an active interest in the discovery of the transmitter in sensory fibers, as illustrated in a letter to Eccles in 1953, written at the age of 78. After reading a preprint of the initial work of Eccles, Fatt & Koketsu (cf. 1954) demonstrating the cholinergic nature of the transmission from motoneuron axon collaterals onto Renshaw cells, Dale wrote:

> It is extremely satisfactory to have the direct evidence of a cholinergic transmission from the ending of the collateral of a cholinergic axon, which, as you say, could have been predicted. I myself emphasize, in 1934, in a *Nothnagel Lecture*[2], which I gave in Vienna, the fact that the chemical function appeared to be a function, not merely of the nerve ending, but of the whole neurone, and speculated at the time concerning the possibility, that the identification of the peripheral transmitter of the so-called "antidromic vasodilatation" might give a clue to the transmitter, at the other, central synaptic ending, of what appeared to be normally an afferent nerve fibre.
>
> A number of people seem now to be taking up this clue, and trying to identify the peripheral transmitter of the antidromic vasodilation, in the hope of identifying one of the missing central transmitters. I suspect, however, that they are overlooking the fact, that Gasser and Hinsey[3] showed, years ago, that this antidromic vasodilation was due to small, so-called "C" fibres in the dorsal roots, which certainly would not be concerned in the monosynaptic reflex effects on large motoneurons with which you have been largely concerned.

[2]Although the formation of "Dale's Principle" first appears in the Nothnagel Lecture (1935a), it is the Dixon Lecture (1935b), which was given a month later, that is usually quoted on this topic.

[3]Dale is referring to the paper of Hinsey & Gasser (1930).

PRESENCE AND LOCALIZATION There were two major laboratories that were "taking up this clue," that of Hellauer & Umrath (1948) and that of Lembeck (1953). Hellauer & Umrath (1948) measured noncholinergic vasodilator activity in extracts of dorsal and ventral roots and found that considerably more activity was present in dorsal than ventral roots. They proposed that this vasodilator activity represented the action of the excitatory transmitter of sensory fibers. Lembeck (1953) obtained similar results and, in addition, found that the extract caused gut contraction. These properties were identical to those of SP, which had been extracted earlier from gut and brain by von Euler & Gaddum (1931) and found to be a peptide (von Euler, 1936). Thus, Lembeck proposed that SP might be the sensory transmitter.

These studies were extended by Otsuka and collaborators who isolated a peptide from bovine dorsal roots that had pharmacological properties similar to SP, but also was found to depolarize motoneurons of the isolated frog spinal cord (Otsuka et al 1972). This was an important finding because until this time no clearly defined CNS effect of SP extracts had been detected. The dorsal root peptide was subsequently shown (Takahashi et al 1974) to have precisely the same pharmacological, chemical, enzymatic, and immunological properties as the undecapeptide isolated by Chang & Leeman (1970) from hypothalamus and identified by them as SP. Using bioassay, Takahashi & Otsuka (1975) found that SP was highly concentrated in the dorsal horn of the spinal cord and that following dorsal root ligation or section, the concentration of SP in the dorsal horn and the segment of the dorsal root nearest the cord fell markedly, whereas the concentration in the segment nearest the cell bodies increased manyfold. Compared to the changes in the dorsal horn, only a slight fall in SP was detected in the ventral horn after dorsal root section. These results suggest that SP is synthesized in the cell body and subsequently transported to the dorsal horn of the spinal cord. Interestingly, years earlier, Holton (1959) had shown with similar ligation experiments that there was transport of SP into the peripheral branch of sensory neurons.

Immunohistochemical studies have shown that SPLI-positive fibers form a dense plexus in the substantia gelatinosa of the spinal cord (Hökfelt et al 1975, Chan-Palay & Palay 1977, Ljungdahl et al 1978a, Cuello & Kanazawa 1978, Barber et al 1979) and that this immunoreactivity is markedly decreased by dorsal root ligation (Hökfelt et al 1977c) or dorsal rhizotomy (Barber et al 1979). Only about 20% of the cells in the dorsal root ganglia of the rat are SPLI-positive (Hökfelt et al 1975, 1976a). A similar percentage has been found for spinal ganglia maintained in culture (Schultzberg et al 1978b). Although it is possible that negative cells may also contain slight, low concentrations of SPLI, the fact that a portion of the unlabeled cells contain another peptide, somatostatin (Hökfelt et al

1976a), suggests that there is a distinct population of SP-containing neurons. These cells have small somas and small, probably unmyelinated or thinly myelinated axons (Hökfelt et al 1977c). This discovery, in addition to the presence of free fibers with SPLI in skin, raises the possibility that SP may be localized in fibers involved in nociception. This association is made stronger by immunohistochemistry done on tooth pulp afferents, which are considered to mediate only the sensation of pain in humans (Anderson et al 1970). Olgart et al (1977b) found SPLI-positive fibers in teeth, which disappeared after sectioning the inferior alveolar nerve, thus supporting their sensory role. At the light microscopic level, it was not possible to establish with certainty whether these fibers were unmyelinated or contained a thin myelin sheath.

Further support for the association of SP with pain sensory fibers comes from studies on the distribution of SP in the trigeminal nucleus. It is generally accepted that the nucleus caudalis of the trigeminal nuclear complex receives nociceptive input (Dubner et al 1976). Immunohistochemical studies have revealed that the highest concentration of SPLI-positive fibers is located in this part of the nucleus (Cuello & Kanazawa, 1978) and that most of this immunoreactivity disappears after section of the trigeminal nerve (Cuello et al 1978a).

Peripheral nerve section is known to produce a marked cell loss in spinal and trigeminal ganglia and a degeneration of central axon branches of primary sensory neurons (Knyihár & Csillik 1976, Gobel & Binck 1977). This cell loss appears to be largely limited to small neurons (Aldskogius & Arvidsson 1978). Recently Jessell et al (1979b) found that following peripheral nerve section, there was a marked fall in SP content measured with radioimmunoassay in the spinal cord, with a time course similar to that of neuronal degeneration. There was no change in the concentration of GAD or CAT activity in the spinal cord after such lesions.

Studies on the action of capsaicin, a derivative of homovanillic acid, also link SPLI-positive afferents to a nociceptive function and a possible role in the axon reflex. Administered acutely, capsaicin produces intense pain and neurogenic plasma extravasation, whereas chronic administration of capsaicin renders animals insensitive to painful chemogenic stimuli (Jancsó 1968).

In newborn rats, capsaicin leads to a selective degeneration of chemosensitive primary afferents (Jancsó et al 1977) and to an irreversible depletion of SPLI in those areas containing primary afferents but not in other regions of the CNS (Gamse et al 1979a). In older rats capsaicin does not lead to a degeneration of primary afferents (Joö et al 1969), but results nevertheless in a decrease in SPLI in the substantia gelatinosa (Jessell et al 1978b). This decrease in SPLI is reversible (Gamse et al 1979a) and may be related to

the ability of capsaicin to induce (Ca^{2+} dependent) release of SPLI from spinal cord in vitro (Theriault et al 1979, Gamse et al 1979c) and in vivo (Jessell et al 1979a). [Interestingly, capsaicin does not release SPLI from slices of hypothalamus or substantia nigra (Gamse et al 1979c).] In the skin, capsaicin inhibits the plasma extravasation that can normally be induced by antidromic stimulation of sensory neurons and decreases the levels of SPLI. Because intra-arterial infusion of SP induces plasma extravasation in the corresponding skin area, SP may play a role in the axon reflex (i.e. Dale's antidromic vasodilatation) (Gamse et al 1979a).

The localization of SPLI in synaptic terminals has also been examined at the ultrastructural level using the peroxidase-antiperoxidase method (Chan-Palay & Palay 1977, Cuello et al 1977, Hökfelt et al 1977c, Pickel et al 1977, Pelletier et al 1977, Barber et al 1979). The most detailed study is by Barber et al (1979), who found immunoreactivity in synaptic terminals containing both small agranular and large granular vesicles. This immunoreactivity appeared to be present over the granular vesicles and in the terminal cytoplasm associated with the exterior surfaces of small, agranular synaptic vesicles (Chan-Palay & Palay 1977). The presence of SPLI in terminals that contain two populations of vesicles raises the interesting possibility that these terminals may contain another neurotransmitter in addition to SP. Alternatively, the smaller vesicles may be a consequence of membrane retrieval subsequent to exocytosis of the large granular ones and hence, may be empty. Because the SP immunoreactivity was not always concentrated at the "active sites" of the synaptic junctions, and the terminals had features resembling neuroendocrine terminals, Barber et al (1979) suggested that SP might be released from nonsynaptic sites as well as from conventional synaptic junctions. SP might also play a role in presynaptic inhibition (or excitation?), as SPLI-positive axo-axonic synapses exist in the spinal cord (Barber et al 1979). This notion is supported by the observation that SP does have a direct depolarizing action on primary afferent fibers (Nicoll 1976).

RELEASE Otsuka & Konishi (1976) have studied the release of SP from the isolated spinal cord of the newborn rat and found that repetitive stimulation of the dorsal root or exposure of the cord to a Krebs solution containing 55 mM K^+ increased the efflux of SPLI. The release of SPLI was abolished in a calcium deficient and magnesium rich Krebs solution. They also found that increasing the stimulus duration, which would activate the smaller fibers, markedly increased the amount of SPLI released. However, it is unclear in these experiments whether release of SP only occurred from unmyelinated C fibers. A calcium dependent release has also been found in slices of trigeminal nucleus (Jessell & Iversen 1977) and in cultures of

dissociated dorsal root ganglion cells (Mudge et al 1979). Release of SPLI from the mammalian spinal cord has also been demonstrated in vivo by perfusion of the spinal subarachnoid space (Jessell et al 1979a). Local perfusion of potassium or capsaicin released SPLI; this release was reduced by cobalt. Sciatic nerve stimulation also released SPLI, but only at stimulus intensities which recruited A delta and C fibers. This finding provides strong physiological support for the presence of SP in, and its release from, small diameter fibers. If SP is the mediator of antidromic vasodilation, as might be predicted from its presence in small cutaneous fibers (Hökfelt et al 1977c) and its known action on blood vessels, then it should be possible to demonstrate its release from peripheral axons. Attempts thus far have failed to show such release (Burcher et al 1977), but preliminary results from tooth pulp afferents (Olgart et al 1977a) suggest that antidromic stimulation can elicit SP release. It would be of interest to know if this release is calcium dependent, as sensory terminals do contain small numbers of vesicles (Iggo 1974).

MIMICRY In a random sampling of dorsal horn neurons, Henry et al (1975) found that the iontophoretic application of SP excited approximately half of the neurons tested. Compared to glutamate, the excitations were slow in onset and considerably outlasted the application. It was argued that the slow time course of action seemed incompatible with SP being the main excitatory transmitter released from primary afferents, and it was suggested that it might modulate activity over a longer period of time (however, cf Guyenet et al 1979). Subsequent studies on dorsal horn neurons have attempted to correlate SP sensitivity to a specific sensory modality. Henry (1976) found that only neurons classified as nociceptive were excited by SP, but that only about half of these units responded. These findings strengthened the idea that SP is involved in the transmission of nociceptive information. As pointed out by Henry, the failure of some cells to respond might be due to the low rate of release of SP from the pipette, or might occur because the neuron to which SP was applied did not receive monosynaptic input from primary afferents. It is perhaps surprising that such a correlation was found, as it is based on the assumption that only those cells that receive an SP input are sensitive to SP; yet, in a previous study on the cuneate nucleus (Krnjević & Morris 1974), with only moderate levels of SP immunofluorescence (Ljungdahl et al 1978a), approximately half of the neurons were excited by SP. Nevertheless, the association of SP sensitivity and activation by nociceptive stimuli has also been made by Randić & Miletić (1977) in the dorsal horn of the spinal cord and in the trigeminal nucleus caudalis in which the neurons were activated by tooth pulp stimulation (Andersen et al 1978). It has also been reported that following dorsal root

section, neurons in the dorsal horn become supersensitive to an SP analogue, an eledoisin-related peptide (Wright & Roberts 1978). These results are compatible either with increased responsiveness of the neurons or with greater access of the peptide to receptor sites following de-afferentation. Because the major site of termination of spinal unmyelinated primary afferents is in the substantia gelatinosa on the dendrites of secondary neurons, one might expect SP to be more effective when applied at this site. Duggan et al (1979) has recorded from lamina IV and V neurons while applying SP, either by iontophoresis or pressure ejection in the substantia gelatinosa. However, when SP was applied at those sites at which enkephalin was effective in blocking nociceptive input (see below), it had a weak action. One explanation for these results is that enkephalin can act at some distance from the nerve terminals, and indeed autoradiographic studies on localization of opiate receptors support this idea (Atweh & Kuhar 1977).

A precise comparison of the mechanism of action of SP and of the transmitter released from nociceptive primary afferents is extremely difficult for a number of reasons. First, the input to neurons in the dorsal horn is thought to occur mainly in the substantia gelatinosa, on the dendrites, and at a considerable distance from the cell body. This anatomical arrangement makes it very difficult to determine whether the primary afferent transmitter alters the conductance of the neuronal membrane and, if so, to determine the reversal potential of the transmitter action. Also, the slow conduction velocity of unmyelinated afferents makes it difficult to conclude conclusively that the neuron under investigation is being activated monosynaptically by afferent fibers. Zieglgänsberger & Tulloch (1979) have recorded the effect of iontophoretically applied SP to dorsal horn neurons with intracellular recording. They found that SP causes a reversible depolarization that parallels the increase in firing rate observed with extracellular recording. They were unable to detect any change in resistance during the SP depolarization. However, the SP was applied 100–160 μm from the recording site, and it is possible that a small change in resistance might not be detected with such a separation between the site of application and recording.

The evidence outlined above provides very strong support for the idea that SP is involved in synaptic transmission from primary afferents that mediate nociception. The slow time course of SP action is not a particularly negative finding because the time course of transmitter action from nociceptive afferents is not well characterized, and the low transport number of SP (Guyenet et al 1979) can result in delayed release from microelectrodes. Other evidence that would strengthen the transmitter role of SP at nociceptive afferents is as follows. It would be desirable to show a selective release of SP in the spinal cord during noxious stimulation. However, this would still not conclusively show that the release is directly from primary afferents

and not from SP-containing interneurons. Such a differentiation would be difficult to make, although it is clear from cell culture studies (Mudge et al 1979) that SP can indeed be released from afferent neurons. The ionic mechanism underlying the action of SP and the transmitter released from nociceptive afferents is unclear, and the technical problems associated with determining the mechanism will be difficult to surmount. The development of potent and selective SP antagonists would greatly advance our understanding of the physiological role of SP. The synthesis of analogues of SP, as has been done so successfully for angiotensin II (cf Khosla et al 1974), might be a fruitful approach.

In their original work on the *dorsal root peptide*, Otsuka and collaborators made the observation that this peptide depolarized motoneurons, and thus it was logical to conclude that it might be present in those primary afferents that end monosynaptically on motoneurons, i.e. the large Ia afferents. Further support for this idea came from the observation that Lioresal blocked the Ia EPSP on motoneurons and the action of SP (Saito et al 1975). Although Lioresal appears to have some specificity toward the action of SP (Otsuka & Yanagisawa 1979), it is not entirely clear whether the specificity is sufficiently good to permit one to conclude that the block of Ia EPSPs is due to an antagonism of SP. The histochemical studies do not entirely exclude the existence of SP in Ia afferents, but strongly suggest that it is not present.

In the context of SP sensitivity of motoneurons, it is of interest that immunohistochemical studies indicate that at least two SP-containing systems, in addition to primary afferents, exist in the spinal cord. In colchicine-treated rats, numerous SP-positive cells were observed in the dorsal horn, immediately lateral to the dorsal horn, and in the dorsal part of the ventral horn (Hökfelt et al 1977d, Ljungdahl et al 1978a). In addition, the ventral horn contains a moderate density of SP-positive fibers (Hökfelt et al 1977c, Ljungdahl et al 1978a). These fibers remain after dorsal root section but disappear following cord transection, indicating that they represent a descending projection to motor nuclei. As mentioned above, SP exerts a depolarizing action on motoneurons but considerable controversy exists concerning the mechanism underlying this depolarization. Bath application of SP to the spinal cord of the newborn rat (Otsuka 1978) and to the frog spinal cord (Nicoll 1976, 1978) causes a depolarization associated with an increased conductance of the motoneuron membrane. The effect in the frog can be seen in preparations bathed in magnesium to block indirect synaptic effects. In the cat, iontophoretically applied SP has been reported to produce either no change in conductance (Zieglgänsberger & Tulloch 1979) or a decrease in conductance (Krnjević 1977). The basis for these conflicting

results is not entirely clear, but species differences and differences in the technique of application might provide an explanation.

ASSOCIATION OF OPIATE ACTION WITH NOCICEPTIVE AFFERENTS

As the association of SP with nociceptive afferents has emerged, it has become increasingly clear that the action of opiates and enkephalin-containing systems are also intimately associated with these afferents. In the dorsal horn, met-enkephalin-positive cell bodies and nerve terminals are in close proximity to SP-positive cell bodies and nerve terminals; lesion experiments suggest that these neurons are interneurons (Hökfelt et al 1977d). Results from a number of studies suggest that opiates and enkephalin act on the primary afferent terminals and fibers. LaMotte et al (1976) have found that opiate receptors are highly concentrated in the dorsal horn of the spinal cord and that dorsal root section results in a marked decrease in binding in this region. Jessell et al (1979b) also found a decrease in opiate receptor binding following sciatic nerve and dorsal root section. Although it is conceivable that transneuronal degeneration might explain these results, the findings suggest that opiate receptors are present on afferent fibers. Autoradiographic localization of opiate receptor sites, utilizing selective binding of a potent opiate antagonist [^{3}H] diprenorphine, demonstrates a narrow band of silver grains in the substantia gelatinosa of the spinal cord (Atweh & Kuhar 1977). Opiate receptor binding was high only in those sensory nuclei that receive C fiber innervation, which suggests that some opiate receptors are associated with the C fiber terminals. The study of Hiller et al (1978) provides direct evidence that opiate receptors are located on primary afferent fibers. Opiate receptor binding was localized primarily in the neuritic outgrowth from sensory dorsal root ganglion cells grown in culture.

The presence of opiate receptors, presumably synthesized in the cell body of sensory neurons, on the intramedullary portion of primary afferents, as for example in the vagus (Atweh & Kuhar 1977), raises the question as to whether these receptors are preferentially transported centrally, or whether they might also be transported into the peripheral axon. There is some evidence that this may indeed be the case for GABA receptors because it can be shown pharmacologically that GABA receptors are present both in the central (Nicoll & Alger 1979) and peripheral portion of sensory fibers (Brown & Marsh 1978). To examine this possibility for opiate receptors, binding studies have been performed on the vagal trunk, which contains a high proportion of unmyelinated C-fiber afferents that are thought to possess opiate receptors on their central terminals (Atweh & Kuhar 1977). The vagus nerve does have some binding sites, whereas the sciatic nerve was

devoid of binding sites (M. J. Kuhar, personal communication). It will be of interest to determine whether these opiate receptors in the vagus nerve are localized on SP fibers. About 10% of the vagal fibers contain SPLI and are mostly unmyelinated (Gamse et al 1979b). Furthermore, cell bodies with SPLI are found in the nodose ganglion, the sensory ganglion of the vagus nerve (Lundberg et al 1978).

A number of pharmacological studies have found an association of opiate action with input from small afferent fibers and/or with SP. In a well controlled study, Duggan et al (1976) have found that iontophoretically applied opiates and enkephalin in the substantia gelatinosa block the activation of lamina IV and V neurons by noxious, but not innocuous, stimulation, and that the effect is reversed by small systemic doses of the opiate antagonist naloxone. Although these experiments do not entirely exclude a postsynaptic site of action, for instance on an interneuron in the substantia gelatinosa, they are most easily explained by a selective presynaptic block of transmitter release from nociceptive afferents. A similar differential effect of enkephalin on responses of nucleus caudalis neurons in the trigeminal nucleus to tooth pulp stimulation was found by Andersen et al (1978). In spinal cord explants with attached sensory dorsal root ganglia, sensory-evoked synaptic activity in the dorsal horn region, but not in the ventral horn region, is blocked by opiates and opioid peptides, and this effect is prevented by naloxone (Crain et al 1977, 1978). These results do not distinguish between pre- or postsynaptic actions, but they do indicate that only the effects of certain primary afferents are blocked as a consequence of opiate receptor activation. MacDonald & Nelson (1978) have simultaneously recorded intracellularly from synaptically coupled dorsal root ganglion cells and spinal neurons and have tested the effect of iontophoretically applied etorphine, a potent opiate agonist, on the EPSP evoked by ganglion cell stimulation. Using the coefficient of variation of the EPSP to determine quantal content and quantal size, they found that etorphine had no effect on the size of the quanta but reduced the quantal content. This result indicates that opiate receptor activation depressed the EPSP solely by reducing the release of excitatory transmitter. Interestingly, all the cells they examined gave a similar result suggesting either that (*a*) they were very fortunate in selecting sensory afferents that have opiate receptors or (*b*) that all of the sensory afferents in their cultures possess opiate receptors, a situation that appears not to be the case in the culture experiments of Crain et al (1977, 1978). Jessell & Iversen (1977) found that the potassium-induced release of SP from slices of the trigeminal nucleus was reduced by opiates and opioid peptides; this block of release was prevented by naloxone. Although the precise site of action of opiates could not be determined in this preparation, these experiments clearly establish a functional association

between opiates and substance P-containing neurons. Mudge et al (1979) also showed that enkephalin can inhibit release of substance P from cultured neurons; because sensory neurons were the only cell types present, enkephalin must have acted directly on the sensory neurons.

Two likely mechanisms by which opiates might inhibit transmitter release are either (*a*) that they cause a conductance increase so that the action potential does not effectively depolarize the terminal membrane or (*b*) that they inhibit the calcium influx which is necessary for transmitter release.

Picrotoxin-sensitive presynaptic inhibition by GABA is associated, at least temporally, with a depolarization of primary afferents (PAD) and there is evidence that an increase in chloride conductance may be the mechanism of both presynaptic inhibition and PAD (Nicoll & Alger 1979). Whether the depolarization itself contributes to the presynaptic inhibition is unclear, but it is likely of secondary importance because at the crustacean neuromuscular junction, presynaptic inhibition is associated with a conductance increase that results in a hyperpolarization of the motor nerve terminal (Kawai & Niwa 1977).

In isolated spinal cord preparations, enkephalin has been found to have a direct hyperpolarizing action which is blocked by naloxone; in these experiments enkephalin had little effect on ventral root potentials elicited by dorsal root stimulation (Evans & Hill 1978, R. A. Nicoll, unpublished observation). Sastry (1978b) has found that iontophoretically applied enkaphalin decreases the excitability of Aδ afferent terminals of the cat, which is consistent with a hyperpolarizing action. Such an action could block transmitter release if the hyperpolarization blocked impulse invasion into the nerve terminals or if the conductance increase reduced the size of the action potential in the terminal.

In the trigeminal nucleus, tooth pulp afferents are depolarized by stimulating the periaqueductal grey matter, a site that elicits a naloxone reversible analgesia. However, naloxone fails to alter this depolarization (Hu et al 1978). These results suggest that naloxone reversible analgesia is not mediated by a depolarization of the afferents.

Mudge et al (1979) suggest that enkephalin may inhibit substance P release from cultured sensory neurons inhibiting Ca^{2+} influx. Using intracellular electrodes, they find that enkephalin can decrease the duration of Ca^{2+}-action potentials recorded in the cell soma. The effect is antagonized by naloxone. It is likely that the calcium channels present in the cell soma membrane share common properties with calcium channels that activate transmitter release from the processes. These channels might, therefore, serve as models for those at the inaccessible release sites (Baker 1972). In these cultured sensory neurons, enkephalin did not alter the resting membrane properties of the neurons; there was no change in membrane

potential or conductance. Rather, enkephalin inhibited a voltage-sensitive conductance.

As reviewed by Fields & Basbaum (1978), descending neuronal pathways that are in part serotonergic can suppress transmission of pain signals at the level of the spinal cord. The finding that serotonin, GABA, and norepinephrine can inhibit the release of SP from cultures of dorsal root ganglion cells (Mudge et al 1979) and decrease the calcium component of the action potential in sensory neurons (Dunlap & Fischbach 1978) raises the possibility that a variety of neurotransmitter systems may be capable of suppressing pain transmission by a presynaptic mechanism.

The results discussed above indicate that a very intimate relationship exists between nociceptive afferents (which in all probability utilize SP as a neurotransmitter) and endogenous opiate systems. Important questions for future research include 1. Do enkephalin terminals, in fact, make axo-axonic synapses on SP-containing afferent terminals? 2. What is the precise mechanism underlying the presynaptic action of opiates? 3. How are the presumed enkephalinergic presynaptic inhibitory pathways activated and can this pathway be demonstrated electrophysiologically?

Substance P in the Striatonigral Tract

The reciprocal striatonigral-nigrostriatal system is part of the extra-pyramidal motor system and is involved in sensory-motor integration. Its clinical importance is emphasized by two major diseases directly linked to defects in this system: Parkinson's disease (paralysis agitans), characterized by tremor, rigidity, and hypokinesis associated with degeneration of nigral and pallidal neurons (Calne 1970); and Huntington's chorea, characterized by involuntary muscular contractions apparently related to degeneration of striatal, pallidal, and cortical neurons (Shoulson & Chase 1975, Lange et al 1976). It has become clear that the striatonigral tract, in addition to the GAD-positive neurons (Kim et al 1971, Fonnum et al 1974, Precht & Yoshida 1971), contains the majority of the SP fibers that give rise to the dense plexus of SPLI in the substantia nigra. Because, in addition, SP is released from nigral tissue upon stimulation, and because it has an excitatory effect upon the dopaminergic nigrostriatal neurons, SP is very likely a transmitter in the substantia nigra, possibly acting directly upon the dopaminergic neurons.

PRESENCE AND LOCALIZATION The substantia nigra contains the highest level of SPLI of any microdissected brain region (Table 1). Within the nigra of a number of species, several laboratories have found, using RIA, that SPLI is more concentrated in the pars reticularis than in the pars compacta (Brownstein et al 1976, Gauchy et al 1979, Kanazawa et al 1977a;

Table 1), although other laboratories have found little difference (Jessell et al 1978a, Gale et al 1978). The reason for this discrepancy is not clear. SP immunofluorescence studies (Hökfelt et al 1977c, Ljungdahl et al 1978a,b, see also Cuello & Kanazawa 1978) reveal an extremely dense network of fluorescent fibers interspersed with occasional nonfluorescent cell bodies that spans the entire pars reticularis. The SP immunofluorescence decreases from pars reticularis to pars compacta except in the rostral part of the substantia nigra. In the pars compacta, a considerably less dense plexus containing SPLI surrounds the many nonfluorescent cell bodies. As is evident from adjacent sections, many of these cell bodies are tyrosine hydroxylase(TH)-positive and hence are most likely the DA-containing nigrostriatal neurons (Ljungdahl et al 1978b).

The dendrites of these neurons extend far into the pars reticularis (Björklund & Lindvall 1975) and comparison of adjacent sections stained for SP and TH, respectively (Ljungdahl et al 1978b), clearly demonstrates the overlap of the dendritic field of the amine-containing neurons and the plexus of the SPLI-positive fibers. Direct synaptic contact, however, remains to be demonstrated at the electron microscope level.

Subcellularly, most of the SPLI is found in the synaptosomal fraction (Duffy et al 1975). No electron micrographs of SP immunoreactive terminals in the nigra have appeared in the literature. Hökfelt et al (1977c) report, however, that SPLI is confined to "nerve endings containing both small agranular and large granular vesicles" when examined under the electron microscope. The reaction product obtained by the peroxidase-antiperoxidase method is reportedly located in the cytoplasm or over the large granular vesicles.

Lesion studies indicate that the majority of the neurons giving rise to the SPLI in the rat substantia nigra have their cell bodies in the anterior striatum and hence are part of the striato-nigral tract (Mroz et al 1977a,b, Brownstein et al 1977, Hong et al 1977, Gale et al 1977, Kanazawa et al 1977b, Jessell et al 1978a). Substance P immunofluorescent cell bodies have indeed been observed in the anterior striatum in the rat (Kanazawa et al 1977b, Ljungdahl et al 1978a), and SP immunofluorescent fibers are seen in the caudal portion of the internal capsule (Ljungdahl et al 1978a,b, Jessell et al 1978a) and in the adjacent medial forebrain bundle (Ljungdahl et al 1978a). Whether there are substance P-containing pallido-nigral neurons as well is still a matter of debate. The problem stems from the fact that the efferents from the anterior striatum pass caudo-medio-ventrally through the globus pallidus on their way to the substantia nigra (e.g. see Fox & Rafols 1976). Brownstein et al (1977) concluded that the anterior striatum constitutes the major afferent input to the substantia nigra pars reticularis and probably the sole afferent input of anterior origin, because lesions immedi-

ately rostral to the substantia nigra (Mroz et al 1977a) produced no greater fall in nigral SPLI than did lesions separating the anterior striatum from the globus pallidus. On the other hand, Kanazawa et al (1977b) found that the fall in nigral SPLI (about 90%) caused by electrolytic lesions of the globus pallidus exceeded the decrease (about 70%) caused by even the largest anterior striatal lesion. In addition, they detected some large SP immunofluorescent cell bodies in the globus pallidus, although Ljungdahl et al (1978a) were unable to confirm this finding in colchicine-treated rats. The lesion experiments of Kanazawa et al (1977b) are somewhat ambiguous because, as they point out, their pallidal lesions "generally involved other structures such as parts of the striatum and the internal capsule."

In addition to SPLI, the striatonigral tract also contains GABA. The two systems have different but overlapping distributions. Whereas most of the SPLI-containing striatonigral neurons originate in the anterior striatum, the GABA-or GAD-positive afferents to the nigra seem to have their cell bodies located more caudally, as determined from lesion studies in the rat (Brownstein et al 1977, Gale et al 1977, Jessell et al 1978a). Whether any neurons in the overlap zone contain both substance P and GABA has not yet been investigated.

The neuronal population in the striatum consists largely (over 95%) of medium sized, spiny neurons, with a minor fraction of large aspiny ones (Kemp & Powell 1971, Fox et al 1972/73a,b, Lange et al 1976). Initial Golgi studies had suggested that only the large aspiny neurons project out of the striatum (Fox et al 1972/73b). From more recent evidence it has become clear, however (see Fox & Rafols 1976, Graybiel & Ragsdale 1979, Kitai et al 1976), that it is the medium sized spiny neurons that constitute the striatal efferents. It can therefore be expected that the SP (and GABA) neurons belong to this latter class. Indeed, as outlined by their immunofluorescence, the cell bodies with SPLI in the striatum appear small to medium sized (Ljungdahl et al 1978a).

In Huntington's chorea, the smaller striatal neurons and some pallidal neurons degenerate (Lange et al 1976). This cell loss is accompanied by a decrease in SPLI in the substantia nigra (Kanazawa et al 1977a, 1979, Gale et al 1978) and in the internal part of the globus pallidus (Kanazawa et al 1979).

RELEASE It has been clearly established, qualitatively, that depolarization of nigral tissue leads to a Ca^{2+} dependent release of SPLI (Schenker et al 1976, Jessell 1978). In superfused slices of substantia nigra, either K^+ or veratridine are effective depolarizing agents. SPLI efflux is approximately linearly related to the external K^+ concentration over the range of 15–60 mM K and, at constant K^+, to the external Ca^{2+} concentration over

a range of 0.1–3 mM (with concomitant decrease of Mg^{2+}) (Jessell 1978). Sequential K^+ pulses in superfused synaptosomes induce progressively less Ca^{2+} dependent SPLI release, which suggests that the readily releasable pool of SPLI is of limited size or that there is inactivation at some step in the depolarization-secretion process (Schenker et al 1976). This is consistent with the observation that in the continued presence of Ca^{2+} and depolarizing amounts of K^+, the SPLI release rises to an early peak and then declines back toward the basal level (see von Euler & Pernow, 1977, p. 216).

Although these studies were designed to optimize the stimulation-induced release and demonstrate the voltage and Ca^{2+} dependence of the release process, they give no quantitative information about the amount, the time course, and the conditions under which SP is released in the intact functioning nigra. It also remains to be determined whether there normally is a spontaneous release of SP, whether the release is quantal, whether release occurs via exocytosis, and whether a single action potential is sufficient or whether a train of spikes is required to induce release over baseline.

Indirect evidence in support of spontaneous release of SP in vivo comes from studies done by Glowinski's group in the cat (Chéramy et al 1978a, Michelot et al 1979). Infusion of anti-SP gamma-globulins into the substantia nigra by means of a push-pull cannula led to a decrease of the spontaneous ^{3}H-DA release in the ipsilateral caudate nucleus, monitored simultaneously as an index of the activity of the nigrostriatal DA neurons. Control experiments done by infusing nonspecific gammaglobulins were without effect. Some of these experiments will be further discussed below. Whether this inferred spontaneous release of SP also occurs in the intact animal or represents an artifact due to local tissue damage will be difficult to determine because completely noninvasive techniques for measuring release of a compound in the brain are not yet available.

Stimulation of nigral tissue induces the release not only of SPLI but also of DA (Geffen et al 1976, Nieoullon et al 1977a), of GABA (Reubi et al 1977) and of 5-HT (Reubi & Emson 1978). These transmitter candidates affect each other's release from slices or in vivo in a complex way (Reubi et al 1978, Jessell 1978, Chéramy et al 1978b, Michelot et al 1979, Starr 1979), probably reflecting their functional interactions in the intact nigra but unfortunately giving few clues about the local circuitry. GABA (10–50 μM) inhibits the K^+-induced release of SPLI from superfused nigral slices but has no effect on the basal SPLI efflux (Jessell 1978). This inhibitory action of GABA is abolished in the presence of picrotoxin (50 μM), indicating that the effect is mediated via specific GABA receptors. It would be interesting to determine whether these GABA receptors are located on SP axon terminals or on some intervening structure. This question could possibly be resolved by repeating these experiments using synaptosomes and high

superfusion rates. The release of SPLI in the nigra seems to be tonically regulated by GABA because high concentrattions (100 μM) of picrotoxin alone induce SPLI release over baseline (Jessell 1978). DA (50 μM), on the other hand, does not affect SP release (Reubi et al 1977).

MIMICRY The cell bodies in the substantia nigra are topographically arranged according to their respective projection to the striatum, the thalamus, and the tectum (Faull & Mehler 1978). Because the terminal field of the SPLI-containing striatonigral neurons covers the entire substantia nigra (see Ljungdahl et al 1978a,b), these neurons can be expected, a priori, to innervate all three nigral efferent projections in addition to possible intrinsic neurons. Whether this is in fact the case has not yet been investigated. Because a transmitter candidate must mimic the action of the natural transmitter, it must first be determined which synaptic interaction SP must mimic. This will prove to be extremely difficult in view of the complexity of the substantia nigra. It is not surprising, then, that only a few studies have examined the effects of SP on nigral neurons.

Davies & Dray (1976) recorded extracellularly from spontaneously active neurons in the nigra and tested the effect of iontophoretic application of SP on firing rate. SP induced a small increase in firing rate in 23/34 neurons (eight neurons did not respond to SP and three were depressed). All of these neurons were also excited by glutamate and ACh and inhibited by GABA. The location of the micropipette was identified histochemically but Davies & Dray fail to indicate where the responsive neurons were located. Dray mentions, however, in a lecture (Dray & Straughan 1976) that neurons in the compacta as well as in the reticularis responded to SP. This would make it likely that at least some dopaminergic nigrostriatal neurons are excited by SP, either directly or indirectly. The paper by Walker et al (1976) is sometimes quoted as evidence that SP may have an excitatory effect on nigral neurons. They indeed observed an increase in firing rate upon iontophoresis of SP in a dilute solution at pH 5–6. At that pH, SP is clearly a cation. Yet Walter et al obtained positive results only with "anodal current." If by "anodal current" they mean ejection of cations, their results confirmed those obtained by Davies & Dray (1976); but if their "anodal current" ejected anions, then their observations are unrelated to any action of SP.

The activity of the dopaminergic nigrostriatal neurons can also be estimated from ^{3}H-DA release in the caudate nucleus by means of push-pull cannulas (Nieoullon et al 1977b). Such an approach has the advantage of monitoring chemically identified neurons in the anesthetized cat in vivo. Unilateral infusion of SP into the substantia nigra via a push-pull cannula leads to an increase in ^{3}H-DA release in the ipsilateral caudate (Chéramy et al 1977, Michelot et al 1979), whereas infusion of anti-SP gammaglobu-

lins decreases the ^{3}H-DA release in the ipsilateral caudate (Chéramy et al 1978a, Michelot et al 1979). These observations strongly suggest that SP increases the firing rate of dopaminergic neurons and seems to do so tonically in the anesthetized cat in vivo. Whether SP acts directly upon these neurons or whether its action is mediated via the local circuitry remains to be determined. It might be interesting, for example, to repeat the above experiments during local transmission block.

Nieoullon et al (1977c) had previously made the puzzling observation that in the anesthetized cat in vivo, an increase in ^{3}H-DA release in the caudate is accompanied by a decrease in ^{3}H-DA release in the ipsilateral nigra; at the same time the converse changes in ^{3}H-DA release occur in the contralateral nigrostriatal system (Nieoullon et al 1977c). Similarly, whereas unilateral nigral infusion of SP increases ^{3}H-DA release in the ipsilateral caudate, it inhibits ^{3}H-DA release in the ipsilateral nigra in the cat in vivo (Michelot et al 1979); nigral application of anti-SP gammaglobulins have the opposite effect (Chéramy et al 1978a). [Interestingly, application of 10^{-5} M SP to superfused nigral slices increases ^{3}H-DA release (Reubi et al 1978).] On the other hand, unilateral nigral application of SP does not affect the contralateral nigrostriatal system in vivo (Michelot et al 1979). It is neither known in what way SP achieves this uncoupling of the two nigrostriatal systems, nor by what pathway they are normally coupled and reciprocally controlled.

How can the overall excitatory effect of SP on nigral neurons be reconciled with the overall inhibitory response observed in nigral neurons during striatal stimulation (Yoshida & Precht 1971, Dray et al 1976)? Striatal electrical stimulation can be expected to activate a heterogeneous population of neurons, many of which are the striatonigral GABA neurons. Indeed, the inhibitory field potential recorded in the nigra upon stimulation of the caudate is blocked by picrotoxin (Precht & Yoshida 1971) and by bicuculline (Dray et al 1976). It is therefore possible that an excitatory response may be masked by the inhibition produced by the striatonigral GABA Neurons. This is consistent with the observation that inhibition is sometimes preceded by brief excitation (Dray et al 1976, Preston et al 1978, S. T. Kitai, personal communication).

Substance P in the Habenulo-Interpeduncular Tract

PRESENCE AND LOCALIZATION SP immunofluorescent cell bodies in the medial habenula (Hökfelt et al 1975, Cuello et al 1978b, Cuello & Kanazawa 1978, Ljungdahl et al 1978a,b) give rise to fibers that can be followed all along the fasciculus retroflexus (Ljungdahl et al 1978a). The SP immunofluorescence becomes particularly strong at the ventral surface of the brain before the fibers enter the lateral nucleus. The very high levels of SPLI detected in the interpeduncular nucleus by RIA in the rat (Brownstein

et al 1976, Kanazawa & Jessell 1976; Table 1) are concentrated in a dense band of immunofluorescent fibers in the ventrolateral part of the nucleus and only occasional patches in the central part of the nucleus (Hökfelt et al 1977c, Ljungdahl et al 1978a,b, Cuello et al 1978b). Lesion of the medial habenula(e) leads to a loss of SPLI in the interpeduncular nucleus, as determined by RIA (Mroz et al 1976, 1977a, Hong et al 1976, Cuello et al 1978b) or by immunofluorescence (Hökfelt et al 1977c, Cuello et al 1978b). Transsection of the fasciculus retroflexus similarly depletes the interpeduncular nucleus of SPLI, and SPLI accumulates proximal to the cut (Emson et al 1977, Cuello et al 1978b). The loss of SPLI after these procedures is not complete, which is consistent with the presence of SP immunofluorescent cell bodies observed in the interpenduncular nucleus (Ljungdahl et al 1978a, Cuello & Kanazawa 1978, Cuello et al 1978b). It is not known whether these SPLI-positive neurons are intrinsic to the nucleus. They do not, however, project to the habenulae since no loss in SPLI is observed in these nuclei following destruction of the interpeduncular nucleus and the ventral tegmental area (Cuello et al 1978b).

In addition to the SPLI neurons, the habenulo-interpenduncular tract also contains cholinergic neurons (Kataoka et al 1973, Léránth et al 1975, Emson et al 1977, Cuello et al 1978b) and these two populations appear to have distinct origins. Separation of the medial from the lateral habenula leads to (*a*) a decrease in CAT activity and AChE staining in the medial habenula and (*b*) a decrease in SPLI in the lateral habenula and the interpenduncular/ventral tegmental area (Cuello et al 1978b). Since no change in SPLI was observed in the medial habenula, or any decrease in AChE staining or CAT activity in the lateral habenula, the majority of SP and cholinergic fibers must have a separate origin. Although the cell bodies of these SPLI neurons are in the medial habenula, those of the cholinergic neurons are most likely in the lateral habenula. It is also likely that they reciprocally project axon collaterals or axons into the lateral and medial habenula, respectively. However, these studies do not resolve whether all of these nuerons project to the interpenduncular nucleus and whether a separate population of habenulo-interpenduncular neurons contain both SP and ACh.

RELEASE Whether SP is released from the interpenduncular nucleus upon stimulation has, to our knowledge, not yet been investigated.

MIMICRY Sastry (1978a) has randomly sampled the firing rate of spontaneously active neurons in the interpeduncular nucleus and tested their response to habenular stimulation and to iontophoretically applied SP and ACh in anesthetized rats. Most cells responded to habenular stimulation (92/98), to SP (83/98), and to ACh (82/98). However, whereas the re-

sponse to habenular stimulation was either excitation, inhibition, or inhibition followed by excitation (as determined from the discharge rates), the response to SP and ACh was almost invariably excitation. Using intracellular electrodes, Ogata (1979) has recorded from cells in slices of the interpeduncular nucleus an SP-induced depolarization, which persists after blockade of synaptic transmission by a low calcium medium. These results suggest that both the SP and ACh pathways have an excitatory action on interpeduncular neurons. In addition, habenular stimulation seems to activate some unidentified inhibitory neurons, possibly local interneurons. The ACh innervation clearly predominates because the excitatory effect of habenular stimulation was almost completely inhibited by doses of atropine that completely blocked ACh responses, but had no effect on SP responses. The residual atropine resistant excitation could conceivably be due to release of endogenous SP. In some neurons SP and ACh applied together gave a potentiated response (not seen with SP + glutamate or ACh + glutamate). Sastry (1978a) suggested that this potentiation could reflect the simultaneous activation of the paired *crest synapses* found in the interpeduncular nucleus (see Lenn 1976). It will be interesting to investigate this proposition with immunohistochemical methods.

Because of the relatively simple, synaptic connections (predominantly axodendritic, a few axosomatic, see Lenn 1976) and the possibility of blocking the muscarinic response, the interpeduncular nucleus may prove a suitable place to compare the excitatory effects of SP and ACh in the same appropriately chosen neurons by intracellular recording. Perfused slices could be used to determine the ionic requirements of the responses.

Substance P in Other Areas of the Central Nervous System

It is not unreasonable to expect that SP will have a transmitter function in all areas where terminals containing SPLI are present. Such areas are quite numerous (see Ljungdahl et al 1978a,b) but, except for the SP systems described above, the cell bodies of these terminals have not been localized and release of SPLI has only been demonstrated in the hypothalamus (Iversen et al 1976). However, iontophoresed SP increases the firing rate of neurons in the cuneate nucleus (Krnjević & Morris 1974), the locus coeruleus (Guyenet & Aghajanian 1977, Guyenet et al 1979), and the amygdala (Le Gal La Salle & Ben-Ari 1977). All of these areas contain at least moderately dense SP immunofluorescent fibers which could be terminals, whereas the amygdala exhibits an extremely dense SPLI-positive plexus. If SP release can be demonstrated in these regions and if the action of SP can be shown to be direct, then a general pattern emerges that SP in the CNS is an excitatory transmitter. Supporting evidence for a direct effect of SP in the locus coeruleus comes from the observation that SPLI-positive terminals make axo-dendritic synapses there (Pickel et al 1979).

Substance P and Synaptic Transmission in Autonomic Ganglia

Recent findings suggest that SP may play a role in autonomic transmission. Immunohistochemical studies (Hökfelt et al 1977b) have shown that fibers in several sympathetic ganglia contain SP. The inferior mesenteric ganglion of the guinea pig is particularly rich in these fibers which surround principal ganglion cells in a basket-like manner. A very dense network of SP-positive nerve terminals also occurs around cell bodies in the myenteric plexus (Hökfelt et al 1977b). No SPLI-positive cell bodies were found in any ganglia and thus the origin of these fibers remains unknown. It is possible that some are derived from primary sensory neurons. However, the presence of SP-positive fibers in organotypic tissue culture of intestine, in which all extrinsic innervation is presumably removed, indicates that SP neurons are present in the gut (Schultzberg et al 1978a). Interestingly, both inferior mesenteric ganglion cells and myenteric plexus neurons receive slow non-cholinergic excitatory synaptic innervation. In inferior mesenteric ganglia the slow potential is associated with a fall in membrane resistance in the majority of cells (Neild 1978). Dun & Karczmar (1979) found that SP (10–100 nM) caused a depolarization in these cells that was unaffected by cholinolytic drugs or by blockade of synaptic transmission with a low Ca^{2+}/high Mg^{2+} solution. In the majority of cells this response was also associated with a fall in membrane resistance. Of particular importance was the finding that the slow synaptic potential was abolished when ganglion cells were desensitized by large doses of SP. The slow synaptic potential in myenteric plexus neurons (Katayama et al 1979) is associated with a marked increase in membrane resistance. Similarly, SP elicits a slow depolarization with an increase in membrane resistance. Experiments in which the concentration of extracellular potassium was altered suggest that SP acts by decreasing resting potassium permeability. Grafe et al (1979) have questioned the involvement of SP in the slow synaptic potential of myenteric plexis neurons because the serotonin antagonist, methysergide, blocks the synaptic potential but not the action of SP. Thus they favor serotonin as the transmitter for the slow synaptic potentials in this ganglion. However, one cannot rule out that with different stimulus parameters SP may contribute to the slow potentials.

These physiological studies of autonomic ganglia provide pharmacological evidence that SP may mediate some forms of non-cholinergic slow synaptic excitation. Of particular interest is the finding that the excitatory action of SP in different types of ganglion cells is generated by distinctly different ionic mechanisms. Furthermore, these studies emphasize the value of simple neuronal systems in elucidating the cellular actions of SP.

SUBSTANCE P AND 5-HYDROXYTRYPTAMINE COEXIST IN SOME MEDULLARY NEURONS

The presence of two or more putative transmitters in the same mature neuron has been documented in invertebrates (Kerkut et al 1967, Brownstein et al 1974, but see also Osborne 1977) and in the mammalian peripheral (Hökfelt et al 1977a) and central nervous system (Chan-Palay et al 1978, Hökfelt et al 1978b). In several nuclei of the lower medulla oblongata in the rat, there are neurons that contain either 5-HT or SPLI, or both 5-HT and SPLI (Chan-Palay et al 1978, Hökfelt et al 1978b). The presence of both 5-HT and DOPA decarboxylase immunofluorescence in the SPLI-containing neurons (Hökfelt et al 1978b) argues against the possibility that the 5-HT derives solely from active uptake; rather, it would seem that there is a distinct population of neurons that can synthesize both 5-HT and SP. All searches for an active uptake system for SP have been negative (Iversen et al 1976, Segawa et al 1976, 1977, A. W. Mudge and S. E. Leeman, unpublished).

Where do these "5-HT + SP" neurons project? A serotonergic raphé-spinal pathway has been described (Dahlström & Fuxe 1965) and, because the SPLI in the ventral horn is of supraspinal origin (Hökfelt et al 1977c), it is possible that the "5-HT + SP" neurons are a component of the raphé-spinal pathway. Hökfelt et al (1978b) and Björklund et al (1979), Singer et al (1979) have examined this possibility. Selective destruction of serotonergic neurons was obtained by means of neurotoxins. Intracysternal or intraventricular administration of 5,6- or 5,7-dihydroxytryptamine (DHT) into rats pretreated with protryptiline to prevent destruction of catecholamine neurons (Hökfelt et al 1978b) resulted in the virtual disappearance of 5-HT immunofluorescence in both the dorsal and ventral horns and a decrease in the SP immunofluorescence in the ventral horn. Radioimmunoassay data indicate that there is a slight decrease in SPLI in the dorsal horn as well (Hökfelt et al 1978b, Björklund et al 1979), but by far the most severe depletion of SPLI (–93%) was seen in the lumbar ventral horn (Björklund et al 1979). These results suggest that the majority of SP neurons projecting to the ventral horn and a smaller population of SP neurons projecting to the dorsal horn contain both 5-HT and SP in their terminals. However, the possibility can not be entirely excluded that destruction of the 5-HT neurons with the neurotoxins may have damaged some SP neurons nonspecifically or may have led to SPLI depletion by a transsynaptic mechanism. The question can only be settled definitively at the ultrastructural level. Multiple action of 5,6-DHT is perhaps suggested by the interesting observation (Björklund et al 1979) that the SPLI levels fail to recover in the spinal cord even after 20 months. By that time, 5,6-DHT treated 5-HT

neurons regenerate some axons that reach all levels of the cord (see Björklund & Stenevi 1979). The SPLI levels in the raphé, on the other hand, did recover to control values by 20 months after the 5,6-DHT treatment (Björklund et al 1979). If it can be verified that a large component of the raphé-spinal projection consists of "5-HT + SP" neurons, then it is unlikely that these neurons represent a transitional state from "5-HT-only" to "SP-only" or vice versa, as transitional neurons would be expected to constitute only a small fraction of the total neuronal population (e.g. see Patterson 1978).

Particularly intriguing is the possibility of a descending "5-HT + SP" projection to the dorsal horn. SP is thought to be the transmitter of some nociceptive primary afferents (as discussed above), whereas 5-HT probably mediates the descending inhibition of nociceptive transmission in the dorsal horn (see Fields & Basbaum 1978). Speculations concerning such an arrangement include the possibility of centrally induced *peripheral pain,* in addition to simple potentiation or depression of incoming signals.

Aside from the synaptic organization of the proposed "5-HT + SP" terminals in the spinal cord, the most interesting question concerns the control of release of the two transmitter candidates. In particular, if both are released, are they always released simultaneously and at the same site or can their release be controlled separately? It is interesting, in this context, that reserpine treatment depletes the ventral horn only of 5-HT while the SPLI levels remain unchanged (Hökfelt et al 1978b). Whether this effect reflects a differential action of reserpine on the release (and hence storage or neuronal origin) of the two compounds, or simply indicates a selective inhibition of the 5-HT reuptake mechanism merits more specific investigation.

It is likely that more instances will be found in which two transmitter candidates are present in the same neuron. It will be important to demonstrate whether such neurons synthesize both compounds, or whether active uptake accounts for the presence of the second transmitter. The two possibilities have different functional implications.

THE SUBSTANCE P RECEPTOR IN SALIVARY GLANDS

SP was isolated on the basis of its sialogogic activity (Chang & Leeman 1970), which is not mediated via the adrenergic or cholinergic input to the salivary glands (Leeman & Hammerschlag 1967). It is likely that salivary glands are innervated by SP fibers. SP immunofluorescence has been reported in the vicinity of secretory structures (Hökfelt et al 1977c). Also, the SPLI levels in the rat submaxillary gland decrease upon section of the chorda tympani (Robinson et al 1979). The precise location of the SP

terminals and their function (sensory or effector) in the glands are not known, however. Direct application of SP to rat parotid gland slices induces K^+ and amylase secretion (Rudich & Butcher 1976). The action of SP in this system is very similar to that of α-adrenergic and muscarinic agonists, but is not mediated by any of these receptors. In particular, all three agonists are more potent secretagogues for K^+ than for amylase, and the K^+ release is Ca^{2+} dependent.

Putney's group has recently accumulated considerable evidence supporting the hypothesis that in rat parotid acinar cells, three distinct receptor systems (α-adrenergic, muscarinic, and SP) regulate the K^+ permeability by interacting with a single population of Ca^{2+} channels (Putney 1977, 1978, Marier et al 1978, Putney et al 1978, see also Putney 1979). They had shown previously that the agonist-induced increase in K^+ permeability, as measured by $^{86}Rb^+$ release from parotid acinar slices, is biphasic: an initial transient surge in secretion is followed by a steady, slowly declining phase (Putney 1976, 1978). The initial transient can occur in the absence of external Ca^{2+}, whereas the sustained phase requires Ca^{2+} in the medium. In the absence of external Ca^{2+} and in the presence of EGTA, the initial transient is a one-shot event: once it has been induced by any one of the three agonists, carbachol, phenylephrine or SP, it can no longer be elicited by any of the other two agonists (Putney 1977). That the three receptors are known to be distinct (Putney 1976, Rudich & Butcher 1976), suggests that all three receptors affect a common site. This cross-receptor inactivation can be prevented if Ca^{2+} is added to the medium before exposing the tissue to the second agonist. Then a second transient can be elicited. The initial transient, therefore, has the features of a capacitative event and Putney (1977) suggested that it might be mediated by the release of bound Ca^{2+} from a site inaccessible to EGTA, but accessible to all three receptors.

Further experiments tested the possibility that the three receptors might also regulate the sustained phase of $^{86}Rb^+$ release by acting at a common site. Because a Ca^{2+} ionophore, in the presence of external Ca^{2+}, can mimic agonist induced K^+ release (Selinger et al 1974) and because the sustained release is blocked by cobalt (Marier et al 1978), it is likely that this phase is triggered by Ca^{2+} influx. Indeed, $^{45}Ca^{2+}$ uptake into dispersed parotid acinar cells is stimulated by carbachol, epinephrine, isoproterenol, and SP (Putney et al 1978). Furthermore, the carbachol and SP-stimulated $^{45}Ca^{2+}$ uptake is blocked by cobalt (cobalt block of adrenergic stimulation was not tested.) If the three receptors acted on different Ca^{2+} channels, then their ability to induce the sustained phase of ^{86}Rb release should be additive. Marier et al (1978) found, however, that in the presence of a submaximal concentration of external Ca^{2+}, supramaximal concentrations of any two agonists were not additive. This strongly suggests that all three receptors

act on the same population of Ca^{2+} channels and individual cells possess all three receptors.

These studies represent the first clear description linking the postulated SP receptor with an identified ionic channel. They further demonstrate that the SP-stimulated increase in Ca^{2+} permeability is cobalt sensitive. Finally, they suggest that in parotid acinar cells a limiting number of these Ca^{2+} channels are shared by three distinct receptors. What still need to be demonstrated are the specific, saturable binding sites for SP in these cells.

Preliminary data from Putney's group (Wheeler et al 1979, J. W. Putney Jr., personal communication) indicates that specific binding of SP and analogs in dispersed parotid acinar cells can be demonstrated by the inhibition of ^{125}I-physalaemin binding that they cause. (Physalaemin is an SP-related peptide of amphibian origin and has a higher affinity for salivary gland tissue than SP.) ^{125}I-physalaemin binding is rapid, reversible, and saturable with an apparent K_m of 1.4 nM. Maximum specific binding appears to be very low (1.67 fmol /mg protein) and is therefore difficult to compare to the higher values (95.7 fmol/mg protein) obtained by Nakata et al (1978) for rabbit brain membranes. From Scatchard analysis it was estimated that there are only about 200 ^{125}I-physalaemin receptors per parotid acinar cell. The apparently excellent agreement between binding and pharmacological potency (^{86}Rb release) of SP and other peptides suggest, however, that these few binding sites may very well represent the physiologically relevant SP receptors. These studies are somewhat preliminary and should be extended to a more complete pharmacological characterization of the SP receptor by means of additional SP fragments and analogs. Also, it would be very useful to carry out ^{125}I-physalaemin binding studies with neuronal tissue for comparison. [Several laboratories have been unsuccessful in repeating Nakata et al's (1978) binding studies (S. H. Snyder, personal communication).]

An interesting feature of the receptors regulating Ca^{2+} permeability in salivary gland cells is the ligand-induced, Ca^{2+} independent turnover of phosphatidyl inositol (Oron et al 1975, Jones & Michell 1975) that they mediate. Preliminary studies suggest that SP also promotes phosphatidyl inositol turnover in parotid acinar cells (Jones & Michell 1978b). This is analogous to a vast number of other instances in various tissues in which a physiological response (e.g. secretion, exocytosis, contraction) seems to depend on Ca^{2+} entry and in which agonist binding leads to phosphatidyl inositol turnover in the absence of external Ca^{2+} (for a review see Michell 1975, Jones & Michell 1978a). Michell (1975) has argued that phosphatidyl inositol breakdown, which perhaps precedes ^{32}P incorporation, may be involved in the coupling of receptor occupation to Ca^{2+} channel opening. This proposition is speculative and somewhat controversial, but merits being tested by direct experiments.

It is an open question whether the characteristics of neuronal SP receptors are the same as those of the SP receptor(s) in salivary glands. By analogy with the nicotinic receptor, which appears to be different in neurons and in skeletal muscle (see Morley et al 1979 for a review), a similar situation may exist for SP receptors. Furthermore, just as there are nicotinic and muscarinic receptors for ACh, SP may have a set of receptors possibly associated with different ionic channels. Hence, a priori extrapolation seems unwise. But Putney's studies do suggest that it is worth investigating whether SP can induce Ca^{2+} currents in neurons as well.

THE INTERACTION OF SUBSTANCE P WITH SOME NICOTINIC RECEPTORS

SP depresses the activity induced by ACh in Renshaw cells (Krnjević & Lekić 1977, Belcher & Ryall 1977, Ryall & Belcher 1977) and in cultured adrenal chromaffin cells (Livett et al 1979).

Renshaw cells, which are innervated by motoneuron collaterals, can be identified by their characteristic high frequency discharge upon ventral root stimulation or upon iontophoresis of ACh. During iontophoresis of SP the nicotinic ACh-induced excitation is markedly reduced or completely abolished in virtually all Renshaw cells tested in the cat (Krnjević & Lekić 1977, Belcher & Ryall 1977, Ryall & Belcher 1977). Spontaneous firing or firing in response to ventral root stimulation is depressed to a lesser extent, possibly because the peptide does not diffuse to all activated synapses. Since excitation by glutamate, aspartate, DL-homocysteate, or a muscarinic agonist remains unaltered in the presence of SP, the SP-induced depression most likely occurs at the level of the nicotinic receptor.

A similar curare-like action of SP has been observed in cultured bovine adrenal cells (Livett et al 1979). Activity of these cells can be estimated from the stimulus-induced, Ca^{2+} dependent release of ^{3}H-NE into the medium. SP inhibits the ACh-or nicotine-induced release of ^{3}H-NE in a dose related manner. Neither the basal efflux nor the K^+-induced release of ^{3}H-NE are affected by SP. Because these cells do not respond to muscarinic agonists, these results are most easily explained by an interaction of SP with the nicotinic receptor-channel complex. The precise site and mechanism of action of SP remains to be determined. Rephrasing the suggestions made by Belcher & Ryall (1977), SP may prevent channel opening, block open channels, or promote channel closing. It could do so by competing with ACh or by acting independently. Cultured adrenal chromaffin cells seem to be a suitable system for further characterization of the interaction of SP with the nicotinic receptor.

In contrast to these cases of postsynaptic activity at a cholinergic synapse, the action of SP at the frog neuromuscular junction seems to be primarily

presynaptic (Steinacker 1977). The transient decrease in miniature endplate potential (mepp) amplitude caused by high (10^{-4} M) concentrations of SP does, however, raise the possibility that SP may also intereact with the nicotinic receptor in muscle; the high dose required may reflect a diffusional barrier. Alternatively, the receptors may be effectively different (see Morley et al 1979); perhaps physalaemin would be a more suitable ligand to test in the frog. A similar SP-induced decrease in mepp amplitude has also been observed at the Mauthner fiber-giant fiber synapse in the hatchetfish where the transmitter is also thought to be ACh (Steinacker & Highstein 1976).

Is the curare-like effect of SP of physiological relevance? Little is known about the synaptic arrangement of SP-containing terminals in the ventral horn, but appreciable amounts of SPLI are clearly present there (Takahashi & Otsuka 1975), and SPLI fibers have been described in the vicinity of motoneurons (Barber et al 1979). Small amounts of SPLI are also present in the rat adrenal gland as well as in the hatchetfish brain stem (A. W. Mudge and S. E. Leeman, unpublished). It is therefore conceivable that the interaction of SP with these nicotinic receptors may be more than just a pharmacological curiosity.

SUBSTANCE P IN ASSOCIATION WITH BLOOD VESSELS

The presence of SP-positive terminals in the walls of arteries and veins or in contact with capillaries suggests that SP plays a role in regulating blood flow in some tissues, whereas at other sites it is secreted into the circulation and may function as a hormone (Sundler et al 1977, Chan-Palay & Palay 1977, Barber et al 1979, Hökfelt et al 1977c). Hökfelt et al (1977c) report SP fibers in association with cerebral and cutaneous blood vessels. A dense plexus of SPLI-positive terminals around hypophyseal portal vessels of the primate median eminence suggest a role for SP in regulating anterior pituitary secretion (Hökfelt et al 1978c). Barber et al (1979) found that although SP fibers form an extensive network around blood vessels in the dorsal horn of the spinal cord, the fibers are in contact with the perivascular astrocytic processes rather than directly with the endothelial cells. The function of this arrangement is not clear, but Barber et al (1979) discuss some possible implications. The finding that endothelial cells can readily degrade SP (Johnson & Erdös 1977) may pose a problem for the passage of SP into the circulation. Chan-Palay & Palay (1977) indicate transport of membrane-bound SPLI granules through endothelial cells.

SPLI has been detected in the plasma of several species including man (Nilsson et al 1975, Skrabanek et al 1977). Extraction of bovine plasma (Leeman & Carraway 1977) or cat plasma (Gamse et al 1978) yields im-

munoreactive material that comigrates with synthetic SP in several chromatographic systems. Cannon et al (1979) find differences in the size and stability of endogenous and exogenous SP and suggest that in plasma, SP circulates in association with a carrier of large molecular weight. Their experiments are also consistent with the presence of a circulating precursor that cross-reacts with their antibody. In the cat, the intestine appears to be an important source of SP in plasma (Gamse et al 1978), which may be derived from the SPLI-containing endocrine-like cells located in intestinal mucosa (Heitz et al 1976). The intestinal origin of plasma SPLI again suggests a hormonal role for SP that is perhaps related to some aspect of gastrointestinal function. Of course the SPLI in plasma may partly or merely represent SPLI that has been released and is en route to excretion.

CONCLUSION

In this review we have focused on a few particularly well characterized systems in which SP might function as a neurotransmitter. Because the biosynthesis and inactivation of SP have not been characterized, the evidence for its role as a neurotransmitter is limited to presence, release, and mimicry. Probably the single most important advance in our understanding of SP, and for that matter, other peptides, has been in the category of presence. The development of extremely sensitive and specific immunoassays and immunohistochemical techniques has permitted a detailed description of the neuronal systems containing SP. In primary afferents, localization has been extended to the ultrastructural level. In addition, in this system studies on release have been very successful as it has been possible to unambiguously demonstrate a calcium dependent release of SP from primary afferents. In the central nervous system, the most difficult criterion to satisfy has been that of mimicry. Virtually all neurons that are affected by SP are excited; where examined, this excitation is associated with a depolarization. However, it has not been possible to compare rigorously the membrane effects of SP with the synaptic potentials that are presumed to be mediated by SP. Some of the technical problems that have prevented this comparison may be overcome with the use of slice preparations. The interpeduncular nucleus provides a favorable site for such an approach. On the other hand, in some autonomic ganglia SP has been shown to mimic the membrane effects of slow synaptic potentials, although a detailed analysis of the ionic mechanism and pharmacology are still required. The lack of a selective SP antagonist is a serious handicap in establishing the membrane effects and, hence, in evaluating the neurotransmitter role of SP. In addition, receptor binding studies have had limited success. The preliminary results using I^{125}-physalaemin in salivary gland tissue are promising, and it will be of interest to see if this approach can be used in the CNS.

Some results with SP may require an expansion of our concept of chemical transmission. The ultrastructural studies on primary afferents reveal terminals that have features resembling those of neuroendocrine cells. The intimate association of SP terminals with blood vessels and perivascular astrocytes is also consistent with a neuroendocrine function. The interaction of SP with some nicotinic responses suggests that it might alter synaptic transmission by directly interacting with the receptors of other neurotransmitters. Finally, the clear demonstration of the coexistence of SP and 5-HT in the same neurons raises interesting questions about the physiology of these neurons.

Research on SP has exploded during the past few years and, although the evidence for a classical neurotransmitter role is now very strong, recent findings indicate that it would be unwise to limit our thinking to such a narrow point of view.

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