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Perspective

Substance P

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Substance P Pharmacology: Possible Therapeutic Applications

The undecapeptide substance P (SP) was first described as a biologically active compound in gut extracts by von Euler and Gaddum[1] more than 50 years ago, and at that time they recognized its existence also in brain. Since the identification of the amino acid sequence by Chang et al.[2] in 1971 (Figure 1) progress has been rapid. The application of immunoassay and immunostaining techniques has provided a wealth of information on the distribution of SP within the mammalian nervous system, where the peptide is found in a number of distinct neuronal populations. One of the most thoroughly studied locations is in a subclass of small sensory neurons in spinal sensory ganglia, where SP may act as one of the "sensory transmitters" released by the terminals of primary afferent neurons in the dorsal horn of spinal cord. Because of its particular location in small sensory neurons possibly of the C-fiber category, as well as other circumstantial evidence, SP has been proposed as a possible transmitter for the sensory fibers that carry pain information from the periphery into the spinal cord and brainstem.[3] SP may also be released from the peripheral terminals of sensory nerves in skin or other organs, where it may play a role in the inflammatory response to tissue injury or infection.[3] Within the central nervous system (CNS), SP is present within numerous intrinsic neural pathways, in which its function is largely unknown. Some of these pathways, however, terminate in particular abundance in regions containing the cell bodies of catecholamine-containing neurons (e.g., substantia nigra, locus coeruleus), suggesting that SP may represent an important excitatory input controlling the activity of central monoaminergic systems.[3]

At present, our knowledge of SP pharmacology is rudimentary. There are few selective compounds available to be used as tools in exploring the possible physiological functions of SP. We know little of the nature of the cellular receptors with which SP interacts in target tissues, and few synthetic agonists or antagonists are available, and none of nonpeptide nature. Similarly, the mechanisms involved in the biosynthesis or metabolic degradation of SP are largely unknown.

It is consequently impossible to predict whether the development of SP pharmacology will lead to useful new therapeutic agents. One might speculate, nevertheless, that such compounds could have valuable applications. Thus, there is considerable interest in the possibility that antagonists of SP might represent a novel means of controlling some forms of pain and could thus potentially lead to the development of new, nonopiate, centrally acting analgesics. SP antagonists might also offer a novel means of depressing central monoaminergic function, and this in the case of dopaminergic systems might represent a novel alternative to neuroleptic drugs, which act by blocking dopamine at the receptor level.

In the periphery, blockade of SP actions could represent a novel approach to the development of antiinflammatory drugs, and SP-mimetic compounds might be valuable as hypotensive agents.

Such predictions are hazardous; what does seem clear is that we could be in a better position to understand just what role SP plays in the CNS and in peripheral organs if we had better pharmacological tools with which selectively to modify its actions. This article represents a highly biased account of some recent developments toward this end.

Substance P Receptors and the Possible Existence of Subclasses

Substance P has a broad spectrum of biological activities in addition to its role as a putative neurotransmitter in the CNS. The peripheral actions of SP include hypotension, vasodilation, salivation, and contraction of various smooth muscles ranging from the urinary bladder to the pupillary sphincter.[4] When administered directly into sites in the mammalian CNS, SP elicits scratching behavior, increased locomotor activity, and antidipsogenic activity.[4] In view of the diverse central and peripheral actions of SP, it seems likely that more than one type of SP receptor exists. This notion has received some support from observations of different rank orders of potencies among SP and its C-terminal fragments[5] or among members of the tachykinin

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Table I. Relative Potencies of Tachykinins and Substance P Analogues[a]

	SP-P			SP-E			CNS
	contraction of guinea pig ileum	guinea pig vas deferens	rat bladder	rat vas deferens	rat duodenum	hamster bladder	rat brain binding
physalaemin	2.43	0.80	1.81	0.60	1.8	0.70	1.9
eledoisin	0.90	0.60	0.96	86	13.9	105	0.3
kassinin	1.10	0.34	0.73	181	11.1	372	0.1
SP-OCH_3	0.95	0.52	0.61	0.01	0.02	0.01	0.95
SP-$NHC_2H_4NH_3^+$	1.1×10^{-3}	1.5×10^{-3}	5.6×10^{-4}	<0.01	9×10^{-3}		7.5

[a] The potencies of tachykinins and analogues are expressed relative to that of substance P (SP = 1) and represent the mean of at least three determinations. Standard errors were less than 40% and usually within 10% of the mean. The bioassays[13] and the rat brain binding assay[16] have been described elsewhere.

												Ref.
SUBSTANCE P	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	$MetNH_2$	
SP-P AGONIST	"	"	"	"	"	"	"	"	"	"	Met-OCH_3	13
CNS AGONIST	"	"	"	"	"	"	"	"	"	"	Met-$NHC_2H_4\overset{+}{N}H_3$	20
ANTAGONIST	"	D-Pro	"	"	"	"	D-Trp	"	D-Trp	"	$MetNH_2$	40

Figure 1. Substance P analogues with selective properties.

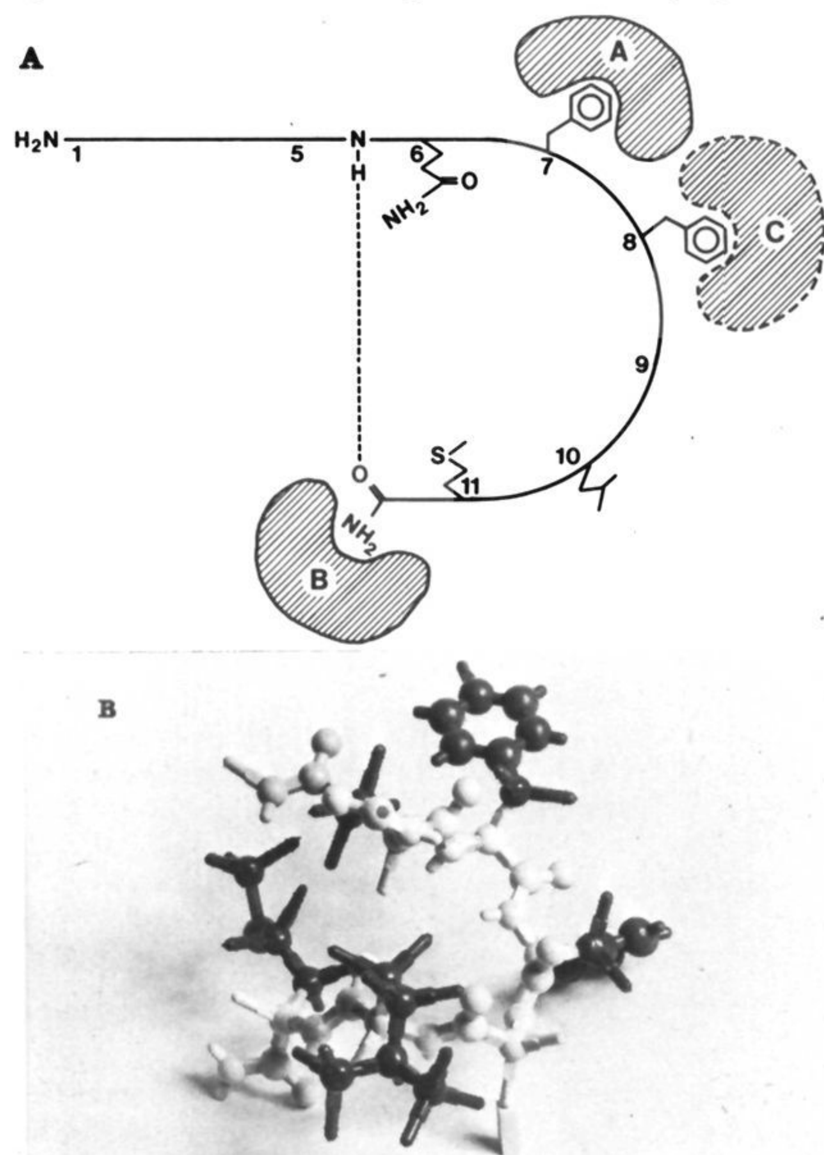

Figure 2. (A) A schematic picture of substance P–receptor interaction. The dashed areas represent binding subsites of the substance P receptor(s). The orientation of the side chains not interacting with any receptor subsites are arbitrary. (B) Nicholson model of a low-energy substance P conformer based on semi-empirical energy calculation.[27] Only the C-terminal hexapeptide is shown on the picture. The C-terminal carboxamide function is in the lower left, and Phe[8] is seen edge-on in the right part of the model.

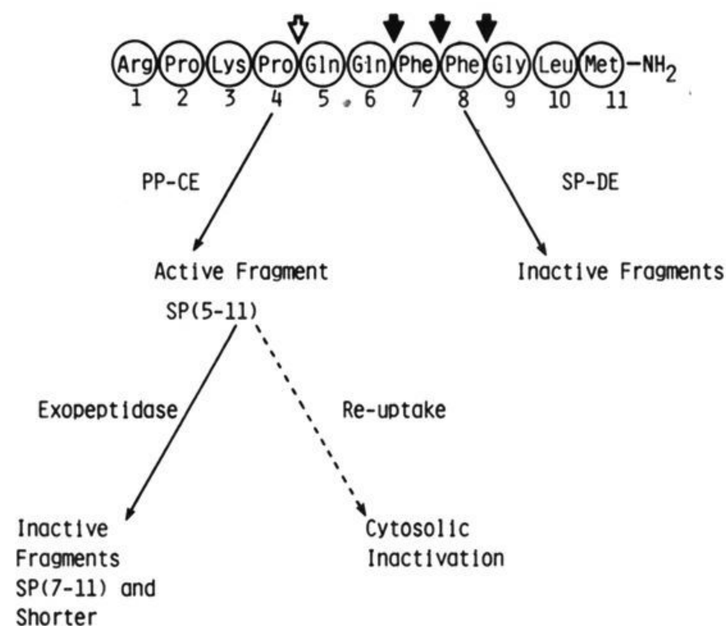

Figure 3. Two possible inactivation mechanisms for substance P. PP-CE = post-proline cleavage enzyme; SP-DE = substance P degrading enzyme.

family of peptides when tested in parallel in various bioassays.[6,7] These peptides share very similar C-terminal sequences (Figure 4). We found that peripheral organs could be divided into two groups with repect to their responses to different tachykinins (Table I).[8,9] In one group, typified by guinea pig ileum, the four peptides tested (physalaemin, substance P, eledoisin, and kassinin[10]) were all approximately equipotent. This class was termed "SP-P" since physalaemin was usually the most potent tachykinin, although all four peptides acted at nanomolar concentrations. In a second group, typified by rat vas deferens, the peptides had the rank order kassinin > eledoisin ≫ substance P ≃ physalaemin, and this was termed "SP-E" (Table I). A major difference between the two

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(10) Kassinin, Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2; Phyllomedusin, Glp-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH_2.

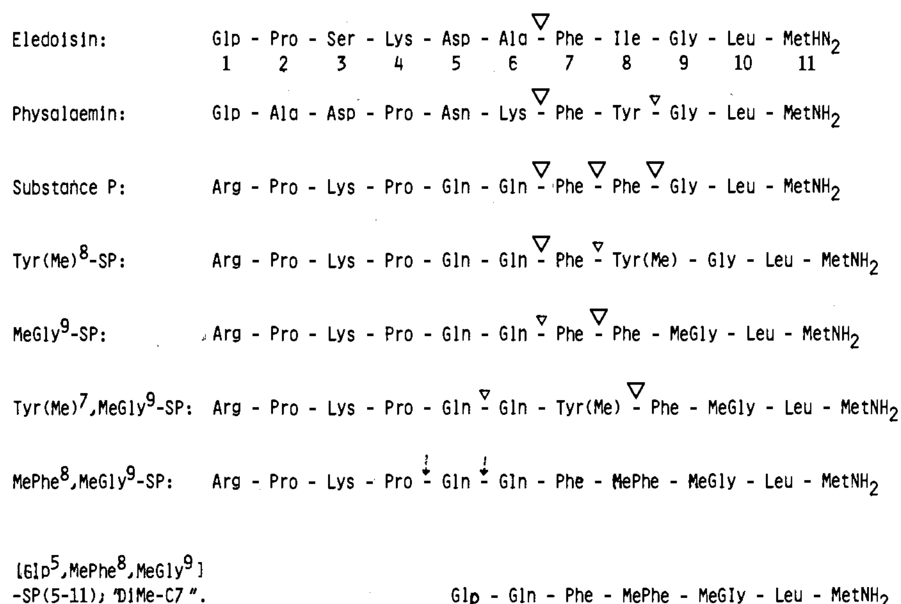

Figure 4. Substrate specificity of substance P degrading enzyme. Major (▽) and minor (▿) cleavage points after 30–60 min incubation with substance P degrading system. Cleavage of [MePhe[8],MeGly[9]]SP was detected only after a substantial longer incubation (>3 h). For DiMe-C7, no degradation could be detected after 7 h incubation with the same amount of enzyme.

classes of response is that whereas eledoisin and kassinin act at nanomolar concentrations on both, the concentrations of substance P and physalaemin needed to elicit responses in SP-E preparations are in the micromolar range.[8] Any subdivision of SP receptors based only on differences in the rank order of potencies of agonists can at best be regarded as tentative, since such factors as different rates of inactivation or differences in access to the receptors[11,12] could lead to apparent differences in the relative potencies of tachykinins. However, if the different patterns of SP tissue responses to tachykinins described above do reflect the presence of multiple receptors with slightly different structural requirements, it will be a challenging task to modify SP in order to increase or decrease activity on these different receptor populations. We found, for example, that replacing the methionine carboxamide with the corresponding methyl ester yielded an analogue with considerably increased selectivity for SP-P receptors (Figure 1, Table I).[13] The selectivity of SP methyl ester for SP-P sites results from a large decrease in the potency of this analogue in bioassays of the SP-E type, while it is approximately equipotent with the native peptide in SP-P systems.[13]

It is not clear whether the postulated subclasses of peripheral SP receptors are also present in the central nervous system. Centrally administered eledoisin has been reported to be considerably more potent than SP in eliciting scratching behavior in mice[14] and in its antidipsogenic activity in rats,[15] suggesting the possible presence of SP-E type receptors in the CNS. On the other hand, the specificity of [^{3}H]SP binding sites in rat brain membranes[16] appears to reflect that of SP-P sites. SP-E receptors, however, would only be expected to have low affinity for [^{3}H]SP and would not be detected in binding experiments using this radioligand.

In general, there is a good agreement between the potencies of SP-related peptides in competing for [^{3}H]SP binding sites in brain and the biological potencies of the same peptides as assessed by their depolarizing activity on spinal neurons or contraction on guinea pig ileum. Thus, in all these tests, the [desamido[11]] analogue (substance P free acid) exhibits a much reduced potency, and progressively shorter C-terminal fragments retain activity until the pentapeptide SP(7–11), which has a considerably lower potency. Results obtained with some N-terminal fragments of SP, however, revealed some interesting discrepancies between central and peripheral tests. All of these fragments were at least 10 000 times less potent than SP in the ileum assay, but some, particularly those which retained a C-terminal carboxamide, had up to 1% of the potency of SP in the rat brain [^{3}H]SP binding assay.[17] These N-terminal fragments of SP have also been reported to exhibit excitatory activities on mammalian neurons.[18,19] These observations suggested that not only is the carboxamide moiety of SP important for activity but also that modification of this moiety might lead to development of CNS-selective SP agonists. This prompted us to synthesize a number of SP analogues with an extended C-terminal amide, and, indeed, these analogues show a remarkable CNS selectivity.[20] For example, [methionyl-2-aminoethylamide[11]]substance P (Figure 1) was 10 times more potent than SP in competing for [^{3}H]SP binding in rat brain (Table I) and is more potent than SP in its ability to excite neurons when applied from microelectrodes placed in rat substantia nigra. Preliminary results indicate that the 2-aminoethyl analogue of SP also has a potency comparable with that of SP in depolarizing rat spinal cord neurons. In peripheral bioassays, however, the 2-aminoethyl derivative is 100–1800 times less potent than SP and shows no selectivity for SP-P or SP-E responses (Table

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I). The existence of CNS-selective agonists, however, does not necessarily imply the existence of different receptors in the CNS and periphery. Factors such as different rates of enzymatic inactivation or the partition of peptide analogues into the biophase could also lead to apparent tissue selectivity. However, the very large differences in potency of the amide-modified analogues of SP in the central and peripheral tests would be difficult to explain in this way, and we are encouraged to believe that the development of CNS-selective compounds is a real possibility.[9,20]

Structure-Activity Studies

In order to develop substance P pharmacology, one clearly needs a detailed knowledge of the structural requirements of biological receptors. So far only fragmentary information is available, based on studies of the chemically related naturally occurring peptides, the tachykinins, and a limited number of synthetic analogues for which published results are available.[4] The tachykinins all share a common C-terminal sequence:

Phe-X-Gly-Leu-Met-NH_2

The phenylalanine at the beginning of this sequence (position 7 in SP) is conserved in all tachykinins and appears to be an essential residue, probably involved in binding to the receptor. Indeed, in a systematic study in which all the amino acid residues of substance P were replaced one by one by L-alanine, position 7 proved to be by far the most important position.[21] Replacement of Phe^7 by Leu or Ile decreases the activity 50- to 500-fold.[22,23] However, the aromatic character of the side-chain residue seems to be less important. SP analogues possessing a strongly electronegative substituent in the para position of Phe^7 or those with electron-donating substituents in this position are equally potent on the guinea pig ileum assay. There is also no apparent correlation between hydrophobicity of para substituents at position 7 and biological activity. If Phe^7 is replaced by L-carboranylalanine, the biological activity is reduced about 1000-fold. Apart from the difference in hydrophobicity between phenylalanine and carboranylalanine, this might also be taken as an indication of restricted rotation of the phenyl nucleus about its C(1)–C(4) axis.[24] Substitution of Phe^7 with L-cyclohexylalanine gives an analogue with reduced activity but still substantially higher than the corresponding Leu^7 and Ile^7 analogues.[22] Since cyclohexylalanine occupies about the same volume as phenylalanine as judged by their molar refractority[25] but lacks the planar structure of an aromatic ring, a planar structure may not be an absolute requirement for high biological activity.

Position 8 in SP is particularly interesting because tachykinins, which are more potent than SP on SP-E systems, like kassinin[10] and eledoisin (Figure 4), have an aliphatic amino acid residue in this position. SP and physalaemin, which have aromatic residues in this position, have relatively low potencies in tissues of the SP-E type. If the nature of this residue is crucial for distinguishing between these two receptor subclasses, then eledoisin with an aromatic residue in position 8 would be expected to possess a pharmacological profile more like SP or physalaemin, and SP analogues containing Val^8 or Ile^8 should become like kassinin or eledoisin in their properties. However, although the published information available is fragmentary, some observations suggest that such an interpretation is too simplistic. Thus, Ala^8-SP retains about 30–40% of the activity of SP both in the guinea pig ileum assay (SP-P) and in the rat vas deferens (SPE),[21] suggesting that the mere presence of an aliphatic residue in position 8 does not by itself make SP more eledoisin- or kassinin-like. Furthermore, one of the tachykinins containing Ile in this position, phyllomedusin,[7,10] does not have a typical eledoisin- or kassinin-like pharmacological profile. It seems likely, therefore, that the N-terminal sequence is also a major determinant of the relative potencies of the tachykinins in SP-E and SP-P systems.

No correlation has been found between the electronegativity of the aromatic residue in position 8 of SP and biological activity, as assessed by the guinea pig ileum, nor does the hydrophobicity or the volume occupied by this side-chain residue have any marked influence on potency in the ileum assay. In contrast to Phe^7, Phe^8 can be replaced by L-cyclohexylalanine without affecting the spasmogenic activity.[22]

However, in the rat brain, [^{3}H]SP binding is more sensitive to modifications of Phe^8, suggesting that this residue may be more important for SP in its interaction with CNS receptors.[8]

NMR studies in solution[26] revealed that the spatial arrangement of the Phe side chains differs between biologically "active" and "inactive" C-terminal fragments of SP. The distribution of different rotational isomers of the C_α–C_β bond was found to be very similar for the longer fragments known to be "active" and differed significantly from that of shorter "inactive" fragments. This observation suggests that at least one of the Phe side chains requires this constrained conformation in order to form a proper binding to the SP receptor. It was not possible to make individual assignments for the β protons of Phe^7 and Phe^8, but since the structural requirement for the residue in position 8 is much less restricted than that of Phe^7, it is likely that the latter is more dependent on this special orientation. The NMR analyses[26] also suggested the existence of a strong hydrogen bond between the amide proton (or amino proton) of Gln^6 and a carbonyl group in the C-terminal amide sequence in "active fragments" of SP, and this hydrogen bond could be an essential element for biological activity. Recently, a set of stable molecular structures of SP based on a semiempirical conformation analysis was reported.[27] It was suggested that the "bioactive" conformation of SP requires the presence of a bend in the peptide backbone structure near Phe^7 of approximately 90° and another bend (although less marked) near Leu^{10}. This arrangement leads to a close proximity of the side chain of Gln^6 and that of Met^{11}. Another characteristic feature of these conformations is the "outward" orientation of the aromatic side chain of Phe^7. This is consistent with the proposed model based

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on NMR[26] as well as structure–activity studies, all leading to the conclusion that Phe[7] is likely to be directly involved in binding to the receptor. Inspection of a model based on these conformations (Figure 2) also reveals that by rotating the ψ^{11} and ϕ^{6} the amide proton of Gln[6] and the carbonyl group of Met[11] could come sufficiently close to form a hydrogen bond, consistent with the NMR data. Recently, another group[28] reported similar conformational features of substance P also based on conformational energy calculations. They favored bioactive structures stabilized by interactions between the C-terminal portion of the substance P molecule and the side chains of either the Gln[5] or Gln[6] residues. All of these proposed structures have one thing in common: they suggest a U shape for the C-terminal part of the molecule, providing a plausible explantation for the observation that full biological activity is retained in SP hexapeptide.

The general features of the structures discussed above are outlined in a schematic model of SP–receptor interactions (Figure 2). We propose that two different binding loci are important for the interaction of SP with peripheral receptors and that an additional/alternative locus may be needed for SP interaction with CNS receptors. We suggest that one important binding locus accommodates the aromatic side chain of Phe[7]. Since the aromatic character is not an absolute requirement, the interaction is probably hydrophobic in nature. This binding locus is probably common to all SP receptors. A sharp bend in the polypeptide backbone in the vicinity of Phe[7] might facilitate a constrained conformation of this residue. The presence of another bend in the vicinity of Gly[9] and Leu[10] might help to explain why these residues are highly conserved in nearly all tachykinins, since this bend is essential for the U shape of the molecule. Gly[9] can be replaced by *N*-methylglycine or L-alanine without affecting bioactivity,[29,21] but it cannot be replaced by D-amino acids.[30,31] Substitution in position 10 with *N*-methylleucine[32] decreases the activity 20-fold, whereas L-Ala in the same position hardly affects the activity at all.[21] A second binding locus (B) has different structural requirements, depending on the receptor subtype. In the SP-P type, this binding subsite can accommodate a C-terminal amide residue or an alkyl ester. Replacement of the C-terminal amide function with a carboxy group will introduce a negative charge as well as increase the polarity of the C-terminal, and it is also likely to break the proposed hydrogen bond. In the SP-E type of receptors, only the amide residue can be accommodated at site B, suggesting that one of the amide protons may be involved in hydrogen binding to this locus and that this bond may be essential for a high affinity to the receptor.[33] A special form of site B appears to be present in CNS and can accommodate a variety of C-terminal moieties, including alkyl esters of Met[11] or a 2-aminoethylamide function. The CNS receptor(s) may also have an additional binding locus (C) recognizing Phe[8], and this could be more important than the B site. This would be supported by the observation that CNS receptors are less capable of tolerating substitutions in Phe[8] and by the finding that N-terminal fragments of SP retain CNS activity.

Substance P Antagonists

The availability of antagonists that specifically block the actions of substance P will be of crucial importance in evaluating the physiological functions of SP. Such compounds might also possess desirable therapeutic properties, e.g., as novel analgesics. Two completely different routes for developing SP antagonists have been explored. One has been based on random screening of nonpeptide structures and has not, to our knowledge, been successful, so far. The only nonpeptide putative antagonist suggested to block the action of SP (baclofen) is not generally regarded as a specific SP antagonist.[4] The other approach, used by Folkers and his colleagues, has been to modify the structure of SP synthetically to yield a competitive antagonist. Insertion of D-amino acids at crucial positions in the sequence of LH-RH has been shown previously to confer antagonistic properties on these peptide analogues. The same strategy was used for SP. Evaluation of the first generation of D-amino acid substituted SP analogues revealed that [D-Phe[7]]SP had some weak antagonistic activity, although it also retained agonist properties.[23,31] Another crucial residue proved to be Gly[9]. The next series of compounds contained an analogue with D-Phe in position 7, D-Trp in position 9, and D-Pro in position 2. On the guinea pig ileum, [D-Pro[2],D-Phe[7],D-Trp[9]]SP produced a dose-dependent specific competitive inhibition of SP-induced contractions, and unlike the first generation of analogues, it possessed virtually no agonist activity.[34] This compound, however, is only weakly active, requiring concentrations of up to 100 μM for full antagonism of SP. More recently described analogues, with D-Trp in positions 7 and 9, are about 10 times more potent as antagonists in guinea pig ileum but still retain partial agonist properties in some other peripheral bioassays

One of the reasons why the development of SP antagonists has been regarded as such an important tool has been that such compounds might help to clarify the role of SP in nociception. Intrathecal injection of [D-Pro[2],D-Phe[7],D-Trp[9]]SP in mice has been reported by one group to be without effect on pain thresholds in the tail-flick or hot-plate reflex tests.[35] Intrathecal administration of the antagonist did, however, block the scratching and biting behavior, suggested to represent nociceptive responses, after capsaicin was applied cutaneously or after intrathecal injection of SP. The effect of the SP antagonist in blocking scratching behavior was also observed by Lembeck et al.[36] They found that intrathecal injections of the SP antagonist in rats lengthened the withdrawal time in the tail withdrawal latency tests, whereas SP itself administered through the same route had the opposite effect. These results support the suggestion that SP may be involved in some types of nociceptive transmission.

The antagonists have also been assessed in other systems in which SP is thought to be involved. It has been demonstrated, for example, that systemic administration of [D-Pro[2],D-Phe[7],D-Trp[9]]SP blocks antidromic vasodila-

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(33) To test this hypothesis we synthesized [Met-NHCH$_3$^{11}]SP. This analogue has high activity both in guinea pig ileum (SP-P) and in rat vas deferens (SP-E).
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tion.[36,37] Engberg et al.[38] found that [D-Pro^2,D-Trp^{7,9}]SP when applied from microelectrodes to locus coeruleus neurons blocked the excitatory effects of iontophoretically administered SP. The antagonist appeared to be specific for SP and did not effect the neuronal excitation elicited by iontophoretically applied acetylcholine or glutamate. It is notable that the ejection current, as well as the peptide concentration, was the same for SP and for the antagonist, suggesting that the antagonist may be more potent in its central actions than in peripheral bioassays. This conclusion was not supported, however, by Salt et al.,[39] who failed to observe any antagonistic effect of this analogue on SP-induced excitation of neurons in rat trigeminal nucleus. [D-Pro^2,D-Trp^{7,9}]SP has also been used successfully to block the action of exogenous substance P in the guinea pig taenia coli and in the rabbit iris pupillary sphincter, as well as the noncholinergic nonadrenergic excitatory responses to electrical stimulation in these tissues.[40]

The availability of specific SP antagonists has already had a considerable impact on SP research. However, the relatively low potency of the present generation of substance P antagonists, which require micromolar concentrations to block the actions of SP, and the fact that some of the compounds retain agonist activity make them far from ideal tools. It is notable that in the present generation of SP antagonists, conversion of agonistic to antagonistic properties was achieved by substitution with D-amino acids in positions that are importantly involved in the two bends suggested by conformational studies (Figures 1 and 2).

Metabolic Inactivation of Substance P and the Development of Stable Analogues

If SP acts as a neurotransmitter in the nervous system it is likely that some mechanism exists for inactivating the peptide after its release from nerve terminals. One such mechanism would be removal of SP by tissue uptake processes, but there is no evidence that a specific uptake system exists for SP or for any of the other neuropeptides likely to act as neurotransmitters in the CNS. Recently, however, it was suggested that a specific uptake mechanism for the C-terminal hepapeptide of SP, but not for the undecapeptide, may be present in rabbit spinal cord and rat brain.[41] Although SP heptapeptide does not occur in any significant amounts in the CNS, it could be generated by cleavage of SP with a postproline cleavage enzyme that is present in the brain.[42] The postproline cleavage enzyme, however, cannot by itself inactivate SP, since the C-terminal heptapeptide fragment generated by this enzyme retains full biological activity. The heptapeptide could, however, be removed by reuptake, as mentioned above, or it could secondarily be degraded by exopeptidases; these possibilities require further assessment (Figure 3). Alternatively, the postproline cleavage enzyme could be regarded as a processing enzyme, with the function of generating N-terminal fragments of SP, which have been reported to possess their own intrinsic biological activities,[43] distinct from those associated with the C-terminal portion of the molecule.

The most likely mechanism for inactivation of SP after its synaptic release appears to be by endopeptidase cleavage directed at the C-terminal portion of the molecule. A number of enzymes have been reported to exist in the brain that are capable of inactivating SP by such cleavages. These include both cytosolic and membrane-bound endopeptidases.[44] One of the cytosolic enzymes partially purified from rat brain was shown to release mainly phenylalanine and leucine from SP and is, therefore, likely to cleave the bonds between Gln^6-Phe^7 or Phe^7-Phe^8 and Gly^9-Leu^{10}. A neutral endopeptidase isolated from bovine hypothalamus also cleaves SP in the bioactive C-terminal region, probably by splitting the Gln^6-Phe^7 and Phe^7-Phe^8 bonds. Furthermore, cathepsin D purified from a calf brain lysosomal fraction cleaves SP between Phe^7-Phe^8. All these endopeptidases, although capable of inactivating SP, have one feature in common: they do not show any specificity for SP. Furthermore, most of them are cytosolic or lysosomal enzymes, and it is difficult to see how they could have access to SP once it had been released into the extracellular fluid. The best candidate for an inactivating enzyme would be a membrane-bound endopeptidase that attacks the C-terminal region of SP and with a high specificity for SP. We recently characterized a novel membrane-bound endopeptidase from human brain that fulfills some of these criteria.[44] The enzyme cleaves SP in one of three positions: Gln^6-Phe^7, Phe^7-Phe^8, or Phe^8-Gly^9. Any of these cleavages yields inactive fragments (Figure 3). The enzyme is a neutral metalloendopeptidase with an apparent molecular weight of 40 000–50 000; the apparent K_m for SP is 29 μM. The most unusual feature of this enzyme is its specificity for SP, hence the trivial name "SP degrading enzyme". Unlike other enzymes reported to degrade SP, this enzyme prefers SP as its substrate among the various other neuropeptides tested.[44] The enzyme requires a substantial portion of the SP sequence for full substrate recognition. Structurally related peptides like physalaemin and eledoisin are much less effective in competing with [^{3}H]SP for the active site of the enzyme; although they are degraded by the enzyme, the cleavage pattern is different from that of SP (Figure 4).

Comparison of the ability of a number of SP analogues to inhibit degradation of [^{3}H]SP by the purified SP-degrading enzyme or to act as substrate themselves for degradation by the enzyme revealed some interesting data. All the full-length SP analogues were virtually equipotent with SP in inhibiting degradation of [^{3}H]SP, even though some analogues, e.g., [(Me)Phe^8,(Me)Gly^9]SP, were completely protected against degradation by the enzyme.[29] Thus, the enzyme appears to recognize parts of the molecule other than those immediately adjacent to cleavage sites. This conclusion was also supported by the finding that C-terminal fragments of SP, particularly the nonapeptide and shorter fragments, were much less potent than SP in inhibiting [^{3}H]SP degradation and were hydrolyzed only slowly, although the amino acid sequence at the cleavage regions was identical with that of SP. The number of peptide bonds available for hydrolysis seemed to be of minor importance in limiting the ability of the enzyme to degrade SP-like substrates. Thus, protecting one bond, as in [(Me)Gly^9]SP, or two bonds, as in [Tyr(Me)^7,(Me)-

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Gly^9]SP, did not confer any overall resistance to degradation. In order to confer an increased resistance toward the enzyme, all three cleavage sites in SP had to be protected. This was most successfully achieved by substitution of *N*-methyl amino acid residues in positions 8 and 9 (Figure 4). Thus, [(Me)Phe^8,(Me)Gly^9]SP exhibited complete resistance to cleavage at any of the three normal sites when incubated with purified SP-degrading enzyme.[29] However, although [(Me)Phe^8,(Me)Gly^9]SP retained biological activity, it was still degraded by N-terminal cleavages when incubated with rat hypothalamic slices, albeit more slowly than native SP.[45] It was, therefore, decided to attempt to design an enzyme-resistant analogue of SP based on a C-terminal fragment of the peptide. The most successful analogue, [Glp^5,(Me)Phe^8,(Me)Gly^9]SP-(5-11) ("DiMe-C7") was equipotent with SP in the rat brain [^{3}H]SP binding assay and completely resistant to degradation when incubated with purified SP-degrading enzyme or with rat brain slices or synaptic membrane preparations[29,45] (Figure 4). Furthermore, this analogue seems to be protected against degradation in the intact rat brain in vivo. When [^{3}H]DiMe-C7 was microinfused into rat brain (ventral tegmentum), very little degradation was observed even after 50 min, compared with [^{3}H]SP, which was completely degraded[45] within 10 min. When DiMe-C7 was injected into the ventral tegmental area of the rat brain, it elicited a hyperactivity response similar to that produced by injecting SP, but of prolonged duration.[46,47] Thus, DiMe-C7 represents the first SP analogue that is protected against degradation in the mammalian CNS; as such, it should prove a useful new research tool.

Conclusions

Although SP pharmacology is still in its infancy, there has clearly been some progress in recent years. We can begin to discern the general properties of SP receptors and to appreciate the possible existence of multiple subclasses in peripheral tissues and in the CNS—a phenomenon that clearly has a potentially important bearing on the possibility of designing agonists or antagonists with tissue-selective actions. The first useful SP antagonists are now available and will certainly be improved upon in the near future. Such compounds will undoubtedly play a crucial role in furthering our understanding of the functions of SP in the periphery and the CNS.

Drugs based on peptide structures, however, have a number of intrinsic disadvantages as potential therapeutic agents: they are not easily absorbed, they do not penetrate readily into the CNS, they are liable to rapid degradation, and they are costly to manufacture. An ideal would be to discover nonpeptide structures with agonist or antagonist properties at SP receptors. This goal, however, is still beyond even the most sophisticated resources of modern medicinal chemistry and is most likely to be achieved by serendipity. In more general terms, the pursuing of SP pharmacology offers many stimulating challenges, as there are few precedents to guide one to the most suitable strategies. It may be that the lessons learned from work on SP will prove in the future to have a more general application to the development of "peptide pharmacology" for the 2 dozen or more other neuropeptides now known to exist in the mammalian nervous system.

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Communications to the Editor

Design, Synthesis, and Testing of Potential Antisickling Agents. 1. Halogenated Benzyloxy and Phenoxy Acids

Sir:

Many classes of organic compounds[1] have been investigated with the hope that a viable treatment for sickle cell anemia could be found. The cause of this disease is a mutation of Glu in normal hemoglobin (HbA) to Val in sickle hemoglobin (HbS) on the β subunits at position 6. This structural change results in the polymerization of HbS in the red blood cell under anaerobic conditions, giving rise to the disorder known as sickle cell anemia. As of yet, no known agents are approved for general use.

We have attempted[2,3] to design antigelling agents in a logical manner by determining their binding sites in the hemoglobin molecule by X-ray analysis. Using carboxyhemoglobin A crystals, we discovered that iodobenzene and *p*-bromobenzyl alcohol bind in a hydrophobic cavity located on the α subunits of Hb in the region of Trp-14α. Both of these agents possess some antigelling activity.[2-4] Previously, Schoenborn[5] has shown that the primary binding site of the antisickling agent dichloromethane is in this cavity. These results prompted us to continue to explore simple halogenated aromatic molecules for their ability to hinder hemoglobin S polymerization.

Our first attention toward further structural modifications of aromatic halogen molecules drew us to consider making derivatives of *p*-bromobenzyl alcohol. Ross and Subramanian[4] have shown that *p*-bromobenzyl alcohol, although very active at 5 mM, has questionable utility because of its limited solubility in aqueous medium. We therefore initiated a study to solubilize *p*-bromobenzyl alcohol via addition of various hydrophilic organic moieties to the alcohol function. The first of these modifications

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