0270-6474/85/0505-1293$02.00/0
Copyright © Society for Neuroscience
Printed in U.S.A.

The Journal of Neuroscience
Vol. 5, No. 5, pp. 1293-1299
May 1985

Characterization and Segmental Distribution of ^{125}I-Bolton-Hunter-labeled Substance P Binding Sites in Rat Spinal Cord[1]

CLIVEL G. CHARLTON[2] AND CINDA J. HELKE

Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

Abstract

Substance P (SP) is widely distributed in the spinal cord and has been implicated as a neurotransmitter in several spinal cord neuronal systems. To investigate SP receptors in the spinal cord, ^{125}I-Bolton-Hunter-SP (^{125}I-BH-SP) was used to identify and characterize spinal cord binding sites for the peptide. The binding of ^{125}I-BH-SP had the following characteristics: high affinity; time, temperature, and membrane concentration dependent; reversible; and saturable. The IC_{50} of SP in whole spinal cord was 0.46 nM as compared with 0.95, 60, and 150 nM for physalaemin, eledoisin, and kassinin. Four putative antagonists of SP were < 0.0001 times as potent as SP in inhibiting ^{125}I-BH-SP binding. IC_{50}s were 5, 7.5, 7.0, and 45 μM for D-Pro^2, D-Trp^{7,9}-SP; D-Pro^2, D-Phe^7, D-Trp^9-SP; D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP; and D-Pro^4, D-Trp^{7,9,10}-SP(4-11), respectively. The lumbosacral section bound 3 times more SP than the cervical and thoracic sections, although IC_{50} for the cervical section was 0.06 of that for the lumbosacral and thoracic sections. The data suggest more than one class of binding site for SP in the spinal cord and indicate a direct role for SP in spinal cord functions.

Substance P (SP) was detected in the equine brain and gut by von Euler and Gaddum (1931). Thirty-nine years later, the peptide was isolated from bovine brain and identified by Chang and Leeman (1970). It was later found that SP is contained in the spinal cord (Hökfelt et al., 1975, 1976, 1977; Takahashi and Otsuka, 1975; Ditirro et al., 1981) where the highest levels were localized in the dorsal horn, lamina X region, the intermediolateral cell column (IML), and the ventral horn. Cell bodies in the dorsal root ganglia (Hökfelt et al., 1975; Takahashi and Otsuka,1975) and probably the Edinger-Westphal nucleus (Phipps et al., 1983) are the sources for the majority of SP varicosites found in the dorsal horn. The caudal brainstem raphe nuclei (de Lanerolle and LaMotte, 1982) and the nucleus interfascicularis hypoglossi of the ventral medulla (Helke et al., 1982) are two sources for SP in the ventral horn. The nucleus interfascicularis hypoglossi also supplies SP-containing nerve terminals to the IML cells of origin for autonomic preganglion fibers (Helke et al., 1982).

Functional studies support the concept of a neurotransmitter role for SP in the spinal cord. Nociception (Piercey et al., 1981; Åkerman et al., 1982; Fasmer and Post, 1983), motor control (Otsuka and Konishi, 1977; Yanagisawa et al., 1982) and certain autonomic functions (Loewy and Sawyer, 1982; Keeler and Helke, 1984) are modulated by spinal cord SP. In addition, SP receptors in the spinal cord have been demonstrated by iontophoretic studies in which SP was shown to excite nociceptive neurons in the dorsal horn (Henry, 1976) and preganglionic sympathetic neurons in the IML (Backman and Henry, 1984).

Specific binding sites for SP were recently described in homogenates of brain (Hanley et al., 1980; Perrone et al., 1983; Quirion et al., 1983), salivary glands (Liang and Cascieri, 1980), and small intestine (Buck et al., 1984). In addition, autoradiographic studies showed the distribution of SP receptors in both forebrain (Shults et al., 1982; Quirion et al., 1983) and hindbrain nuclei (Helke et al., 1984). However, SP receptors in the spinal cord are not as well characterized. Whereas specific binding of SP to whole cord synaptosomes (Torrens et al., 1983) and the autoradiographic distribution of binding sites in the thoracic spinal cord (Maurin et al., 1984) were reported, the characterization and segmental distribution of SP binding sites in spinal cord membranes were not studied. In addition, the binding properties of putative SP receptor antagonists in the spinal cord are unknown. Therefore, this report will address the kinetics and pharmacology of ^{125}I-Bolton-Hunter-labeled substance P (^{125}I-BH-SP) binding to spinal cord membrane homogenates with emphasis on segmental binding, and the effects of SP-like peptides and putative SP antagonists.

Material and Methods

Animals and chemicals. Sprague-Dawley male rats weighing 250 to 300 gm were obtained from Taconic Farms (Germantown, NY). The rats were housed in a colony room with a 12-hr light/12-hr dark cycle and maintained at 23°C. Food and water were supplied *ad libitum*, and the animals were allowed to acclimatize at least 1 week before being utilized.

Synthetic SP, physalaemin; eledoisin; kassinin; D-Pro^2, D-Trp^{7,9}-SP; D-Pro^2, D-Phe^7, D-Trp^9-SP; D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP; and D-Pro^4, D-Trp^{7,9,10}-SP(4-11) were purchased from Peninsula Laboratory (San Carlos, CA). Trisma, bovine serum albumin (BSA), bacitracin, leupeptin, chymostatin, manganese chloride, ethylenediamine tetra-acetate (EDTA), and dextrose were from Sigma Chemical Co. (St. Louis, MO). Radiolabeled monoiodinated Bolton-Hunter-SP (^{125}I-BH-SP; 2000 μCi/μmol) was purchased from New England Nuclear (Boston, MA) or was prepared in this laboratory, utilizing the method of Bolton and Hunter (1973). Purification of the latter was achieved by fractionating on a Sephadex G-25 (fine) column using 0.025 M NaCl, 0.01 M acetic acid containing 0.02% sodium azide and 0.2% BSA as elution medium. The second peak eluted from the column contained ^{125}I-BH-SP and was further purified on SP-Sephadex C-25 cation exchange resin.

Received July 26, 1984; Revised November 5, 1984;
Accepted November 9, 1984

[1] We wish to thank Mrs. Jean Bartlett for typing this manuscript and Mrs. Elaine Phillips for her technical assistance. This work was supported by National Institutes of Health Grant NS-19317.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Animal Resources, National Research Council, DHEW Publication No. (NIH) 74-23.

[2] To whom correspondence should be addressed.

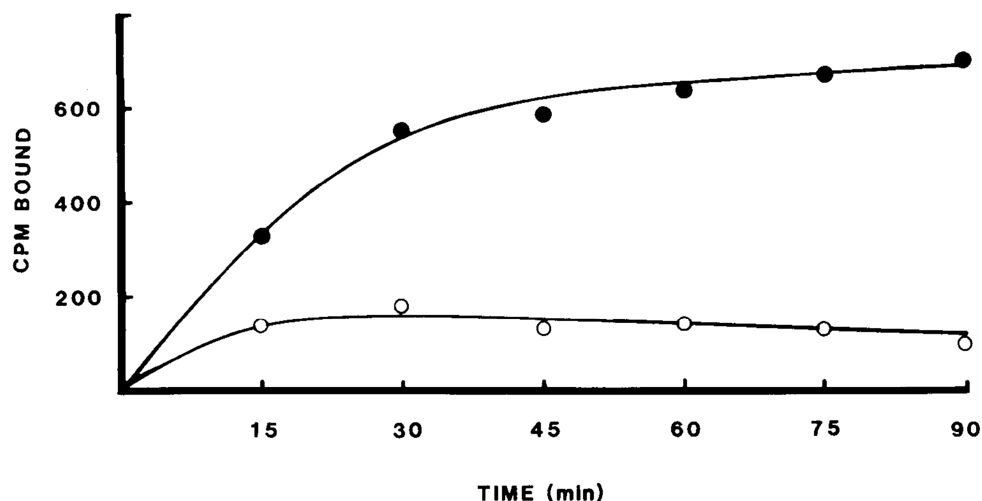

Figure 1. Time course of ^{125}I-BH-SP binding to rat spinal cord cell membranes. *Open circles* represent nonspecific binding, and *solid circles* represent specific binding (total minus nonspecific). ^{125}I-BH-SP (26 fmol) was incubated with (nonspecific binding) or without (total binding) 1 μM SP for varying times, up to 90 min, before centrifugation and the determination of the quantity of ^{125}I-BH-SP bound to membranes.

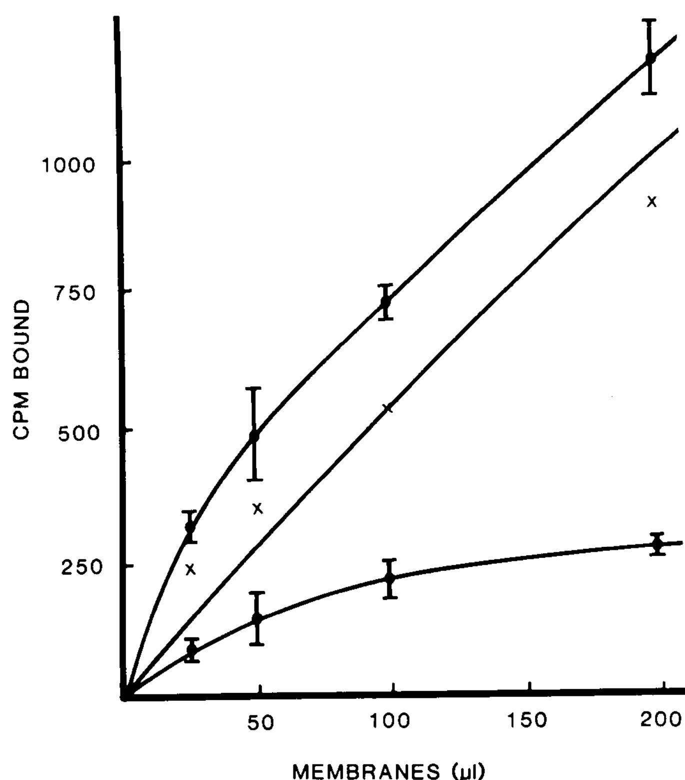

Figure 2. Binding of ^{125}I-BH-SP as a function of spinal cord cell membrane concentrations. The *lower curve* represents nonspecific binding, the *middle curve* represents specific binding, and the *upper curve* represents total binding. ^{125}I-BH-SP was incubated with or without 1 μM SP for 75 min.

The SP-Sephadex C-25 packed column was washed with the above medium after the radioactive material was loaded, and ^{125}I-BH-SP was eluted using 0.5 M NaCl in the medium instead of the 0.025 M concentration.

Cell membrane preparation. Rats were decapitated and spinal cords were removed by applying air pressure to one end of each section (about 2.5 cm) of the vertebral column removed from the rat. Entire spinal cords or sections of the cord were placed in 50-ml polypropylene centrifuge tubes containing about 40 vol of ice-cold Tris-HCl buffer (50 mM Tris-HCl, pH 7.4, plus EDTA, 2.5 mM; BSA, 0.02%; bacitracin, 40 μg/ml; leupeptin, 4 μg/ml; chymostatin, 2 μg/ml). The tissue was homogenized with a Polytron (Brinkman Instruments, Westbury, NY) for 90 sec at a setting of 3. After 1 hr standing in the cold (ice), the tubes were centrifuged at 5000 × *g* for 10 min and the supernatant was discarded. The pellets were resuspended and washed twice in about 40 vol of the buffer and three more times in a similar buffer containing 3.0 mM Mn^{2+} instead of EDTA. The final pellets (termed spinal cord cell membranes) were suspended in 10 vol of the latter buffer and contained about 520 μg of protein/100 μl.

^{125}I-BH-SP binding assay. The binding of ^{125}I-BH-SP to rat spinal cord cell membranes was performed at room temperature for 75 min (or as otherwise stated) in 50 mM Tris-HCl, pH 7.4, containing 0.02% BSA, 40 μg/ml of bacitracin, 4 μg/ml of leupeptin, 2 μg/ml of chymostatin, and 3.0 mM Mn^{2+}. Incubation was performed in 12 × 75 mm borosilicate glass tubes in a final volume of 0.5 ml containing assay buffer, ^{125}I-BH-SP (26 fmol), with or without unlabeled peptides (e.g., SP, SP antagonists, physalaemin, eledoisin, and kassinin). Incubation was started by adding membranes (100 μl) to the pre-equilibrated mixture. After 65 min the incubate was layered over a 3-ml volume of cold 0.1 M dextrose solubilized in the Tris-HCl buffer, and contained 0.20% BSA instead of 0.02% in 12 × 75 mm borosilicate glass tubes. At 75 min, the tubes were centrifuged at 5000 × *g* for 8 min in the cold and the supernatant was aspirated. The radioactivity in the pellet was determined in a Micromedic 4/600 gamma counter.

A small quantity of the membrane suspension was used for protein determination (Lowry et al., 1951).

Results

Binding of ^{125}I-BH-SP as a function of time, membrane concentrations, and temperature. Figure 1 illustrates the time course for the binding of ^{125}I-BH-SP to rat spinal cord cell membranes. The binding of 26 fmol to 100 μl (520 μg of protein) of membrane increases linearly for 15 min and plateaus at about 60 min (Fig. 1, *solid circles*). In the presence of 1 μM SP (nonspecific binding), the binding of ^{125}I-BH-SP was much lower and does not increase during the 90-min incubation period. The binding of ^{125}I-BH-SP as a function of membrane concentration is shown in Figure 2. The quantities bound were proportional to the membrane concentration. The temperature dependence of binding was also tested, and it was shown that, although nonspecific binding at 4°C and room temperature (25°C) was equal, the specific binding of ^{125}I-BH-SP to spinal cord membranes at 4°C was only 18.9% of the specific binding that occurred at 25°C.

Inhibition of ^{125}I-BH-SP binding by SP. A representative experiment illustrated in Figure 3 shows the dose-dependent inhibition of ^{125}I-BH-SP binding to spinal cord cell membranes by unlabeled SP. A steady-state inhibition of binding was achieved with 0.1 to 1.0 μM SP, but 1.0 μM was routinely used to determine specific binding. Nonspecific binding represents 5 to 20% of the total membrane binding throughout the study; the higher quantities occurred with aging of the ^{125}I-BH-SP preparations. Twenty percent inhibition of specific binding occurred with 0.014 nM and 80% with 4.0 nM of

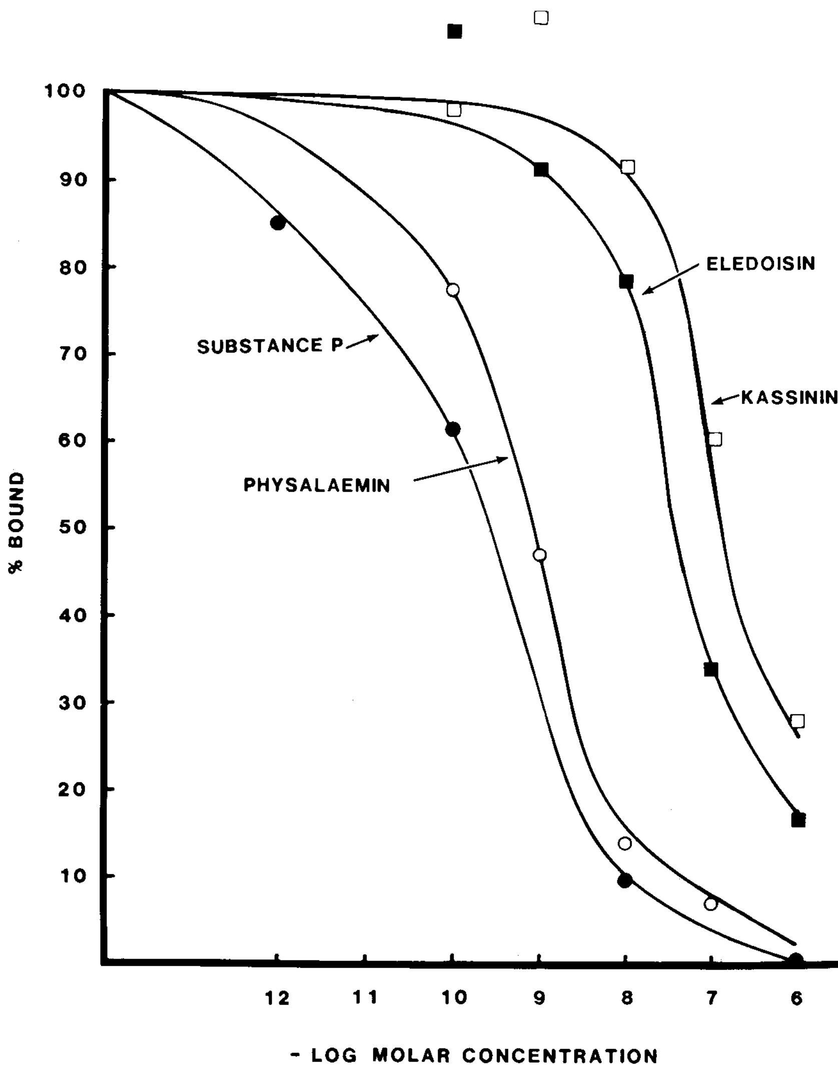

Figure 3. Inhibition of the binding of ^{125}I-BH-SP to spinal cord cell membranes by unlabeled SP as compared with physalaemin, eledoisin, and kassinin. ^{125}I-BH-SP (26 fmol) was incubated with various concentrations of the peptides or without peptides for 75 min. The specific binding is shown.

unlabeled SP. The IC_{50} (50% inhibition of specific binding) was 0.4 nM. A Scatchard transformation (Scatchard, 1949) of the inhibition binding of ^{125}I-BH-SP by SP (Fig. 4) gave a concave curve which indicates a lower binding affinity at higher ligand concentrations. Analysis of the Scatchard plot assuming two independent classes of sites (Klotz and Hunston, 1971) produced a curve with a K_d of 0.162 nM and B_{max} of 2.89 fmol/mg of protein (Fig. 4*A*) and another with a K_d of 3.69 nM and B_{max} of 17.4 fmol/mg of protein (Fig. 4*B*).

Inhibition of ^{125}I-BH-SP binding by physalaemin, eledoisin, and kassinin. A dose-dependent inhibition of ^{125}I-BH-SP binding was also examined for three members of the tachykinin family of peptides (physalaemin, eledoisin, and kassinin). Figure 3 shows the inhibition curves as compared with SP. The order of potency was SP > physalaemin > eledoisin > kassinin. The IC_{50}s were 0.45, 0.95, 60, and 150 nM, respectively. SP was twice as potent as physalaemin, 133 times more potent than eledoisin, and 333 times as potent as kassinin. The potencies of SP and physalaemin are closer and are to the left of eledoisin and kassinin, whose potencies are different by less than a multiple of two. This reflects an apparent structure-activity relationship, because SP is more similar to physalaemin and eledoisin is closer to kassinin in their amino acid sequences (Table I).

Inhibition of ^{125}I-BH-SP binding by putative SP antagonists. Four D-amino acid analogues of SP were also examined for the inhibition of the binding of ^{125}I-BH-SP to spinal cord cell membranes. Figure 5 shows the inhibition curves for these putative SP antagonists as compared with SP. All analogues were less than 0.0001 as potent as SP in inhibiting ^{125}I-BH-SP binding. The IC_{50}s were 5, 7.5, 7, and 45 μM for D-Pro^{2}, D-Trp^{7,9}-SP, D-Pro^{2}, D-Phe^{7}, D-Trp^{9}-SP, D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}-SP, and D-Pro^{4}, D-Trp^{7,9,10}-SP(4-11), respectively.

Binding of ^{125}I-BH-SP to membranes from spinal cord sections. The binding of ^{125}I-BH-SP to membranes prepared from the cervical, thoracic, and lumbosacral sections of the spinal cord was investigated. Table II shows that the specific binding per milligram of protein in the lumbosacral section was more than 3 times the specific binding observed in the cervical and thoracic sections, which showed approximately equal binding. However, comparative inhibition curves (Fig. 6*A*) for the three sections showed equal IC_{50}s (0.7 nM) for the thoracic and lumbosacral sections, whereas the IC_{50} for the cervical section was 15.5 times lower. The Scatchard transformation of the inhibition binding gave concave curves for the cervical and lumbosacral sections and an almost straight-line curve for the thoracic section (Fig. 6, *B* to *D*). The concave Scatchard curves indicate heterogeneous binding interaction and suggest different SP binding sites or different states of homogeneous SP binding sites in the spinal cord. The slope of the initial part of the curve is steepest for the cervical section and is indicative of binding sites with highest affinity. The point of interception of the curves on the abscissa substantiate a higher binding capacity for the lumbosacral section.

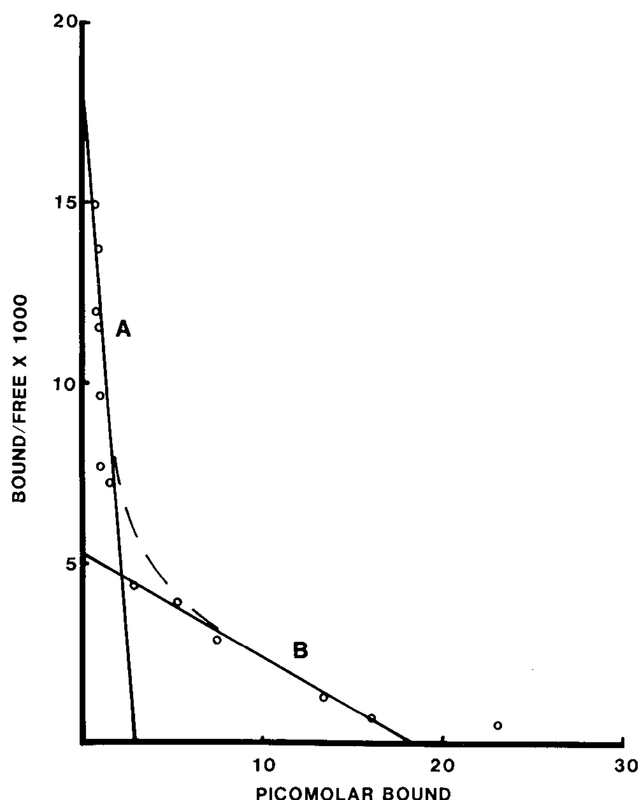

Figure 4. A Scatchard transformation of the inhibition of the binding of ^{125}I-BH-SP to spinal cord cell membranes in the presence of increasing concentrations of SP. A *concave curve* is shown, which suggests decreases in binding with increasing concentration of SP. *A*, Scatchard analysis produced a curve with a K_d of 0.162 nM and a B_{max} of 2.89 fmol/mg of protein. *B*, Scatchard analysis produced a curve with a K_d of 3.69 nM and a B_{max} of 17.4 fmol/mg of protein.

TABLE I

Molecular structures of four tachykinins

The underlines show the amino acid identity. The aromatic amino acids in position 8 for SP and physalaemin as against the hydrophobic residues (isoleucine and valine) for eledoisin and kassinin and the neutral amino acids at position 5 for SP and physalaemin verus the acidic aspartate for eledoisin and kassinin seem to be important in the reactivity of the peptides, since in the binding study the former two were more potent than the latter two.

Amino acid units	1 2 3 4 5 6 7 8 9 10 11
Substance P	Arg- Pro-Lys-Pro-Gln- Gln-Phe-Phe-Gly-Leu-Met-NH2
Physalaemin	pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr- Gly-Leu-Met-NH2
Kassinin	pGlu-Pro-Ser- Lys-Asp-Ala-Phe-Ile- Gly-Leu-Met-NH2
Eledoisin	Asp-Val- Pro-Lys-Ser-Asp-Gln-Phe-Val- Gly-Leu-Met-NH2

Discussion

The results showed that binding of ^{125}I-BH-SP occurred in rat spinal cord cell membranes. It appeared that ^{125}I-BH-SP was the only tracer ligand bound to the membranes because tests showed that, of the radiolabeled substances produced during the conjugation of SP with ^{125}I-Bolton-Hunter reagent, only the peak that coeluted with ^{125}I-BH-SP from gel filtration and cation exchange columns bound to the membranes. The binding of the Bolton-Hunter derivative of SP should represent a component of SP physiochemistry in the spinal cord, because the binding properties of BH-SP and SP are apparently similar. Liang and Cascieri (1980) reported that the K_ds for I-BH-SP and SP for the inhibition of ^{125}I-BH-SP binding to parotid cells were 4 and 5 nM, respectively. They also showed that the inhibition curves were superimposable. Beaujouan et al (1982) found that the IC_{50}s for the inhibiton of the binding of ^{125}I-BH-SP to cultured mesencephalic cells by BH-SP and SP were 0.26 and 0.23 nM, respectively. Thus, the Bolton-Hunter derivative of SP resembles SP binding to cell membranes. However, it was reported that in spinal cord membranes peptidase rapidly inactivated the ^{125}I-BH-SP ligand (Torrens et al., 1983). In this study, we overcame this problem by repeatedly washing the membranes to remove peptidase and by including peptidase inhibitors in the reaction medium.

The Scatchard plot of the inhibition binding for SP indicates a lower binding affinity at higher ligand concentrations. This, in turn, may be due to heterogeneity of binding sites or differences in states of the sites, negative cooperative interactions, or a two-step reaction resulting in the formation of a ternary complex (Molinoff et al., 1981). This heterogeneity in binding raises the possibility that SP binding sites may show regional or segmental differences in the spinal cord. By studying the inhibition of the binding of ^{125}I-BH-SP in the cervical, thoracic, and lumbosacral spinal cord, it was shown that the inhibition of ^{125}I-BH-SP from the cervical spinal cord was much more sensitive to cold SP than the inhibition from the thoracic and lumbosacral sections. The concentration of SP that inhibited 10% of ^{125}I-BH-SP binding in the cervical spinal cord was 50 times less than the concentrations required for the thoracic and lumbosacral segments. Similarly, the IC_{50} for the cervical section was 15.5 times less than the IC_{50} for the thoracic and lumbosacral sections. This, along with the Scatchard transformation of the binding, indicates that SP binding sites of a higher affinity occurred in the cervical section as compared with the thoracic and lumbosacral sections. Densitometric quantification of autoradiograms of the inhibition binding of ^{125}I-BH-SP by SP in spinal cord slices also revealed a higher affinity binding site in the cervical spinal cord (Charlton and Helke, 1985).

The existence of more than one class of binding sites for SP indicated in this study contrasts the single class of noninteracting sites reported to occur in brain and spinal cord synaptosomes (Torrens et al., 1983), rat brain membranes (Hanley et al., 1980), mouse mesencephalic cells (Beaujouan et al., 1982), and brain synaptic membrane (Nakata et al., 1978). In peripheral organs, however, the existence of more than a single class of SP binding sites has been suggested (Erspamer, 1981; Teichberg et al., 1981; Gater et al., 1982; Lee et al., 1982). Piercey et al. (1982) and Stewart et al. (1982) further suggested that two classes of SP receptors occurred in both the CNS and in the peripheral systems. One class of receptors in the CNS recognizes the carboxyl terminal region of SP and regulates pain sensation in the spinal cord and dopamine release in the nigrostriatal system, while the other class of receptors in the CNS mediates behavioral actions and recognizes primarily the amino terminal region of SP (Stewart et al., 1982).

The classification of SP binding sites on the basis of preference toward SP and physalaemin versus eledoisin and kassinin has been suggested by Lee et al. (1982). This suggestion was based primarily on the physiological actions of the peptides. Physalaemin was more potent than eledoisin in depolarizing spinal motor neurons (Otsuka and Konishi, 1977), but eledoisin was more potent than physalaemin and SP in inhibiting angiotensin II-induced drinking after intracerebroventricular injections into the rat (DeCaro et al., 1980). The selective effects of the two pairs of tachykinins (SP and physalaemin versus eledoisin and kassinin), taken together, further suggest that there is probably more than one class of receptors for SP in the CNS. It would appear, therefore, that the higher affinity receptors identified in this study were of the SP-physalaemin type because, although all four tachykinins inhibited the binding of ^{125}I-BH-SP, the rank order of potencies was SP $>$ physalaemin $\gg$ eledoisin $>$ kassinin, and the curves for SP and physalaemin were far to the left of the curves for eledoisin and kassinin. This selectivity may also reflect the closer structural similarity between SP and physalaemin as against eledoisin and kassinin (Lee et al., 1982). SP and physa-

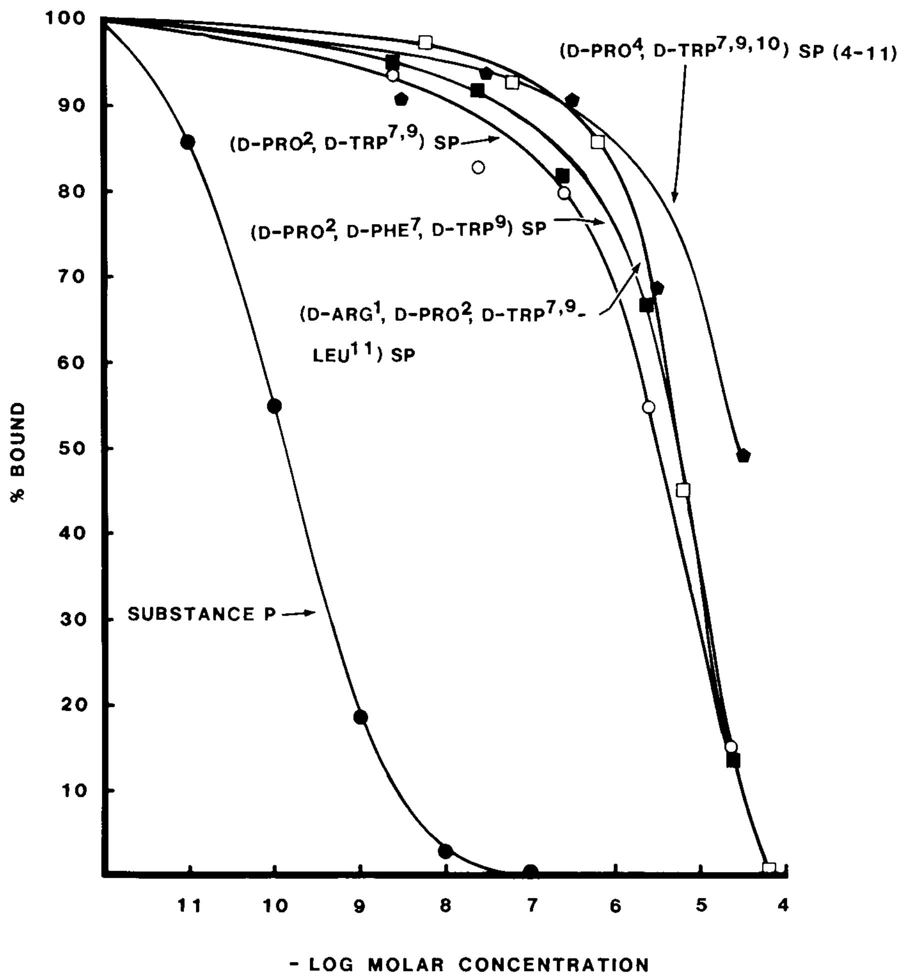

Figure 5. Inhibition of the binding of ^{125}I-BH-SP by putative SP antagonists as compared with SP.

TABLE II

Binding of ^{125}I-BH-SP to membranes from spinal cord sections

Spinal Cord Sections	CPM ^{125}I-BH-SP Bound/mg of Protein		
	Total	Specific	Relative Specific Binding
Cervical	921 ± 68	853	1.0
Thoracic	928 ± 52	805	0.94
Lumbosacral	2862 ± 212	2754	3.22

laemin contain aromatic amino acids (phenylalamine, tyrosine), and eledoisin and kassinin contain hydrophobic residues (isoleucine, valine) in position 8 in Table I. In position 5, neutral amino acids (asparagine, glutamine) occur in SP and physalaemin versus an acidic amino acid (aspartate) for eledoisin and kassinin.

The close similarity between the distribution of the concentrations of SP in the spinal cord (Takahashi and Otsuka, 1975; Hökfelt et al., 1976; Pickel et al., 1977; Ditirro et al., 1981; Phipps et al., 1983; Helke et al., 1984) and the density of the binding sites for the peptide (Charlton and Helke, 1985) suggest that the binding sites may be located proximal to SP-containing nerve terminals and serve as receptors for SP as a neurotransmitter. The binding sites may also serve as receptors for other tachykinins, because immunoreactivities of physalaemin (Lazarus et al., 1980) and kassinin (Hunter and Maggio, 1984) have been identified in the spinal cord, and these two peptides were able to inhibit ^{125}I-BH-SP binding in this study. The order of potency was SP > physalaemin > eledoisin > kassinin. A similar order of potency was observed by Quirion et al. (1983) and Torrens et al. (1983) in rat brain, by Beaujouan et al. (1982) in cultured mouse mesencephalic cells and by Buck et al. (1984) in guinea pig small intestine (but using ^{3}H-SP as ligand). Hanley et al. (1980), however, observed that physalaemin > SP > eledoisin in their ability to inhibit the binding of ^{3}H-SP to rat brain membranes. This order of potency also occurred in the inhibition of ^{3}H-SP binding to salivary gland cell membranes (Lee et al., 1983), but contrary to that, Nakata et al. (1978) showed that SP ≫ eledoisin > kassinin in inhibiting the binding of ^{3}H-SP in rabbit CNS.

On a molar basis, the putative SP antagonists were poor inhibitors of ^{125}I-BH-SP binding to spinal cord cell membranes, yet their IC_{50}s, in which D-Pro^2, D-Trp^{7,9}-SP; D-Pro^2, D-Phe^7, D-Trp^9-SP; and D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP were more potent than D-Pro^4, D-Trp^{7,9,10}-SP(4-11) correlate with the ability of the antagonists to inhibit the cardiovascular responses elicited from the ventral medulla (Keeler and Helke, 1984), the origin of a proposed bulbospinal SP pathway (Helke et al., 1982; Helke, 1982). Only D-Pro^4, D-Trp^{7,9,10}-SP(4-11) inhibited the action of SP in contracting guinea pig isolated trachea; the former three acted instead as agonists (Mizrahi et al., 1984). This phenomenon indicates differences in the spinal cord and trachea binding sites for SP.

Finally, this and other studies (Piercey et al., 1982; Stewart et al., 1982; Lee et al., 1983) suggest that heterogeneous SP binding sites occurred in cellular membranes. Because physalaemin and kassinin interacted with the same binding sites and are located in the CNS also (Lazarus et al., 1980; Hunter and Maggio, 1984), the specific relationship of the receptors to each peptide is unknown. The localization of SP binding sites further indicates a direct physiological role for SP in the spinal cord.

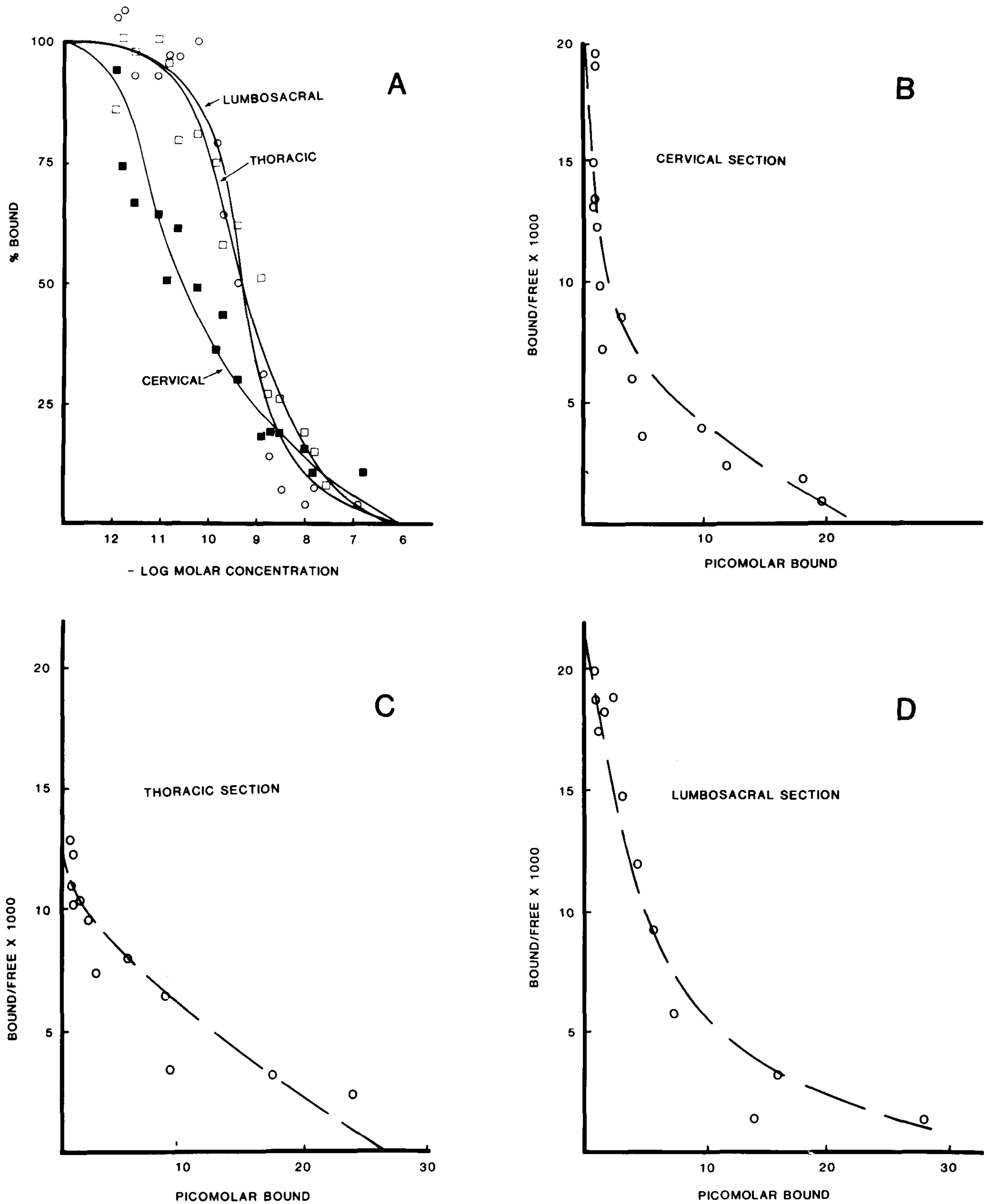

Figure 6. Inhibition binding curves of ^{125}I-BH-SP to membranes from cervical, thoracic, and lumbosacral spinal cord sections (*A*), and the Scatchard plots for the respective sections (*B*, *C*, and *D*). Each *point* represents the mean of triplicate results.

References

Åkerman, B., S. Rosell, and K. Folkers (1982) Intrathecal (D-Pro^2, D-Trp^{7,9})-SP elicits hypoalgesia and motor blockade in the rat and antagonizes noxious responses induced by substance P. Acta Physiol. Scand. *114:* 631–633.

Backman, S. B., and J. L. Henry (1984) Effects of substance P and thyrotropin-releasing hormone on sympathetic preganglionic neurons in the upper thoracic intermediolateral nucleus of the cat. Can. J. Physiol. Pharmacol. *62:* 248–251.

Beaujouan, J. C., Y. Torrens, A. Herbet, M. C. Dagnet, J. Glowinski, and A. Porchiantz (1982) Specific binding of an immunoreactive and biologically active ^{125}I-labeled substance P derivative to mouse mesencephalic cells in primary culture. Mol. Pharmacol. *22:* 48–55.

Bolton, A. E., and W. M. Hunter (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J. *133:* 529–539.

Buck, S. H., Y. Maurin, T. F. Burks, and H. I. Yamamura (1984) High-affinity ^{3}H-substance P binding to longitudinal muscle membranes of the guinea pig small intestine. Life Sci. *34:* 497–507.

Chang, M. M., and S. E. Leeman (1970) The isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J. Biol. Chem. *245:* 4784–4790.

Charlton, C. G., and C. J. Helke (1985) Autoradiographic localization and characterization of spinal cord substance P binding sites: High densities in sensory, autonomic, phrenic and Onuf's motor nuclei. J. Neurosci., in press.

DeCaro, G., M. Massi, and L. G. Micossi (1980) Modifications of drinking behavior and of arterial blood pressure induced by tachykinins in rats and pigeons. Psychopharmacology *68:* 243–247.

de Lanerolle, N. C., and C. C. LaMotte (1982) The human spinal cord: Substance P and methionine-enkephalin immunoreactivity. J. Neurosci. *2:* 1369–1386.

Ditirro, F. J., R. H. Ho, and G. F. Martin (1981) Immunohistochemical localization of substance P, somatostatin and methionine-enkephalin in the spinal cord and dorsal root ganglia of the North American opossum, *Didelphis virginiana*. J. Comp. Neurol. *198:* 351–363.

Erspamer, V. (1981) The tachykinin peptide family. Trends Neurosci. *4:* 267–269.

Fasmer, O. B., and C. Post (1983) Behavioural responses induced by intrathecal injection of 5-hydroxytryptamine in mice are inhibited by a substance P antagonist, D-Pro^2, D-Trp^{7,9}-substance P. Neuropharmacology *22:* 1397–1400.

Gater, P. R., C. C. Jordon, and D. G. Owen (1982) Relative activities of substance P-related peptides in the guinea-pig ileum and rat parotid gland, *in vitro*. Br. J. Pharmacol. *75:* 341–351.

Hanley, M. R., B. E. B. Sandberg, C. M. Lee, L. L. Iversen, D. E. Brundish, and R. Wade (1980) Specific binding of ^{3}H-substance P to rat brain membranes. Nature *286:* 810–812.

Helke, C. J. (1982) Neuroanatomical localization of substance P: Implications for central cardiovascular control. Peptides *3:* 479–483.

Helke, C. J., J. J. Neil, J. V. Massari, and A. D. Loewy (1982) Substance P neurons project from the ventral medulla to the intermediolateral cell column and ventral horn in the rat. Brain Res. *243:* 147–152.

Helke, C. J., C. W. Shultz, T. N. Chase, and T. L. O'Donohue (1984) Autoradiographic localization of substance P receptors in rat medulla: Effect of vagotomy and nodose ganglionectomy. Neuroscience *12:* 215–223.

Henry, J. L. (1976) Effects of substance P on functionally identified units in cat spinal cord. Brain Res. *114:* 439–451.

Hökfelt, T., J. Kellerth, G. Nilsson, and B. Pernow (1975) Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. Brain Res. *100:* 235–252.

Hökfelt, T., R. Elde, O. Johansson, R. Luft, G. Nilsson, and A. Arimura (1976) Immunohistochemical evidence for separate populations of somatostatin containing and substance P containing primary afferent neurons in rat. Neurosci. Lett. *1:* 131–136.

Hökfelt, T., A. Ljungdahl, T. Terenius, R. Elde, and G. Nilsson (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia. Enkephalin and substance P. Proc. Natl. Acad. Sci. U. S. A. *74:* 3081–3085.

Hunter, J. C., and J. E. Maggio (1984) Pharmacological characterization of a novel tachykinin isolated from mammalian spinal cord. Eur. J. Pharmacol. *97:* 159–160.

Keeler, J. R., and C. J. Helke (1984) Pharmacologic evidence that substance P is involved in bulbospinal cardiovascular control in the rat. Soc. Neurosci. Abstr. *10:* 712.

Klotz, I. M., and D. L. Hunston (1971) Properties of graphical representations of multiple classes of binding sites. Biochemistry *10:* 3065–3069.

Lazarus, L. H., R. I. Linnoila, O. Hernandez, and R. P. DiAugustine (1980) A neuropeptide in mammalian tissues with physalaemin-like immunoreactivity. Nature *287:* 555–558.

Lee, C. M., L. L. Iversen, M. R. Hanley, and B. E. G. Sandberg (1982) The possible existence of multiple receptors for substance P. Naunyn Schmiedebergs Arch. Pharmacol. *318:* 281–287.

Lee, C. M., J. A. Javitch, and S. H. Snyder (1983) ^{3}H-Substance P binding to salivary gland membranes. Regulation by guanyl nucleotides and divalent cations. Mol. Pharmacol. *23:* 563–569.

Liang, T., and M. A. Cascieri (1980) Specific binding of an immunoreactive and biologically active ^{125}I-labelled N(1) acylated substance P derivative to parotid cells. Biochem. Biophys. Res. Commun. *96:* 1793–1799.

Loewy, A. D., and W. B. Sawyer (1982) Substance P antagonist inhibits vasomotor responses elicited from ventral medulla in rat. Brain Res. *245:* 379–383.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Maurin, Y., S. H. Buck, J. K. Wamsley, T. F. Burks, and H. I. Yamamura (1984) Light microscopic autoradiographic localization of [^{3}H]substance P binding sites in rat thoracic spinal cord. Life Sci. *34:* 1713–1716.

Mizrahi, J., E. Escher, P. D'Orléans-Juste, and Regoli, D.(1984) Undeca- and octa-peptide antagonists for substance P. A study on the guinea pig trachea. Eur. J. Pharmacol. *99:* 193–202.

Molinoff, P. B., B. B. Wolfe, and G. A. Weiland (1981) Quantitative analysis of drug-receptor interactions. II. Determination of the properties of receptor subtypes. Life. Sci. *29:* 427–443.

Nakata, Y., Y. Kusaka, T. Segawa, H. Yajima, and K. Kitagawa (1978) Substance P: Regional distribution and specific binding to synaptic membranes in rabbit central nervous system. Life. Sci. *22:* 259–268.

Otsuka, M., and S. Konishi (1977) Electrophysiological and neurochemical evidence for substance P as a transmitter of primary sensory neurons. In *Substance P*, U. S. von Euler and B. Pernov, eds., pp.207–214, Raven Press, New York.

Perrone, M. H., R. E. Diehl, and D. R. Haubrich (1983) Binding of [^{3}H] substance P to putative substance P receptors in rat brain membranes. Eur. J. Pharmacol. *95:* 131–133.

Phipps, B. S., R. Maciewicz, B. B. Sandrew, C. E. Poletti, and W. E. Foote (1983) Ehdinger-Westphal neurons that project to spinal cord contain substance P. Neurosci. Lett. *36:* 125–131.

Pickel, V. M., D. J. Reis, and S. E. Leeman (1977) Ultrastructural localization of substance P in neurons of rat spinal cord. Brain Res. *122:* 534–540.

Piercey, M. F., P. J. K. Dobry, L. A. Schroeder, and F. J. Einspahr (1981) Behavioral evidence that substance P may be a spinal cord sensory neurotransmitter. Brain Res. *210:* 407–412.

Piercey, M. F., P. J. K. Dobry, F. J. Einspahr, L. A. Schroeder, and N. Masiques (1982) Use of substance P fragments to differentiate substance P receptors of different tissues. Regul. Pept. *3:* 337–349.

Quirion, R., C. W. Shults, T. W. Moody, C. B. Pert, T. N. Chase, and T. L. O'Donohue (1983) Autoradiographic distribution of substance P receptors in rat central nervous system. Nature *303:* 714–716.

Scatchard, G. (1949) The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. *51:* 660–672.

Shults, C. W., R. Quirion, R. T. Jensen, T. W. Moody, T. L. O'Donohue, and T. N. Chase (1982) Autoradiographic localization of substance P receptors using ^{125}I-substance P. Peptides *3:* 1073–1075.

Stewart, J. M., M. E. Hall, J. Harkins, R. C. A. Frederickson, L. Terenius, T. Hökfelt, and W. A. Krivoy (1982) A fragment of substance P with specific central activity: SP-(1-7). Peptides *3:* 831–857.

Takahashi, T., and M. Otsuka (1975) Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. Brain Res. *87:* 1–11.

Teichberg, V. I., S. Cohen, and S. Blumberg (1981) Distinct classes of substance P receptors revealed by a comparison of the activities of substance P and some of its segments. Regul. Pept. *1:* 327–333.

Torrens, Y., J. C. Beaujouan, A. Viger, and J. Glowinski (1983) Properties of a ^{125}I-substance P derivative binding to synaptosomes from various brain structures and the spinal cord of the rat. Naunyn Schmiedebergs Arch. Pharmacol. *324:* 134–139.

von Euler, U. S., and J. H. Gaddum (1931) An unidentified depressor substance in certain tissue extracts. J. Physiol. (Lond.) *72:* 74–87.

Yanagisawa, M., M. Otsuka, S. Konishi, H. Akagai, K. Folkers and S. Rossel (1982) A substance P antagonist inhibits a slow reflex response in the spinal cord of the newborn rat. Acta Physiol. Scand. *116:* 109–112.