at 85 °C under a reflux condenser. After 15 h, the reaction mixture was cooled, diluted with water, and extracted with chloroform. The chloroform extracts were washed with 10% sodium bicarbonate, treated with Norit to remove tar, and then dried over magnesium sulfate. After concentration in vacuo, the crude product was flash column chromatographed with 30% pentane in methylene chloride on silica gel. The major component (single spot by TLC) was a mixture of 7 and 8; we were unable to find a satisfactory method (chromatographic or gas phase) to resolve the two components, so the compounds were both partially characterized and employed in the next step as a mixture: 4.5 g; 7:8 = 1:0.875.

7: NMR ($CDCl_3$) δ 7.55 (1 H, d, J = 7 Hz, CH), 6.66 (1 H, d, J = 7 Hz, CH), 4.4 (2 H, s, CH_2); HRMS ($C_7H_4ClF_3O_2$).

8: NMR ($CDCl_3$) δ 7.6 (1 H, s, CH), 4.45 (4 H, s, CH_2); HRMS ($C_8H_5Cl_2F_3O_2$).

3-Benzyl-6-(trifluoromethyl)-2-pyranone (4). The phenyl Grignard formed from phenyl bromide (2.40 g, 15.3 mmol) and magnesium (0.372 g, 15.3 mmol) in THF (50 mL) was cooled to 0 °C under nitrogen. CuBr-SMe_2 (3.14 g, 15.3 mmol) was sifted in and the mixture stirred for 15 min. Then, the mixture of 7 and 8 (4.50 g of a 0.875:1 mixture of 8 to 7; 10.2 mmol of 7) in 10 mL of THF was added as rapidly as possible. The mixture was then slowly warmed to 50 °C for 4 h. The reaction was then quenched with saturated sodium chloride and extracted with ether. The ether extracts were washed with acidic saturated sodium chloride and dried over magnesium sulfate, and the solvent was removed in vacuo. The crude material was chromatographed first with 40% pentane in methylene chloride on silica gel to remove biphenyl and 8; the fractions containing 4 were combined and rechromatographed with 5% ethyl acetate in hexane, giving pure 4 (0.8492 g, 32.8%) as an oil: NMR ($CDCl_3$) δ 7.1-7.3 (5 H, m, Ph), 6.95 (1 H, d, J = 6 Hz, CH), 6.47 (1 H, d, J = 6 Hz, CH), 3.70 (2 H, s, CH_2). Anal. ($C_{13}H_9F_3O_2$) C, H.

Biochemistry. The inactivation assays were performed on a Varian 635 UV-vis double-beam spectrometer or a Hewlett-Packard 8451A diode array single-beam spectrometer. α-Chymotrypsin (three times crystallized and free of autolysis products and low molecular weight contaminants) was obtained from Worthington Biochemical. *N*-Benzoyltyrosine ethyl ester (BTEE) was obtained from Sigma. *N*-Acetyltyrosine *p*-nitroanilide was prepared according to Bundy.[19] *N*-Acetyltyrosine *p*-nitrophenyl ester[20] was obtained from Sigma. The phosphate buffer used in the inactivation studies and competitive assays was 0.1 M KH_2PO_4/K_2HPO4, pH 7.5.

Time-Dependent Loss of Activity Assay. A 5 mM stock solution of the inhibitor was prepared in Me_2SO, and depending upon solubility limits, 25-50 μL of this solution was added to 1.9 μM chymotrypsin (0.950-0.975 mL) in buffer at 25 °C. A 50-μL aliquot was removed from this mixture starting from t = 0, and at regular intervals afterward to determine the activity. The activity was determined by adding the aliquot to 0.95 mL of a 50 mM solution of BTEE in 15% acetonitrile/buffer. For **18a**, the cosolvent of BTEE was changed to Me_2SO. The rate of hydrolysis was followed at 256 nm and the percent activity at time t found by dividing the slope of the hydrolysis curve by that of t = 0. The assay was repeated with increasing concentrations of inhibitor until no further increase in rate of inactivation was observed. The exception was **18a**, whose rapid inactivation and reactivation of enzyme required the simultaneous substrate-inhibitor assay of Main,[21] as described by Katzenellenbogen.[11]

Competition Assays. Except for **18a**, time-dependent inhibition was sufficiently slow to allow standard competitive inhibition assays to be performed. The competitive K_i was determined for each compound, using either *N*-acetyltyrosine *p*-nitrophenyl ester or *N*-acetyltyrosine *p*-nitroanilide in 15% Me_2SO/buffer, depending upon the UV absorbances of the inhibitor. The K_m of the substrate was determined each time a competitive inhibitor was tested, and the assays were run in triplicate.

Hydrolysis Assays. Where feasible, the hydrolysis constants were determined by the loss of the pyrone chromophore (290-315 nm).[1]

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Supplementary Material Available: Full analytical compound characterizations (5 pages). Ordering information is given on any current masthead page.

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Analogues of Substance P. Peptides Containing D-Amino Acid Residues in Various Positions of Substance P and Displaying Agonist or Receptor Selective Antagonist Effects

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Agonist and antagonist analogues of substance P were synthesized by replacing at least two of the amino acid residues with D-Trp, D-Phe, D-Val, or D-Pro residues. The syntheses of these compounds were achieved by solid-phase methodology using the hydroxymethyl resin. The analogues were tested for agonist and antagonist activity on guinea pig ileum and rat spinal cord preparations. Two types of antagonists were obtained. The first type of compounds, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (1), antagonized SP and SP(6-11)-hexapeptide on the ileum but only SP(6-11)-hexapeptide on the spinal cord. The second type of antagonists, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (17), were inactive on the ileum but were potent antagonists of the hexapeptide on the spinal cord. Two of the antagonists, [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (3) and [D-Trp^{7,8,9}]-SP (43), were also tested in vivo. Both of these depressed hypotensive responses to SP and SP(6-11)-hexapeptide in rabbits.

Substance P (SP) has been implicated in the transmission of primary afferent nociceptive signals to the dorsal horn of the spinal cord.[1] It has therefore been hypothesized that antagonism of its actions on dorsal horn

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neurons may lead to analgesia. This hypothesis has been supported not only by the existence of SP in the spinal cord and afferent C-fibers[1] but also by the facts that substance P like immunoreactive material is released by noxious stimuli at central and peripheral ends of the primary afferents[2,3] and that the release can be inhibited by opiates.[2-4] Active uptake of SP(5–11)-heptapeptide has also been demonstrated in rat brain and rabbit spinal cord slices.[5]

The main aim of the present work was to obtain specific antagonists of SP that could be used in exploring the pharmacology of SP and may ultimately lead to novel analgesics. Although at the start of this work no antagonists of SP were known, a number of such compounds have since been reported. Folkers et al. have published[6-8] antagonists based on the entire undecapeptide sequence of SP and these have been obtained by substituting D-amino acid residues in various positions of SP. Regoli et al. have reported antagonists based on the C-terminal (4–11)-octapeptide sequence.[9-11] Antagonist activity in a modified C-terminal hexapeptide sequence was reported by Baizman et al.[12]

During the last few years various tachykinin receptor types have also been identified. These have been classified by studying the contractile responses of either the naturally occurring tachykinins[13,14] (physalaemin, eledoisin, kassinin, etc.) or SP and its fragments[15-17] on various in vitro tissue preparations in the presence or absence of antagonists.[18,19] A few in vivo test systems, e.g., inducing salivation in rats or scratching in mice, have also been used.[17] This type of work led Iversen et al. to suggest the existence of two distinct types (SP-P and SP-E) of receptors,[13] while Buck et al. have suggested three types (SP-P, SP-E, SP-K) of tachykinin receptors.[20] This information on different types of tachykinin receptors has resulted in a need for SP receptor antagonists specific for various receptor subtypes.

In our search for SP antagonists, the available structure–activity information on all the tachykinin analogues was taken into consideration, and this led us to divide the SP molecule into two parts—an N-terminal (1–6) region and a C-terminal (7–11) region. Major structural changes in the N-terminal region do not result in serious losses of biological activity.[21] This may be predicted from the large number of differences existing in the N-terminal regions of various tachykinins (SP, physalaemin, phyllomedusin, eledoisin, uperolein, kassinin). Despite the major structural differences, these still have similar agonist properties, although the relative potencies vary a great deal in various tissue preparations.[22-24] Some of the fragments of SP with residues from the N-terminal region missing have been shown to be more potent than the undecapeptide.[25-27] The C-terminal (7–11) region appears to be more sensitive to changes. Replacement of these C-terminal residues individually by alanine residues shows position 7 to be most important for biological activity and position 9 to be the least important.[21]

Since single amino acid substitutions (including D-amino acid residues) in the C-terminal region and multiple changes in the N-terminal region lead to analogues with considerable agonist activity,[28-30] it was decided to carry out at least two D-amino acid residue substitutions in the SP molecule at any time. These amino acid substitutions were limited to either the 1–6 region or the 7–11 region. D-Trp, D-Phe, D-Val, and D-Pro residues were substituted in various positions, and the analogues synthesized are listed in Table I. The biological activities of these analogues on guinea pig ileum and rat spinal cord preparations are listed in Tables II and III.

Synthesis. All the analogues described in Table I were prepared by the solid-phase method[31] with Beckman Model 990 automated peptide synthesizer.

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The recently reported analogues of SP have been prepared by the solid-phase method using a benzyhydrylamine resin support.[7] This strategy has the advantage that, when the desired peptide has been assembled on the resin, all of the protecting groups can be cleaved by a single liquid HF treatment. The same treatment also cleaves the peptides from the resin and provides directly the peptide amides. This method was not applicable in our case because we wanted to prepare peptides containing a C-terminal methyl ester group and were also interested in retaining the benzyloxycarbonyl groups in the final compounds and both of these were not possible by using the above procedure. It was therefore decided to use the hydroxymethyl resin as the solid support. Due to the low reactivity of the hydroxyl group, the first amino acid was coupled to the resin by DCC in presence of 4-(dimethylamino)pyridine.[32] Further amino acids were then coupled by using programs described in the Experimental Section. The *tert*-butyloxycarbonyl group was used for the protection of α-amino groups. The N^{α}-amino group of the N-terminal Arg residue and the N^{ϵ}-amino group of the Lys residue were protected by benzyloxycarbonyl groups. The guanidino function of the arginine residue was protected by protonation (HCl salt). Since all of the analogues contain methionine and tryptophan residues that can easily be alkylated during acid deblocking steps, 5% 1,2-ethanedithiol was added to the 40% TFA–CH_2Cl_2 mixture used for cleaving the N^{α}-*tert*-butyloxycarbonyl protecting groups. This reagent (TFA–ethanedithiol) could also cleave the N^{ϵ}-benzyloxycarbonyl groups of Lys, but the rate of cleavage is much slower in comparison to the rate of cleavage of the *tert*-butyloxycarbonyl group.

The protected bis(benzyloxycarbonyl) peptides were removed from the resin either as the methyl ester by transesterification reactions or in a few examples as the amides by direct ammonolysis of the resin. The protected peptide amides were usually obtained from the purified methyl esters by ammonolysis. Removal of the Z protecting groups from the peptide esters or amides was performed with trifluoroacetic acid–thioanisole.[33] Extensive use was made of the counter-current distribution technique for the purification of protected peptide esters and amides.[34] Suitable solvent systems were designed for each analogue, and in this way compounds were obtained that showed a single spot in several TLC systems. It was sometimes necessary to introduce a second purification step, another CCD system, or gel filtration on LH-20 Sephadex in organic solvents. Fully deprotected peptides were purified by ion-exchange chromatography on Whatman CM-52 resin with use of a gradient elution system based on acetic acid or by gel filtration on Bio-Gel P4 resin in dilute acetic acid or hydrochloric acid. All peptides were characterized by TLC and amino acid analysis after acid or base hydrolysis, and the ratios were consistent with the desired structures.

Biological Activity. The analogues reported in Table I were initially tested for agonist activity in a guinea pig ileum preparation. The compounds that showed significant agonist activity (>0.005% of SP) are listed in Table II, and these were not tested for antagonist activity. Agonist activity in Table II is expressed as a percentage of the SP activity by comparing EC_{50} (effective concentration producing 50% of the maximum response) values. The remaining analogues were tested for antagonist activity with use of SP, SP(6–11)-hexapeptide, and acetylcholine as the agonists, and the results are summarized in Table III. Since the hexapeptide has earlier been shown to act mainly at the E-type of receptors,[35] the use of the two peptide agonists was expected to separate the antagonists into various receptor selective types. The results in Table III are expressed as dose ratios; the figures were calculated from the ratio for the EC_{50} values of the agonist in the presence of the antagonist to the EC_{50} value of the agonist alone. Responses to acetylcholine were not affected by any of the antagonists listed in Table I. At least two ileum preparations were used for each compound, and dose ratios of less than 2 were considered insignificant. The analogues were also tested for antagonist activity on a neonatal rat hemisected spinal cord preparation[36] with SP, SP(6–11)-hexapeptide, carbachol, and glutamate as agonists. All of these agonists caused depolarizations of the ventral roots, that were measured as amplitude of response and area of response. The effects of the antagonists were expressed as percentage change in response amplitude and area, but since both of the changes were similar, only results for amplitude changes are given in Table III. At least two tissue preparations were used for each compound, and less than 10% change in response amplitude was not considered significant.

Results and Discussion

(a) In Vitro Results. The compounds listed in Tables I and III have been arranged in such a manner that position 11 modified compounds appear first and these are followed by analogues with one of the modifications in positions 10, 9, and so on. D-Trp[7,8,9] analogues are listed at the end of the table together with [D-Pro[2],D-Trp[7,9]]-SP.

In the first set of compounds (1–12) the C-terminal methionine residue has been replaced by a D-methionine residue and the phenylalanine residues in positions 7 and 8 have been replaced with D-Trp, D-Phe, D-Pro, or D-Val residues. The α-amino group of Arg[1] and the ϵ-amino group of Lys[3] were either free or as benzyloxycarbonyl derivatives. An amide or a methyl ester group was present at the C-terminus of these position 7, 8, and 11 modified compounds. [N^{α}-Z-Arg[1],N^{ϵ}-Z-Lys[3],D-Trp[7,8],D-Met[11]]-SP-OMe (1) was the most potent antagonist in this series of compounds. Although it antagonized SP and the hexapeptide on the guinea pig ileum, it was much more potent against the hexapeptide (dose ratio 90 at 10 μM). On the rat spinal cord preparation, only the hexapeptide was antagonized up to a concentration of 25 μM; at 50 μM antagonist effects against carbachol were also seen. The response to SP was either potentiated or unchanged. The corresponding amide (3) was only tested on guinea pig ileum and was found to be much less potent. When the benzyloxycarbonyl groups were removed (2, 4), all the activity was lost. The [D-Phe[7,8],D-Met[11]] analogues (5–9) were inactive on the guinea pig ileum, but on the rat spinal cord preparation [N^{α}-Z-Arg[1],N^{ϵ}-Z-Lys[3],D-Phe[7,8],D-Met[11]]-SP-OMe (5) and [N^{α}-Z-Arg[1]-N^{ϵ}-Z-Lys[3],D-Phe[7,8],D-Met[11]]-SP (8) showed significant antagonist activity (78% and 75% reduction in response amplitude at 33.5 and 36 μM, respectively) against SP(6–11)-hexapeptide; SP and glutamate responses were only slightly affected. As in the [D-Trp[7,8],D-Met[11]] series of compounds, when the benzy-

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Table I. Physical and Chemical Characteristics of Substance P Analogues

compound	TLC[c] purification[a,b]	R_f(A)	R_f(B)	R_f(C)	R_f(K)	amino acid analysis[d] (16-h acid digest) Arg	Pro	Lys	Glu	Phe	Gly	Leu	Met	Trp	Val
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (1)	CCD (A) (K 1.09)	0.60	0.65	0.18	0.60	1.05	2.1	1.04	2.05		0.98	0.99	0.90	2.09	
[D-Trp^{7,8},D-Met^{11}]-SP-OMe (2)	Bio-Gel P-4, 0.01 M HCl		0.26			1.02	1.94	1.03	1.99		0.96	1.04	0.94	1.92	
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (3)			0.36			1.03	1.96	1.04	1.95		0.96	1.01	0.94	1.95	
[D-Trp^{7,8},D-Met^{11}]-SP (4)	Bio-Gel P-4, 0.01 M HCl	0.06	0.30	0.0	0.0	0.97	2.02	1.01	2.01		0.97	1.01	0.98	1.85	
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe (5)	CCD (A) (K 1.0)	0.63	0.75	0.18	0.60	1.01	2.03	1.04	2.04	2.02	1.03	1.04	0.95		
[D-Phe^{7,8},D-Met^{11}]-SP-OMe (6)	Bio-Gel P-4, 0.01 M HCl		0.29			1.03	2.1	1.03	2.04	2.0	0.99	0.97	0.96		
[N^{α}-Boc,D-Phe^{7,8},D-Met^{11}]-SP(4–11)-OMe (7)	silica column chromat[e]	0.70	0.83	0.62	0.74		1.02		2.1	2.1	0.95	0.98	0.95		
N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP (8)		0.65	0.72	0.06	0.62	1.0	1.99	1.01	2.01	1.90	0.99	1.01	0.94		
[D-Phe^{7,8},D-Met^{11}]-SP (9)			0.36			1.04	2.01	1.02	2.04	1.98	0.97	1.03	0.92		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{7,8},D-Met^{11}]-SP-OMe (10)	CCD (B) (K 1.0)	0.63	0.75	0.18	0.60	0.98	3.96	1.0	2.06		1.01	1.0	0.95		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Val^{7,8},D-Met^{11}]-SP-OMe (11)	CCD (B) (K 1.2)	0.60	0.65	0.18	0.56	1.01	1.95	1.0	1.96		1.0	1.0	0.95		1.92
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Val^{7,8},D-Met^{11}]-SP (12)		0.35	0.70	0.16	0.81	1.0	1.94	1.03	2.10		1.02	1.03	0.97		1.89
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{9,10}]-SP-OMe (13)	CCD (C) (K 1.37)	0.39	0.67	0.0	0.86	1.04	1.93	1.03	1.92	4.09			0.94		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{9,10}]-SP (14)		0.0	0.0	0.0	0.79	1.01	2.02	1.04	1.95	3.99			0.95		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{9,10}-SP-OMe (15)	CCD (C) (K 1.18)				0.88	0.99	2.01	1.01	1.97	2.03			0.96	1.92	
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Val^{9,10}]-SP-OMe (16)	CCD (D) (K 2.67)	0.41	0.65	0.20	0.80	0.98	2.03	1.01	1.95	2.06			0.99	1.94	
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (17)	CCD (C) (K 0.34)	0.35	0.64	0.16	0.82	0.99	4.16	1.01	1.98	2.02			0.95		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{2,9,10}]-SP-OMe (18)	CCD (C) (K 0.2)	0.34	0.58	0.18	0.74	0.99	4.08	1.02	1.98	2.0			0.96		
N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{5,6}]-SP-OMe (19)	CCD (A) (K 1.78)	0.36	0.65	0.14	0.93	0.97	3.89	0.98		2.06	1.03	1.02	0.95		
[D-Pro^{5,6}]-SP-OMe (20)	Bio-Gel P-4, 0.01 M HCl	0.17	0.55	0.0	0.55	0.99	3.96	1.01		1.99	1.0	1.02	0.95		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{5,6}]-SP (21)		0.68	0.72	0.14	0.64	0.96	3.92	0.99		1.97	1.0	1.02	0.96		
[D-Pro^{5,6}]-SP (22)	Bio-Gel P-4, 0.01 M HCl	0.12	0.40	0.08	0.06	1.04	3.91	1.04		1.96	0.99	1.04	0.96		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Val^{5,6}]-SP-OMe (23)	CCD (A) (K 0.7)	0.62	0.69	0.25	0.80	1.0	1.91	1.0		2.07	0.99	1.01	0.95		1.94
[D-Val^{5,6}]-SP-OMe (24)	Bio-Gel P-4, 0.01 M HCl	0.20	0.51	0.0	0.0	0.99	1.94	1.0		1.99	1.02	1.0	0.95		1.96
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Val^{5,6}]-SP (25)		0.47	0.63			1.03	1.95	1.02		2.05	1.0	1.03	0.97		1.94
[D-Val^{5,6}]-SP (26)	Bio-Gel P-4, 0.1 M acetic acid		0.43			0.99	1.94	1.03		1.96	0.99	1.04	0.97		1.92
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{5,6}]-SP-OMe (27)	DMF–H_2O precip	0.61	0.67	0.5	0.95	1.02	1.96	1.03		4.0	0.98	1.01	0.97		
[D-Phe^{5,6}]-SP-OMe (28)	Bio-Gel P-4, 0.1 M acetic acid	0.16	0.53	0.0	0.60	1.05	2.1	1.04		3.92	0.96	1.04	1.96		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{2,4}]-SP-OMe (29)	CCD (A) (K 1.02)	0.68	0.72	0.19	0.55	1.04	1.96	1.04	1.97	2.0	0.98	1.02	0.98		
[D-Pro^{2,4}]-SP-OMe (30)	Bio-Gel P-4, 0.01 M HCl	0.07	0.35	0.0	0.0	1.0	1.94	1.04	2.05	2.03	0.96	1.0	0.96		
N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{2,4}]-SP (31)	LH-20, DMF	0.41	0.66	0.14	0.78	1.04	1.95	1.02	2.07	1.96	0.97	1.02	0.96		
[D-Pro^{2,4}]-SP (32)	Bio-Gel P-4, 0.01 M HCl		0.33			1.04	1.96	1.03	1.96	2.01	0.97	1.02	0.97		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3]-SP-OMe (33)	CCD (A) (K 1.35)	0.39	0.68		0.48	1.03	1.97	1.03	1.96	2.07	0.98	1.02	0.96		

SP-OMe (34)	Bio-Gel P-4, 0.1 M CH_3COOH	0.14	0.35	0.0	0.0	1.03	1.96	1.02	2.0	2.05	1.01	0.99	0.97		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3]-SP-NHEt (35)		0.67	0.77	0.17	0.63	f	2.0	1.04	2.1	1.96	1.0	1.0	0.98		
SP-NHEt (36)	Bio-Gel P-4, 0.01 M HCl	0.12	0.38	0.05	0.05	f	2.04	1.03	2.04	1.96	1.03	1.01	0.95		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3]-SP-NHBu^n (37)		0.71	0.77	0.18	0.60	1.04	2.02	1.03	2.04	1.96	1.04	0.98	0.96		
SP-NHBu^n (38)	Bio-Gel P-4, 0.01 M HCl	0.13	0.36	0.0	0.0	1.04	2.02	1.04	2.0	2.0	1.01	1.0	0.96		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9}]-SP-OMe (39)	CCD (C) (K 0.88)	0.46	0.68	0.28	0.69	1.02	2.03	1.02	2.0			1.01	0.96	2.87	
[N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9}]-SP-OMe (40)	CM52 (0.01–2.0 M AcOH); Bio-Gel P-4 (10% AcOH)	0.23	0.61	0.10	0.10	1.0	2.01	1.02	2.0			0.98	0.98	2.90	
[D-Trp^{7,8,9}]-SP-OMe (41)	CM52 (0.01–2.0 M acetic acid)	0.20	0.45			1.03	2.0	1.01	2.0			0.96	0.95	2.86	
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9}]-SP (42)		0.37	0.66	0.10	0.78	1.01	1.96	1.03	1.96			1.01	0.96	2.85	
[D-Trp^{7,8,9}]-SP (43)	Bio-Gel P-4, 0.1 M acetic acid	0.17	0.38	0.0	0.0	1.01	1.98	1.02	1.97			1.0	0.97	2.87	
[D-Pro^2,D-Trp^{7,9}]-SP^h (44)	Bio-Gel P-4 (0.01 M HCl)	0.13	0.33	0.0	0.0	1.03	2.0	1.0	2.04	0.99		1.03	0.96	1.94	

[a] Details of the counter-current systems are described in the Experimental Section. The partition coefficient values (K) were obtained from the actual counter-current run. [b] The bis(benzyloxycarbonyl)-substituted amides listed in the table were prepared from the parent esters, and no further purification was necessary at this stage (see general procedure). [c] See Experimental Section for the TLC systems. [d] Trp figures were obtained by base hydrolysis. [e] Methanol (2%, 5%, 10%) in chloroform was used as eluting solvent. [f] Arginine and ethylamine peaks overlapped. [g] The compound was obtained from the N^{α}-Boc-Arg^1 derivative, which was purified by CCD (C) and the Boc group was then cleaved by TFA/1,2-ethanedithiol treatment. [h] N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3 derivative was prepared by solid phase and purified by counter current method using system A.

Table II. Agonist Activity of Substance P Analogues on Guinea Pig Ileum

compd	agonist activity (% SP) (guinea pig ileum)	compd	agonist activity (% SP) (guinea pig ileum)
20	0.066	**32**	15
21	1.6	**33**	0.075
22	1.5	**34**	16[a]
24	0.005	**35**	10.5
26	6.4	**36**	16
29	7.0	**37**	1.1
30	13.6	**38**	32.5
31	63		

[a] On rat spinal cord preparation the compound was as active as substance P.

loxycarbonyl groups in compound **5** were removed, the resulting compound, [D-Phe^{7,8},D-Met^{11}]-SP-OMe (**6**), was less active than **5** (only 75% reduction in response amplitude against the hexapeptide at 100 μM). The octapeptide analogue [N^{α}-Boc,D-Phe^{7,8},D-Met^{11}]-SP(4–11)-OMe (**7**) antagonized SP, the hexapeptide, carbachol, and glutamate. Compounds with D-Pro or D-Val residues in positions 7 and 8 (**10–12**) were inactive as antagonists on the guinea pig ileum.

In the second set of compounds (**13–18**) the Gly^9 and Leu^{10} residues were both replaced with D-Phe, D-Trp, D-Val, or D-Pro residues. Except for compound **14** all the others had a methyl ester at the C-terminus. All of these analogues were inactive as antagonists on the guinea pig ileum. [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (**17**) showed antagonist activity against SP(6–11)-hexapeptide (67% and 100% reduction in response amplitude at 5 and 50 μM, respectively) on the rat spinal cord preparation; the response to carbachol was not affected at concentrations up to 50 μM. [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{2,9,10}]-SP-OMe (**18**) was somewhat less potent (86% reduction in response amplitude at 50 μM concentration) and also showed some effect against carbachol and glutamate. The SP response was potentiated by these two compounds.

Compounds modified in positions 5 and 6 by replacing the two glutamine residues with D-Pro, D-Val, or D-Phe residues (**19–28**) were all inactive as antagonists of SP and SP(6–11)-hexapeptide, but a number of these showed agonist activity (Table II). In general, compounds with benzyloxycarbonyl groups were less potent as agonists compared to the corresponding compounds containing unprotected amino groups. Similarly the C-terminal methyl ester analogues were less potent than the corresponding amides. Only [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{5,6}]-SP (**21**) and [D-Pro^{5,6}]-SP (**22**) were equipotent as agonists. [D-Val^{5,6}]-SP (**26**) was the most potent agonist of this series (6.4% of SP).

In compounds **29–32** the two proline residues in positions 2 and 4 were replaced with D-proline residues. All four of these analogues showed significant agonist activity (7–63% of SP) on guinea pig ileum (Table II), [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{2,4}]-SP (**31**) being the most potent.

All of the C-terminal methyl ester and substituted amide analogues of SP (**33–38**) showed various levels of agonist activity (0.075–32.5% SP). As above, the bis(benzyloxycarbonyl) derivatives (**33**, **35**, **37**) were less active as agonists than the corresponding unprotected compounds (**34**, **36**, **38**). SP-NHBu^n (**38**) was the most potent (32.5% SP) agonist on the guinea pig ileum from this set of compounds. SP methyl ester (**34**) had only 16% of the potency of SP on the guinea pig ileum, but on the rat spinal cord preparation the two compounds were equipotent. Regoli et al. have also reported the SP methyl ester and some

Table III. Biological Activities of Substance P Analogues

	guinea pig ileum				rat spinal cord				
			antagonist activity (dose ratio)			antagonist activity (% change in amplitude response)			
compd	concn, μM	agonist activity (% SP)	SP	SP(6–11)	concn, μM	SP	SP(6–11)	Glut	Carb
1	10	0	10.5	90	10	+19	−33	−3	−6
					25	+18	−77	−13	
					50	+8.3	−75	+11	−24
2	100	<0.0001	<2	<2					
3	1	0	1.1	1.8					
	10	0	14.6	18					
4	10	<0.0001	<2	<2					
5	4.1	0	2.7	<2	33.5	+10	−78	−14	
	21	0	3.5	<2					
6	10	<0.0001	<2	<2	5	+15	−22		
					50	+27	−55		
					100	+59	−75		
7	12	0	<2	<2	50	−37	−58	−5	−23
					100	−50		−26	−46
8	10	<0.0001	<2	<2	36	+18	−75	+16	
9	10	<0.0001	<2	<2					
10	10	<0.0001	<2	<2					
11	10	<0.0001	<2	<2					
12	10	<0.0001	<2	<2					
13	10	<0.0001	<2	<2					
14	10	<0.0001	<2	<2					
15	5	0	<2	<2					
16	50	0	<2	<2	50	−30	−22	−36	
17	50	0	3	<2	5	+29	−67		
					50	+39	−100	+2	
18	5	0	<2	<2	5	+12	−46		
					50	−9	−86	−37	−29
19	14	0.004	<2	<2					
23	10	0.001	<2	<2	10		+31	−5	
					50	−13	−21	−17	
25	10	<0.0001	<2	<2					
27	10	<0.0001	<2	<2					
28	10	0	<2	<2					
39	10	0	<2	<2					
40	5		<2	<2	5	+11	−13		
	10		10	5.6	50	−29	−62	−18	−47
41	5		3.9	8.1	5	+32	−42	−3	
	10		7.2	9.9	25	+21	−87	+29	
					50	−19	−87	+27	
42	10	<2	<2		25	−12	−49	−40	
43	6.4	0	3.1	2	25	+30	−55	+7	−10
	47		10.3	16.1					
44	5		6.3	3.4	5	+21	−62	−20	
	10		5.3	4.3	50	+16	−100		

substituted amides to be less potent than SP on the guinea pig ileum.[30,37]

The effect of bis(benzyloxycarbonyl) groups on the antagonist activity of D-Trp^{7,8,9} series of compounds (**39–43**) was opposite to the effect on the other antagonists mentioned above. In this series of analogues the bis(benzyloxycarbonyl) derivatives (**39**, **42**) were inactive. [N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9}]-SP-OMe (**40**) had some antagonist activity on the guinea pig ileum and the rat spinal cord preparation, but in the latter preparation glutamate and carbachol were both also antagonized. The deprotected analogues [D-Trp^{7,8,9}]-SP-OMe (**41**) and [D-Trp^{7,8,9}]-SP (**43**) showed antagonist activity on the guinea pig ileum and the rat spinal cord preparation. The less potent analogue **43** had no significant effect against glutamate and carbachol, but the methyl ester analogue (**41**) potentiated the effects of glutamate. [D-Pro^2,D-Trp^{7,9}]-SP (**44**), reported to be an antagonist of SP by Folker et al.,[7] was also examined in our test systems. In comparison to [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (**1**) and [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (**3**), it was less potent on the guinea pig ileum preparation, but it was somewhat more potent than compound **1** as an antagonist of the hexapeptide on the rat spinal cord preparation (100% and 75% reductions in response amplitude, respectively, at 50 μM). Both of these compounds showed some effect against carbachol or glutamate at 50 μM and also potentiated the effects of SP. [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (**17**), which like [D-Pro^2,D-Trp^{7,9}]-SP was a potent antagonist of SP(6–11)-hexapeptide on the rat spinal cord (100% reduction in response amplitude at 50 μM), showed no effect against glutamate.

On the basis of the above results, the antagonists reported here can be divided into two types. One of these types, [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (**1**), [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (**2**), [D-Trp^{7,8,9}]-SP-OMe (**41**), [D-Trp^{7,8,9}]-SP (**43**), and [D-Pro^2,D-Trp^{7,9}]-SP (**44**), antagonized SP and SP(6–11)-hexapeptide on the guinea pig ileum but only SP(6–11)-hexapeptide on the rat spinal cord preparation; the response to SP was potentiated. The other type of antagonists, [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe (**5**), [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP (**8**), [D-Phe^{7,8},D-Met^{11}]-SP-OMe (**6**), and [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (**17**), had no effect against SP or the

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hexapeptide on the guinea pig ileum preparation, but the hexapeptide was antagonized by these on the rat spinal cord. The response to SP on the rat spinal cord was potentiated by these antagonists, and in this respect both of these types of antagonists were similar.

Since the compounds have only been tested in two in vitro preparations, no attempt has been made to classify them as P or E type of antagonists. On the spinal cord preparation where the analogues only antagonize the hexapeptide they may be considered to be acting on E or K type of receptors.

The reasons for the potentiation of the response to SP are not clear. However, a fragment of SP, SP(1–9)-NH_2, which is almost devoid of activity at tachykinin receptors in some experiments,[38] is also able to potentiate the responses to SP. In contrast to the earlier conclusions, we now consider that the simplest explanation of the potentiation of SP by SP(1–9)-NH_2 and by the antagonist peptides is that SP is susceptible to rapid degradation by peptidases;[39] thus responses to SP are normally reduced because the concentrations of SP reaching receptors are lowered. The coadministration of related peptides in higher concentrations causes substrate competition for the degradative peptidases, resulting in reduced destruction of SP and hence larger responses. It is also worth noting that, in the presence of the antagonist, the responses to SP were greater in amplitude but shorter in duration, whereas with SP(1–9)-NH_2, SP responses were greater and longer.[38] This may indicate that the degradation products of SP are active at tachykinin receptor sites and contribute to the "tail" of the response to SP. The C-terminal fragments of SP have been shown to be selective for the tachykinin E receptor types,[40] and it therefore seems likely that the antagonist peptides block the E receptor actions of the fragments produced by peptidase action on SP and this results in shortening the "tail" of the response.

The reasons for the antagonistic effects of some of the antagonists against carbachol are unclear. However, there have been reports of selective interactions between SP and nicotinic cholinergic responses: Belcher and Ryall[41] found that SP inhibited Renshaw cell responses to nicotinic agonists; Akasu et al.[42] described the suppression by SP of the sensitivity of nicotinic acetylcholinic receptors on sympathetic ganglia and skeletal muscle. Clapham and Neher[43] studied acetylcholine-induced ion-channel currents in chromaffin cells and concluded that SP inhibits acetylcholine responses either by increasing the rate of desensitization or by inducing ion-channel blockade, which would enhance desensitization. Furthermore, Eardley and McGee[44] found that several SP antagonists were even more potent than SP in inhibiting carbachol-induced $^{86}Rb^+$ uptake in phaeochromocytoma (PC12) cells, in a noncompetitive way. These findings suggest that our results showing interactions between carbachol and some of the SP antagonists on the rat spinal cord may be due to actions of the antagonists on the ion channels via which carbachol effects are mediated rather than to specific effects on cholinergic receptors. However, in the spinal cord, 5 μM atropine caused a 63% reduction of carbachol (5 μM) responses, so muscarinic rather than nicotinic receptors are a major target for carbachol in this tissue. Nevertheless, an action of the SP antagonists on muscarinic receptor-operated ion channels remains a possible explanation of their reduction of carbachol responses.

Table IV. Blockade of the Hypotensive Responses of SP and SP(6–11)-hexapeptide during a 20-min Infusion of the Antagonists

compound	infusion rate, mg/kg^{-1} min^{-1}	% reduction of hypotensive responses	
		SP	SP(6–11)-hexapeptide
[D-Trp^{7,8,9}]-SP (**43**)	0.5	60	80
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (**3**)	0.5	75	100
	0.25	57	80
	0.05	43	75
	0.025	33	66
	0.005	25	40

(b) In Vivo Results. [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (**3**) and [D-Trp^{7,8,9}]-SP (**43**) were tested for their ability to block the hypotensive responses of SP and SP-(6–11)-hexapeptide in rabbits. Dose–response curves were constructed for SP and the hexapeptide, and suitable doses giving submaximal effects on the blood pressure were selected. The antagonists were infused for 20 min at a dose of 0.005–0.5 mg kg^{-1} min^{-1}, and during this period SP (20 pM/kg) and the hexapeptide (200 pM/kg) were injected iv. The results are summarized in Table IV. Both of the antagonists, **3** and **43**, blocked the hypotensive responses to SP and the hexapeptide. At an infusion rate of 0.5 mg kg^{-1} min^{-1}, compound **3** was more potent than compound **43**; it was effective down to 0.005 mg kg^{-1} min^{-1}. At all the dose levels tested both of the antagonists were more potent against the hexapeptide.

Since the hypotensive responses to both SP and the hexapeptide are antagonized, the receptors on rabbit blood vessels appear similar to the receptors on the guinea pig ileum.

Conclusions

The above results indicate that selective antagonists of SP and SP(6–11)-hexapeptide on the guinea pig ileum can be obtained by D-amino acid substitutions in positions 7–11 of SP. In some cases where the substitution of D-amino acids alone was not enough to generate antagonist activity, a combination of D-amino acids and benzyloxycarbonyl groups resulted in potent antagonists. In most cases, except in the D-Trp^{7,8,9} series of compounds, the potency of the antagonists was increased by substituting a methyl ester group at the C-terminus and benzyloxycarbonyl groups at the N^{α}-amino group of Arg^1 and N^{ϵ}-amino group of Lys^3 residues. It is interesting to note that both of these changes in the agonist series of compounds lead to a reduction in the agonist activity. On the rat spinal cord preparation all the antagonists mentioned above antagonized only the hexapeptide.

The amino acid residues in positions 7–11 of SP and the nature of the amino acid residues substituted in these positions appear to be playing an important part in the selectivity of these antagonists. When a D-Trp residue is present in positions 7 and 8 or 7 and 9, the compounds antagonize SP and the hexapeptide on the ileum and the hexapeptide on the spinal cord, but when D-Phe residues are present in positions 7 and 8 or when L-Phe residues

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are present in position 7 and 8 together with Pro residues in positions 9 and 10, the resulting compounds are inactive on the ileum but still are potent antagonists of the hexapeptide on the spinal cord.

The significance of the individual D-amino acid residues on antagonist activity and on further amino acid replacements in various other positions has also been investigated. Some of these results are reported in an accompanying paper and the others will be reported later.

Experimental Section

Symbols and abbreviations used follow the IUPAC–IUB recommendations;[45] other abbreviations used are as follows: AAA, amino acid analysis; Boc, *tert*-butyloxycarbonyl; CCD, countercurrent distribution; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; DMAP, 4-(dimethylamino)pyridine; HOBT, 1-hydroxybenzotriazole; TEA, triethylamine; TLC, thin-layer chromatography; Z, benzyloxycarbonyl. The following solvent systems were used for TLC on precoated plates of silica gel (Merck Kieselgel 60 F254); A, butan-1-ol–acetic acid–water (4:1:5, v/v); B, butan-2-ol–acetic acid–water–pyridine (15:3:12:10, v/v); C, butan-2-ol–ammonia (3% aqueous) (3:1, v/v); K, chloroform–methanol–water (55:40:10, v/v). Systems used for CCD purifications referred to in Table I are A, ethanol–acetic acid–water–ethyl acetate–petrol ether (60–80 °C) (2.5:1:5:8:1, v/v); B, ethanol–acetic acid–water–ethyl acetate (2.5:1:1:8, v/v); C, methanol–acetic acid–water–ethyl acetate–petrol ether (60–80 °C) (2.5:1:5:8:1, v/v); D, methanol–acetic acid–water–ethyl acetate (3.5:2:10:15, v/v). Amino acid analyses were performed on an LKB 4400 amino acid analyzer with a 4400 Ultra data integrator system following hydrolysis with constant boiling hydrochloric acid containing phenol (1%) or with saturated barium hydroxide solution in evacuated and sealed ampules at 110 °C for 16–48 h.

Peptide Synthesis. Since the analogues described in Table I were prepared by similar procedures, full synthetic details for each compound are not given; instead experimental details are given under each subheading for one particular compound to serve as an illustration of general procedures used.

Preparation of Protected Amino Acid Resin. Example: Boc-D-Met-resin. Chloromethylated resin (Lab Systems Inc., 1% cross-linked styrene–divinylbenzene, 0.75 mmol of Cl/g) was converted to the hydroxymethylated resin[46,47] and then esterified with Boc-D-Met-OH with DCC and DMAP.[32] Unreacted hydroxyl groups were acetylated with use of acetic anhydride and TEA in DMF, and the incorporation of methionine was determined after acid hydrolysis (12 N HCl–propionic acid, 1:1, v/v; 24 h, 110 °C). Over several different preparations the incorporation of this first amino acid was routinely found to be between 0.2 and 0.4 mmol of amino acid/g of resin.

Preparation of Protected Peptide Resin. The protected peptides listed in Table I were synthesized by the solid-phase procedure[31] with a Beckman 990 automated peptide synthesizer. Syntheses were performed on the 0.2–2.5-mmol scale, and the program for a typical synthesis on a 0.5-mmol scale is shown in Table V.

Example: Z-Arg(HCl)-Pro-Lys(Z)-Pro-Gln-Gln-D-Phe-D-Phe-Gly-Leu-D-Met-resin. Boc-D-Met-resin (1.32 g, 0.5 mmol) was placed in the Teflon reaction vessel, and the series of operations were carried out as listed in Table V. Each amino acid residue was incorporated with 2.5 equiv of the Boc-amino acid and of DCC followed by a repeat coupling on the same scale (double-coupling procedure).

The couplings were performed in CH_2Cl_2 or with added DMF for those amino acid derivatives not soluble in CH_2Cl_2. For the incorporation of the glutamine residues, HOBT (1.25 mmol) was added to the Boc-Gln-OH (1.25 mmol) solution in the amino acid reservoir. The N-terminal amino acid was incorporated as Z-Arg(HCl)-OH [obtained as a freeze-dried solid by treating Z-Arg-OH (30.8 g, 100 mmol) with an equivalent of 1 N HCl (100 mL, 100 mmol)], and the protected peptide resin was washed with propan-2-ol and methanol and dried in a vacuum oven at 40 °C (2.63 g).

Table V. Program for Automated Solid-Phase Synthesis of Substance P Analogues (Double-Couple Program)

step	reagents and operations	no. of times	approx vol of solvents, mL	mixing time, min
1	CH_2Cl_2 wash	3	15	1.5
2	40% TFA–CH_2Cl_2 prewash	1	12	1.5
3	40% TFA–CH_2Cl_2 (5% ethanedithiol) deblocking	1	12	30
4	CH_2Cl_2 wash	3	15	1.5
5	propan-2-ol	3	15	1.5
6	CH_2Cl_2 wash	3	15	1.5
7	10% TEA–CH_2Cl_2 neutralization	2	20	4
8	CH_2Cl_2 wash	5	15	1.5
9	Boc-amino acid	1	10	
10	0.25 M DCC–CH_2Cl_2	1	5	
11	coupling reaction			120
12	DMF wash	5	15	1.5
13	propan-2-ol	3	15	1.5
14	CH_2Cl_2 wash	3	15	1.5
15	10% TEA–CH_2Cl_2 neutralization	1	20	4
16	CH_2Cl_2 wash	3	15	1.5
17	Boc-amino acid	1	10	
18	0.25 M DCC–CH_2Cl_2	1	5	
19	coupling reaction			150
20	DMF wash	5	15	1.5
21	search next program			

Preparations of Protected Peptide Methyl Esters. Example: Z-Arg(HCl)-Pro-Lys(Z)-Pro-Gln-Gln-D-Phe-D-Phe-Gly-Leu-D-Met-OMe (5). The peptide resin obtained above (2.6 g) was suspended in freshly distilled DMF (26 mL)–methanol (26 mL). Triethylamine (2.6 mL) was added and the suspension gently stirred at room temperature overnight. The methyl ester thus obtained was purified by CCD with use of system A and was obtained as a solid (320 mg, 40%) for which the analytical data are shown in Table I.

Preparation of Protected Peptide Amides. Example: Z-Arg(HCl)-Pro-Lys(Z)-Pro-Gln-Gln-D-Phe-D-Phe-Gly-Leu-D-Met-NH_2 (8). The protected peptide ester 5 from above (150 mg, 0.09 mmol) was dissolved in methanol (20 mL), and dry gaseous ammonia was bubbled through the stirred solution for 3 h. The flask was then stoppered and allowed to stand for 3 days. Evaporation of the solvent gave the protected peptide amide (125 mg, 84%), which required no further purification.

The two di-Z-substituted substance P alkyl amides (compounds **35** and **37**, Table I) were prepared from the corresponding methyl ester [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3]-SP-OMe (**33**) by treatment of a DMF solution with an excess of the corresponding amine (ethylamine and butylamine, respectively) at room temperature for 2–3 days.

Preparation of Peptide Esters and Amides. Example: H-Arg-Pro-Lys-Pro-Gln-Gln-D-Phe-D-Phe-Gly-Leu-D-Met-NH_2 (9). The di-Z-peptide amide 8 from above (60 mg) was dissolved in TFA (3.6 mL)–thioanisole (1.3 mL) at room temperature and the mixture allowed to stand overnight. The product was precipitated on addition of ether. The ether was decanted off and the residue was partitioned between water (50 mL) and ether (20 mL). The aqueous layer was further extracted with two more aliquots of ether and lyophilized. The residue was dissolved in 0.01 M HCl and chromatographed on a column (100 × 3.3 cm) of Bio-Gel P4 in 0.01 M HCl. The product containing fractions were combined and lyophilized (42 mg, 84%).

Rat Spinal Cord Assay. Wistar rats of the Alderley Park strain (2–7 days old) were anesthetized with urethane (25% w/v) administered intraperitoneally (2.5 mg/kg). After decapitation, the vertebral column was removed and placed in a dish of oxy-

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genated Krebs-Henseleit medium containing 0.5% urethane at room temperature.

The vertebrae were dissected away from the cord and the dura removed. The dorsal and ventral roots were separated by hemisection of the spinal ganglia. The cord was hemisected sagittally, and each half was placed on a small square of disposable nappy-linear material mounted on a wire mesh grid and transferred to the superfusion block. The cord was superfused (1 mL/min) with medium, and DC and evoked potential differences between the cut end of a ventral root (usually L4 or L5) and the body of the cord were recorded. The system was earthed via a third electrode. Bipolar stainless steel hook electrodes were used to stimulate the corresponding dorsal root to evoke ventral root potentials (VRP), which were displayed after amplification, on an oscilloscope (Gould 4100) and pen recorder (Kipp and Zonen BD9). Supramaximal square wave stimuli were used at a rate of 1 pulse/60 s.

Agonists were applied to the cord in superfusion fluid in 2-mL doses, and antagonists were superfused continuously in the medium for at least 15 min before further testing of agonists. The responses to the agonists were observed as changes in the DC potential between the ventral root and the body of the cord.

Guinea Pig Ileum Assay. Guinea pigs (Dunkin-Hartley, 300-350 g) of both sexes were killed by stunning and exsanguination. The distal ileum was dissected out and suspended in a 5-mL organ bath containing Krebs solution at 37 °C and gassed with 95% oxygen-5% carbon dioxide. Agonist dose-response curves were constructed with use of a 3-min cycle allowing 1-min agonist contact time, doses being washed from the bath following application. To assess antagonist activity, agonist dose-responses curves were constructed in the presence of antagonist (1-10 μM, 15-min contact time) and the contractile responses were recorded with an isotonic tranducer connected to a Kipp and Zonen BD9 chart recorder.

Studies of Substance P Analogues on Rabbit Blood Pressure Responses. Rabbits of either sex (1.5-3.0 kg) were anesthetized with halothane. The trachea was cannulated, and cannulae were also inserted into a carotid artery and femoral vein for blood pressure recording and drug injections, respectively. Dose-response relations were constructed for iv bolus doses of substance P and substance P(6-11)-hexapeptide and also acetylcholine chloride (ACh). The hypotensive responses were more markedly apparent as reductions of diastolic blood pressure than of systolic blood pressure. Suitable agonist doses were selected for examination of the effects of the antagonist peptides, the latter being administered iv as slow infusion, over 20-min periods. The percentage changes in the maximal hypotensive responses to the agonists following antagonist doses are shown in Table IV.

Registry No. 1, 101809-76-1; 2, 101809-77-2; 3, 101809-78-3; 4, 101809-79-4; 5, 101809-80-7; 6, 101914-71-0; 7, 101809-81-8; 8, 101834-52-0; 9, 101914-72-1; 10, 101809-82-9; 11, 101809-83-0; 12, 101809-84-1; 13, 101809-85-2; 14, 101809-86-3; 15, 101809-87-4; 16, 101809-88-5; 17, 101809-89-6; 18, 101809-90-9; 19, 101809-91-0; 20, 101809-92-1; 21, 101809-93-2; 22, 101809-94-3; 23, 101809-95-4; 24, 101809-96-5; 25, 101809-97-6; 26, 101809-98-7; 27, 101809-99-8; 28, 101810-00-8; 29, 101810-01-9; 30, 101810-02-0; 31, 101810-03-1; 32, 101810-04-2; 33, 101914-73-2; 34, 76260-78-1; 35, 101810-05-3; 36, 101810-06-4; 37, 101810-07-5; 38, 101810-08-6; 39, 101810-09-7; 40, 101810-10-0; 41, 101810-11-1; 42, 101810-12-2; 43, 101810-13-3; 44, 80434-86-2; SP, 33507-63-0; SP(6-11)-hexapeptide, 51165-07-2; carbachol, 51-83-2; L-glutamic acid, 56-86-0.

Antagonists of Substance P. Further Modifications of Substance P Antagonists Obtained by Replacing either Positions 7, 9 or 7, 8 and 11 of SP with D-Amino Acid Residues

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Antagonists of SP and the C-terminal (6-11)-hexapeptide have been obtained by multiple D-amino acid substitutions in various positions of SP and by protecting the N^{α}-Arg^1 and N^{ϵ}Lys^3 amino groups with benzyloxycarbonyl groups. On the guinea pig ileum a number of these antagonized both SP and the hexapeptide. Except [N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**4**) and the corresponding amide **7**, which were more potent antagonists of SP than the hexapeptide, all the others, e.g., [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-OMe (**9**), [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (**11**), were more potent antagonists of the hexapeptide. On the rat spinal cord preparation, most of the antagonists were only active against the hexapeptide. A few antagonized SP, but these also reduced carbachol or both carbachol and glutamate responses. Two of the antagonists, [D-Pro^2,Asn^5,Lys^6,D-Phe^7,D-Trp^9]-SP-OMe (**2**) and [Boc-D-Pro^4,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP(4-11)-OMe (**10**), were inactive on the ileum but still antagonized the hexapeptide on the spinal cord. The smallest peptides to antagonize SP and the hexapeptide were two heptapeptides, **6** and **21**, [Z-Asn^5,Arg^6,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP(5-11)-OMe (**21**) being more potent than **6**. None of the antagonists showed significant analgesic activity without side effects. Some of the antagonists were shown to release histamine from isolated rat peritoneal cells.

Since the discovery of substance P (SP),[1,2] a number of physiological and pharmacological effects have been ascribed to this peptide and these have recently been reviewed.[3-5] Specific antagonists of SP are therefore needed to investigate the pharmacology of SP and also to define the role of various receptors that have recently been proposed.[6,7]

A number of SP antagonists have recently been reported.[8-11] These have been obtained by substituting

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