a variety of conditions. Under mildly acidic conditions (0.1 N HCl) at various concentrations or temperatures in various aqueous methanolic solutions either no reaction was observed or the aglycon was formed. With a variety of concentrations of K_2CO_3 or NaOH in aqueous alcohols or DMF at a variety of times and temperatures, aromatization was the only reaction. In a typical experiment, the glycoside (**16**, 2 mg) was dissolved in 5.0 mL of THF and this was cooled to 0 °C in an ice bath before ice cold 0.1 N NaOH was added dropwise over a 15-min interval whereupon the initially yellow solution turned blue. After 5 h the reaction was complete as judged by TLC examination and 0.5 M citric acid (1.38 mL) was added dropwise to achieve a reddish solution of pH about 6. This solution was adjusted to pH 8 with 10% sodium bicarbonate solution with use of a syringe and then extracted with chloroform–MeOH (9:1). The organic layer was dried with sodium sulfate and evaporated to dryness to produce **21** as a brownish crystalline powder identical with a sample synthesized by Friedel–Crafts condensation between 5,8-dimethoxyisoquinoline and phthalic anhydride with $AlCl_3$–NaCl: HRMS, m/z 291.05226 ($C_{17}H_9NO_4$ requires 291.05309). This was the base peak, and only very small fragment peaks were otherwise observed. The product was too insoluble in normal solvents for ^1H NMR examination, so it was acetylated with acetic anhydride and pyridine in the usual fashion for further characterization. The product was a yellow powder: mp 240 °C; MS, m/Z 375 ($M^+ - CH_2CO$), 333 ($M^+ - 2CH_2CO$), 291 (100); ^1H NMR ($CDCl_3$) δ 2.66 (s, 6 H, $COCH_3$), 7.5–8.38 (m, 4 H, Ar H), 8.63–9.75 (m, 3 H, PyH).

Testing in Mice against the P388 Lymphocytic Leukemia Model. 4-Demethoxydaunomycin (**20**), (7*S*)-9-aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin (**17**), and (7*R*)-9-aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin (**16**) were dissolved in Cremophor/saline to the final concentrations given below. Female CDF_1 and DBA_2 mice (Harlan Laboratory, Indianapolis, IN) housed in gang cages were fed Purine Laboratory Chow and water ad libitum and adapted to this regime for at least 1 week before use. The P388 tumor was maintained by continuous passage in DBA_2 mice. On day 0, ascitic fluid was removed and diluted with Hank's balanced salt solution, cells were counted, and 10^6 tumor cells were implanted ip in a total volume of 0.2 mL. Twenty-four hours later, mice were randomly segregated into treatment groups and drug was given ip to groups of nine mice for each dilution. Dosing was repeated on days 5 and 9 also. The mice were observed for 30 days and T/C (percent) values were determined from the survival rate as compared to the controls.[22]

4-Demethoxydaunorubicin: 3 mg/kg (T/C 82); 1.5 (97); 0.75 (160); 0.375 (121).

(7*S*)-9-Aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin: 100 mg/kg (T/C 98); 50 (102); 25 (97); 12.5 (97); 6.25 (100); 3.72 (97); 1.56 (95).

(7*R*)-9-Aza-4-demethoxy-*N*-(trifluoroacetyl)daunomycin: 100 mg/kg (T/C 105); 50 (96); 25 (90); 12.5 (98); 6.25 (97); 3.72 (97); 1.56 (95).

Acknowledgment. We at Kansas University are grateful to Adria Laboratories and the National Institutes of Health Biomedical Research Support Program (RR 5601) for generous financial support of this work.

Notes

Synthesis and Biological Activity of Substance P C-Terminal Hexapeptide Analogues: Structure–Activity Studies

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A series of analogues of the C-terminal hexapeptide of substance P, modified at the glutaminyl residue, was synthesized and their relative activities as spasmogens were determined in the guinea pig ileum and rat colon muscularis mucosae preparations in vitro. In general, when compared to SP_{6-11}, the loss of the carboxamide group has little effect on activity in the colon and reduces activity on the ileum. The exception to this is the Orn^6 analogue which retains activity on both preparations and is proposed as a useful tool for structure–activity studies. It is concluded that the hydrogen-bonding potential of the position 6 substituent may be an important determinant of biological activity.

In most pharmacological test systems which respond to the undecapeptide tachykinin, substance P (SP, **1**) biological activity is retained in C-terminal fragments as small as the hexapeptide Gln-Phe-Phe-Gly-Leu-Met-NH_2 (SP_{6-11}, **2**). This applies to the gastrointestinal smooth muscle[1,2] and salivary secretion[3] and to the hypotensive effects of this group of peptides.[4] In neuronal preparations (rat spinal cord and rat superior cervical ganglion, in vitro), the C-terminal hepta- and hexapeptides are somewhat more active than the parent undecapeptide.[5,6] However, the N-terminal residues should not be considered redundant since they may well contribute to the potency of the compound in some test systems, while in others, there is an absolute requirement for the N-terminal basic residues.[7,8] In view of the importance of the C-terminal hexapeptide for biological activity, this sequence should provide a basis for examining some aspects of the structure-activity relationship for tachykinin agonists.

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(1) Bury, R. W.; Mashford, M. L. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 633.
(2) Bailey, S. J.; Jordan, C. C. *Br. J. Pharmacol.* 1984, *82*, 441.
(3) Hanley, M. J.; Lee, C. M.; Jones, L. M; Michell, R. H. *Mol. Pharmacol.* **1980**, *18*, 78.
(4) Traczyk, W. Z. *Substance* P. (Nobel Symposium 37); von Euler, U. S., Pernow, B., Eds.; Raven: New York, 1977; pp 297–309.
(5) Brown, J. R.: Hawcock, A. B.; Hayes, A. G.; Tyers, M. B.; Hill, R. G. *J. Physiol.* **1983**, *334*, 91P.
(6) Otsuka, M.; Konishi, S. *Nature (London)* **1976**, *264*, 83.
(7) Fewtrell, C. M. S.; Foreman, J. C.; Jordan, C. C.; Oehme, P.; Renner, H.; Stewart, J. M. *J. Physiol.* **1982**, *330*, 393.
(8) Bar-Shavit, Z.; Goldman, R.; Stabinsky, Y.; Gottliels, P.; Fridkin, M.; Teichberg, V. I.; Blumberg, S. *Biochem. Biophys. Res. Commun.* 1980, *94*, 1445.

0022-2623/86/1829-1281$01.50/0

Table I. Relative Potencies of Position 6 Substituted Analogues of SP_{6-11} on Guinea Pig Ileum and Rat Colon

no.	compd	rel potencies (SP = 1) 95% confidence limits: guinea pig ileum	rat colon
1	SP	1	1
2	SP_{6-11}	0.30 [0.24–0.39]	0.076 [0.058–0.096]
15	[Orn^6]-SP_{6-11}	0.39 [0.32–0.51]	0.36 [0.3–0.43]
16	[Thr^6]-SP_{6-11}	0.021 [0.014–0.034]	0.056 [0.048–0.066]
17	[$Thr(OCH_3)^6$]-SP_{6-11}	0.066 [0.052–0.081]	0.16 [0.08–0.25]
18	[Val^6]-SP_{6-11}	0.067 [0.045–0.093]	0.15 [0.12–0.17]

In previous studies, it has been shown that replacement of the Gln^6 by Gly or Ala yieled compounds with reduced activity[9,10] while substitution with the acyl group (4-hydroxy-phenyl)acetyl resulted in a potent analogue.[11,12] These findings prompted us to investigate the role of the glutamine carboxamide group and the general properties of the side chain in order to evaluate their contributions to the biological activity of the hexapeptide.

In the present work, a series of analogues of SP_{6-11} were synthesized in which the N-terminal amino acid glutamine was replaced successively by Orn, Thr, Thr (OCH_3), and Val. The analogues were then tested in two bioassay preparations, the guinea pig ileum and rat colon muscularis mucosae in vitro, preparations which are respectively representative of "SP-P" and "SP-E" receptor containing tissues.[13,14]

Results and Discussion

The analogues of the C-terminal hexapeptide amide of substance P were prepared by conventional solution-phase synthesis as follows: The N-terminal tetrapeptide benzyl esters were built up by stepwise N-terminal elongation using the DCC/HOBt method except for the peptide Boc_2Orn-Phe-Phe-Gly-OBzl which was obtained by an active ester coupling of Boc_2Orn-OTcp to the tripeptide H-Phe-Phe-Gly-OBzl. Hydrogenation of the N-terminal tetrapeptide benzyl esters over 10% Pd/C yielded the corresponding acids which were coupled to the dipeptide H-Leu-Met-NH_2 with the DCC/HOBt method to yield the corresponding protected hexapeptides. Final products were obtained by deprotection of the hexapeptides with HCl in acetic acid.

All of the hexapeptides tested were found to be full agonists relative to SP_{1-11} in both preparations. In the guinea pig ileum, compounds lacking the carboxamide group at position 6 exhibited reduced activity relative to the parent hexapeptide SP_{6-11} (Table I). The exception to this was [Orn^6]-SP_{6-11} which was approximately equipotent with the Gln^6 compound.

It has been shown previously that modifications of the side chain of Gln^6 in the C-terminal hexapeptide of SP reduce activity especially when the carboxamide group of Gln is omitted.[15] It appears, therefore, that the carboxamide group may participate in hydrogen bonding either (a) with elements of the C-terminal peptide backbone to stabilize the active conformation[16,17] or (b) with the receptor, so facilitating drug–receptor interactions.

The effect on activity of a hydrogen-bonding donor (OH in Thr) or a hydrogen-bonding acceptor (OCH_3 in Thr (OCH_3)) in the side chain of the amino acid at position 6, in the absence of the carboxamide group, indicates that the interaction of the carboxamide hydrogens with H-bonding acceptors may be a specific one. With the above modifications, we may have an inappropriate or weak interaction.

It would appear also that only a limited degree of side-chain lipophilicity is tolerated-increased lipophilicity (as in the Val^6 analogue) seems to be undesirable. However, the altered activity of this analogue may also be due to the effect of Val on the peptide backbone conformation, as acylated analogues of SP which have increased lipophilicity at position 6 also have high relative activities.[11,12]

Since omission of the carboxamide group of Gln^6 in the full SP sequence has no effect on the potency[18] we suggest that the C-terminal hexapeptide of SP may not interact with the receptor in the same way as the parent undecapeptide.

The effects of position 6 substitutions on activity on the rat colon is somewhat different. The relative activity of the Gln^6 parent compound is low in comparison to that in the guinea pig ileum, and this is further emphasized by the fact that the EC_{50} for SP itself is some 100 times higher in the rat colon than in the guinea pig ileum.

In the series of compounds discussed here, all are roughly equiactive with SP_{6-11}. The exception to this generalization is [Orn^6]-SP_{6-11}, which is 4.7 times more active than the parent compound. This may reflect the hydrogen-bonding potential of the Orn^6 side-chain amine group although it is perhaps surprising that its activity is so markedly different from its Gln^6 counterpart. Nevertheless, it appears that the nature of the side chain of the position 6 substituent is an important determinant of biological activity in this preparation as well as the guinea pig ileum.

It is concluded that the biological properties of the [Orn^6]-SP_{6-11} analogue make it a useful alternative to SP_{6-11} as a parent sequence for structure-activity studies, being more soluble and not prone to contamination with the corresponding pyroglutamyl derivative.

Experimental Section

Chemistry. Capillary melting points were determined on a Buchi SMP-20 apparatus and are reported uncorrected. Optical rotations were measured with the Carl Zeiss precision polarimeter (0.005°). Analysis by TLC was on precoated plates of silica gel F254 (Merck) with the following solvent systems: (A) chloroform–methanol (6:1), (B) 1-butanol–acetic acid–water (4:1:1), (C)

(9) Chorev, M.; Gilon, C.; Wormser, U.; Fridman, Z. Y.; Selinger, Z. *Peptides*, Proceedings of the 16th European Peptide Symposium; Brunfeldt K., Ed.; Scriptor: Copenhagen, 1981; pp 451–456.
(10) Bernardi, L.; Bosisio, G.; Castiglione, F.; Erspamer, V.; Glaesser, A.; Goffredo, O. *Experientia* **1964**, *20*, 306.
(11) Niedrich, M.; Bergmann, J.; Bienert, M.; Albrecht, E.; Oehme, P. *Peptides: Chemistry, Structure, Biology*, Proceedings of the 4th American Peptide Symposium; Watler, R., Meinhofer, J. eds.; Ann Arbor Press: Ann Arbor, 1975; pp 565–570
(12) Niedrich, M.; Bienert, M.; Vergmann, J.; Kuehne, R.; Franke, R.; Oehme, P. *Peptides* Proceedings of the 14th European Peptide Symposium: Loffet, A., Ed.; Editions de l'Universite de Bruxelles: Belgium, 1976; pp 407–413.
(13) Lee, C. M.; Iversen, L. L; Hanley, M. R.; Sandberg, B. E. B. *Naunyn*-Schmedeberg's *Arch Pharmacol.* **1982**, *318*, 281.
(14) Bailey, S. J.; Gater, P. R.; Jordan, C. C. *Br. J. Pharmacol.* **1982**, *75*, 114.
(15) Chorev, M.; Gilon, C.; Wormser, U.; Friedman, Z. Y.; Selinger, Z. *Peptides*, Proceedings of the 16th European Peptide Symposium; Brunfeld, K., Ed.; Scriptor: Copenhagen, 1981; pp 451–456.
(16) Nikiforovich, G. V., Balodis, Y.; Chipens, G. I. *Peptides*, Proceedings of the 16th Europena Peptide Symposium; Brunfeld, K., Ed.; Scriptor: Copenhagen, 1981; pp 631–636.
(17) Manavalan, P.; Momany, F. A. *Int. J. Peptide Protein Res.* **1982**, *20*, 351.
(18) Couture R.; Fournier, A.; Morgan, J.; St-Pierre, S.; Regoli, D. *Can. J. Physiol. Pharmacol.* **1979**, *57*, 1427.

Table II. Physical Constants of Peptides Boc-X-Phe-Phe-Gly-Y

no.	X	Y	formula	mp, °C	recrystn solvent	yield, %	$[\alpha]^{22}_D$, deg	TLC, R_f (solv)		anal.
3	Orn(Boc)	OBzl	$C_{44}H_{55}N_5O_9$	147–149	EtOAc/pet. ether[b]	84	−17.8[f]	0.88 (A)	0.83 (B)	C, H, N
4	Thr(OBzl)	OBzl	$C_{45}H_{50}N_2O_8$	194–197	EtOAc/pet. ether[c]	81	−37.2[g]	0.75 (A)	0.82 (B)	C, H, N
5	Thr(OCH_3)	OBzl	$C_{39}H_{46}N_4O_8$	177–178	EtOAc/*n*-hexane[d]	86	−23.5[h]	0.79 (A)	0.71 (B)	C, H, N
6	Val	OBzl	$C_{39}H_{46}N_4O_7$	173–174	EtOH	78	−9.5[i]	0.90 (A)	0.85 (B)	C, H, N
7	Orn(Boc)	OH	$C_{37}H_{49}N_5O_9$	165–167		95	−8.9[f]	0.80 (A)	0.83 (B)	C, H, N
8	Thr	OH	$C_{31}H_{38}N_4O_8$	193–202		89	−23.5[h]	0.64 (B)	0.61 (C)	C, H, N
9	Thr(OCH_3)	OH	$C_{32}H_{40}N_4O_8$	165–170		92	−28.5[h]	0.68 (B)	0.65 (C)	C, H, N
10	Val	OH	$C_{32}H_{40}N_4O_7$	190–191		90	−19.2[j]	0.72 (A)	0.83 (B)	C, H, N
11	Orn(Boc)	Leu-Met-NH_2	$C_{48}H_{70}N_8O_{10}S$	211–214[a]	EtOH	72	−23.0[f]	0.72 (B)	0.66 (C)	C, H, N
12	Thr	Leu-Met-NH_2	$C_{42}H_{59}N_7O_9S$	219–220[a]	EtOAc	66	−34.0[j]	0.45 (A)	0.64 (C)	C, H, N
13	Thr(OCH_3)	Leu-Met-NH_2	$C_{43}H_{61}N_7O_9S$	227–230[a]	EtOH	75	−28.5[j]	0.73 (A)	0.73 (C)	C, H, N
14	Val	Leu-Met-NH_2	$C_{43}H_{61}N_7O_8S$	226–230[a]	EtOH	68	−40.8[j]	0.84 (A)	0.82 (B)	C, H, N

[a] Decomposition. [b] 4:1 v/v. [c] 5:1 v/v. [d] 5:2 v/v. [e] 1:3 v/v. [f] 0.5% DMF. [g] 0.25% MeOH. [h] 0.5% MeOH. [j] 1% DMF.

Table III. Physical Constants of Peptides X-Phe-Phe-Gly-Leu-Met-NH_2

no.	X	formula	mp,[a] °C	yield, %	$[\alpha]^{22}_D$, deg	TLC, R_f (solv)			anal.	amino acid anal.						
										Met	Leu	Gly	Phe	Orn	Thr	Val
15	Orn	$C_{38}H_{56}N_8O_8S$ ·2HCl	170–175	49	−44.0[b]	0.22 (B)	0.25 (C)	0.68 (D)	C, H, N	1.0	1.01	1.02	1.96	*e*		
16	Thr	$C_{37}H_{52}N_7O_7S$ ·HCl	160–161	51	−22.2[c]	0.40 (B)	0.45 (C)	0.76 (D)	C, H, N	0.95	1.05	1.01	2.02		0.96	
17	Thr(OCH_3)	$C_{38}H_{54}N_7O_7S$ ·HCl	167–168	55	−48.4[d]	0.38 (B)	0.40 (C)	0.73 (D)	C, H, N	0.9	0.99	1.02	1.98		*f*	
18	Val	$C_{38}H_{54}N_7O_6S$ ·HCl	180–183	45	−23.5[d]	0.49 (B)	0.52 (C)	0.65 (D)	C, H, N	1.01	1.01	1.06	1.95			0.96

[a] Decomposition. [b] 1% 1 M acetic acid. [c] 0.2% DMF. [d] 0.5% DMF. [e] Ornithine present but not estimated. [f] Thr(OCH_3) present but not estimated because of partial hydrolysis to threonine.

1-butanol–acetic acid–water (4:1:5, upper phase), and (D) 1-butanol–acetic acid–water–pyridine (30:6:24:20). The products on TLC plates were detected by UV light, and either chlorination followed by *o*-tolidine or ninhydrin. The elemental analyses are within ±0.4% of the calculated value when specified by symbols. Amino acid analysis of the final products was performed by using an LKB 4400 amino acid analyzer. Samples were hydrolyzed by boiling in 6 N HCl containing 0.1% phenol at 110 °C for 18 h in an evacuated, sealed ampule. All amino acids are of the L configuration.

The following abbrevaitions are used: DCC, *N,N*-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; EtOAc, ethyl acetate; pet. ether, petroleum ether (bp 60–80 °C); EtOH, ethanol; MeOH, methanol; Et_2O, diethyl ether; DMF, dimethylformamide; NMM, *N*-methylmorpholine.

Removal of the N^{α}-*tert*-Butyloxycarbonyl Group. General Procedure. N^{α}-*tert*-Butyloxycarbonyl peptides were deprotected with 1 N HCl in acetic acid for 1 h at room temperature. The solvent was then removed in vacuo and the residue was solidified by the addition of dry ether. The resulting hydrochloride salt was filtered, washed with dry ether, and dried under vacuum over KOH pellets.

Preparation of Boc-X-Phe-Gly-OBzl. A sample of Boc-Phe-Phe-Gly-Obzl[19] (3 mmol) was deprotected with 1 N HCl in acetic acid. The resulting hydrochlorides salt was dissolved in DMF (6 mL), neutralized with NMM, and allowed to react with a sample of Boc-X-OH (3 mmol) dissolved in DMF (8 mL) and preactivated at 0 °C for 0.5 h with HOBt (5.4 mmol) and DCC (3 mmol). The reaction mixture was left to stand for 2 h at 0 °C and then for 24 h at room temperature. The precipitated DCU was filtered, and the solvent was removed in vacuo. The remaining residue was solidified by trituration with 5% $NaHCO_3$. The resulting solid was filtered, washed several times with 5% $NaHCO_3$, water, 10% citric acid, and water, and dried (P_2O_5). The peptide **3** (X = Orn(Boc)) was obtained by an active ester coupling of Boc-Orn(Boc)-OTcp[20] to the tripeptide Boc-Phe-Phe-Gly-OBzl after its deprotection with 1 N HCl in acetic acid and neutralization with NMM in DMF. The reaction was completed in 48 h at room temperature, and isolation of the product was accomplished according to the procedure discribed above. The products were further purified by recrystallization (Table II, Y = OBzl).

Preparation of Boc-X-Phe-Phe-Gly-OH. A sample of Boc-X-Phe-Phe-Gly-OBzl (2 mmol) was dissolved in 40 mL of DMF–H_2O (9:1 v/v) and hydrogenated over 10% Pd/C at atmospheric pressure. The progress of the reaction was monitored by TLC. At the end of the reaction the solvent was removed in vacuo and the residue was solidified by the addition of ether. The solid was filtered, washed with ether, and dried (P_2O_5). These products were used in the next step of the synthesis without further purification. For physical constants of the obtained peptides see Table II (Y = OH).

Preparation of Boc-X-Phe-Phe-Gly-Leu-Met-NH_2. A portion of Boc-Leu-Met-NH_2[20] (1 mmol) was deprotected with 1 N HCl in acetic acid and the resultant hydrochloride salt was dissolved in DMF (6 mL), neutralized with NMM, and allowed to react with a sample of Boc-X-Phe-Phe-Gly-OH (1 mmol) dissolved in DMF (8 mL) and preactivated at 0 °C for 0.5 h with HOBt (1.8 mmol) and DCC (1 mmol). The reaction mixture was left to stand for 2 h at 0 °C and then for 24 h at room temperature. The precipitated DCU was filtered, and the solvent was removed in vacuo. The remaining residue was solidified by trituration with 5% $NaHCO_3$. The resulting solid was washed several times with 5% $NaHCO_3$, water, 10% citric acid, and water and dried (P_2O_5). The products were further purified by recrystallization (Table II, Y = Leu-Met-NH_2).

Preparation of H-X-Phe-Phe-Gly-Leu-Met-NH_2 Hydrochlorides. A sample of Boc-X-Phe-Phe-Gly-Leu-Met-NH_2 (150–250 mg) was deprotected with 1 N HCl in acetic acid. The isolated hydrochloride salt was dissolved in water, filtered through a Millipore filter, and lyophilized. The products were further purified by gel filtration on Sephadex G-15 (2 × 80 cm) using as the eluents 0.5 M acetic acid for peptides **15**, **16**, **17** and 1 M acetic acid for peptide **18** followed by partition chromatography on Sephadex G-25F (2 × 80 cm) with 1-butanol–acetic acid–water (4:1:5 v/v, upper phase). Yields of the final products were 45–55% based on the protected peptides. For physical constants see Table III.

Bioassays. Sources of drugs were as follows: substance P and C-terminal hexapeptide of substance P, SP_{6-11}, Merseyside Laboratories, U.K. [The tendency for Gln to cyclize to form

(19) Jakubke, H. D.; Klessen, C.; Neubert, K. *J. Prakt. Chem.* **1977**, *319*, 640.
(20) Mehlis, B.; Apelt, H.; Bergmann, J.; Niedrich, H. J. *Prakt. Chem.* **1972**, *314*, 390.

pyroglutamic acid means that the compound SP_{6-11} is a mixture of the glutaminyl and pyroglutaminyl forms. Estimates based on HPLC measurements suggest that the ratio of the two forms is approximately 65:35]; atropine sulfate and indomethacin Sigma; methysergide bimaleate, Sandoz; mepyramine maleate, generous gift of May and Baker Ltd. All other compounds were of Analar quality.

Indomethacin was dissolved in 10% $NaHCO_3$, all other drugs were dissolved in physiological salt solution.

Bioassays were performed on the guinea pig ileum and rat colon muscularis mucosae preparations as previously described[2,21] but with the following modifications: (i) atropine (1 μM), mepyramine (1 μM), methysergide (1 μM), and indomethacin (1 μM) were included in the bathing media for all experiments; (ii) the time cycle for administration of agonist doses was arranged such that there was a 7-min interval between doses in the guinea pig ileum and a 10-min interval in the rat colon. Pilot experiments had demonstrated that between dose interactions were minimized for SP when these schedules were used.

Formal 3 + 3 assays (guinea pig ileum) or 2 + 2 assays (rat colon) were performed on all analogues by using a randomized block design.[21,22] Results were analyzed by analysis of variance, and only those assays which satisfied tests for parallelism, linearity, difference of curvature, and regression were accepted for estimates of relative activities. All results are expressed as relative activities, taking SP_{1-11} as 1.0.

Samples of peptides were prepared in 0.01 M acetic acid or dimethyl-sulfoxide (the latter solvent being used for the less soluble analogues), at a stock concentration of 1–10 mM. This solution was divided into aliquots which were stored at -30 °C until required for use. A sample of solution was dried under nitrogen and prepared for amino acid analysis to provide an accurate estimate of peptide concentration. A further sample of each compound was subjected to FAB spectrometry to confirm its structure.

During the experiments, dilutions were made in physiological salt solution. Control experiments with Me_2SO showed that, at the concentrations used in the present experiments, this compound did not effect the response of the tissue to any of the peptides tested.

Acknowledgment. Thanks are due to Prof. K. Barlos for providing us with a sample of Boc-Thr(OCH_3)-OH. This work was partially supported by the 1984 Research Fund of the Royal Society of Chemistry. We wish to acknowledge Mrs. T. A. Crees for excellent technical assistance and Miss A. Ward and Mrs. A. Brett for typing the manuscript.

(21) Gater, P. R.; Jordan, C. C.; Owen, D. G. *Br. J. Pharmacol.* 1982, *75*, 341.

[pGlu^6,Pro^9]SP_{6-11}, a Selective Agonist for the Substance P P-Receptor Subtype

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Substitution of a single amino acid residue, proline for glycine-9 in [pGlu^6]SP_{6-11}, a hexapeptide analogue of substance P, confers on the peptide selective agonist activity toward the SP-P receptor subtype. [pGlu^6,Pro^9]SP_{6-11} had 20% and 75% of the activity of [pGlu^6]SP_{6-11} in stimulating, respectively, K^+ release from rat parotid slices and contraction of the isolated guinea pig ileum, via the SP-P receptor subtype. In contrast, [pGlu^6,Pro^9]SP_{6-11} had substantially reduced activity on SP-E systems such as the hamster urinary bladder and rat duodenum, being about 20-fold less potent than [pGlu^6]SP_{6-11} and 200–670-fold less potent than neurokinin B. In the guinea pig ileum [pGlu^6,Pro^9]SP_{6-11} had very low activity on the neuronal tachykinin receptor, being 325 times less potent than [pGlu^6]SP_{6-11} and 1000 times less potent than neurokinin B. Because of its discrimination between the muscular and neuronal receptors in the guinea pig ileum (muscular/neuronal potency ratio = 600), [pGlu^6,Pro^9]SP_{6-11} can be used to specifically desensitize the muscular receptor of this tissue. This procedure enables a selective and sensitive bioassay of the neuronal receptor.

Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) is an undecapeptide that acts as a neurotransmitter/modulator in the mammalian peripheral and central nervous systems.[1,2] Substance P is part of a family of peptides known as tachykinins that share the common C-terminal sequence Phe-X-Gly-Leu-Met-NH_2.[3] This family includes the peptides eledoisin, physalaemin, and kassinin, which are all of nonmammalian origin.[3] Recently, two additional mammalian tachykinins were isolated and termed neurokinin A and neurokinin B.[4,5]

Although the tachykinins exert similar biological actions, their rank order of potencies varies between different pharmacological preparations, indicating that there may be more than one type of tachykinin receptor.[6,7] three main types of tachykinin receptors have been suggested to exist in the periphery, the SP-P, SP-E, and SP-N receptors.[7-9] While on SP-P systems such as the guinea pig ileum and the rat parotid[8-10] all tachykinins have similar potencies, SP-E systems such as the urinary bladder of hamster and mouse and the rat duodenum are characterized by a much higher potency of eledoisin, kassinin, neurokinin A, and neurokinin B than substance P and physalaemin.[8,9,11] In addition to the classical SP-P re-

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(1) Nicoll, R. A.; Schenker, C.; Leeman, S. E. *Annu. Rev. Neurosci.* 1980, *3*, 227.
(2) Pernow, B. *Pharmacol. Rev.* 1983, *35*, 85.
(3) Erspamer, V. *Trends Neurosci.* 1981, *4*, 267.
(4) Kimura, S.; Okada, M.; Sugita, Y.; Kanazawa, I.; Munekata, E. *Proc. Jpn. Acad. 59 Ser. B* 1983, 101.
(5) Kangawa, K.; Minamino, N.; Fukuda, A.; Matsuo, H. *Biochem. Biophys. Res. Commun.* 1983, *114*, 533.
(6) Falconieri-Erspamer, G.; Erspamer, V.; Piccinelli, D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1980, *311*, 61.
(7) Lee, C. M.; Iversen, L. L.; Hanley, M. R.; Sandberg, B. E. B. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1982, *318*, 281.
(8) Watson, S. P.; Sandberg, B. E. B.; Hanley, M. R.; Iversen, L. L. *Eur. J. Pharmacol.* 1983, *87*, 77.
(9) Laufer, R.; Wormser, U.; Friedman, Z. Y.; Gilon, C.; Chorev, M.; Selinger, Z. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(10) Hanley, M. R.; Lee, C. M.; Jones, L. M.; Michell, R. M. *Mol. Pharmacol.* 1980, *18*, 78.
(11) Nawa, H.; Doteuchi, M.; Igano, K.; Inouye, K.; Nakanishi, S. *Life Sci.* 1984, *34*, 1153.

0022-2623/86/1829-1284$01.50/0