Synthesis of Peptides by the Solid-Phase Method. 7. Substance P and Analogues[1]

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Substance P and 21 related peptides containing isosteric or isofunctional groups were prepared by the solid-phase method. After purification by gel filtration and ion-exchange chromatography, the compounds were characterized by thin-layer chromatography, paper electrophoresis, and amino acid and elemental analysis. The biological activities of the peptides were evaluated in vitro on the guinea pig ileum, the rabbit mesenteric vein, and the dog common carotid artery and in vivo on the rat blood pressure. It is shown that the replacement of some residues in the undecapeptide substance P causes variable losses of apparent affinity with a little or no change in the intrinsic activity. All the analogues used in the present study were found to be inactive as antagonists.

Since the elucidation of the amino acid sequence of substance P (SP),[3] a few structure–activity studies[4a-c,5] have been performed to pinpoint the residues that are essential for the biological activity of this peptide. It has been shown that the amidation of the terminal carboxyl function is essential for affinity[6] and that the aromatic residues Phe[7] and Phe[8] might play an important role in the activity of the peptide.[7,8] Methionine in position 11 also seems implicated either in binding or in stimulation of receptors.[9] Our recent results[10,11] with SP-COOH and Ala analogues obtained by simple substitution of each amino acid with L-alanine support this interpretation. Moreover, we noticed a possible participation of Leu[10] in the biological action of SP, which is well reflected by the marked decrease in affinity of [Ala[10]]SP in all pharmacological preparations.[11]

In the present experiment we have studied more precisely positions 7, 8, 10, and 11 by synthesizing a series of analogues containing isosteric or isofunctional residues. We have also verified the effect of incorporating a D-amino acid in position 9, as previously suggested,[12] in order to check the antagonistic activity of such analogues.

Results and Discussion

Synthesis of the Analogues. All the peptides were synthesized by the solid-phase method, using a Beckman Model 990B synthesizer, according to procedures previously described.[10] The C-terminal amino acid was coupled to the benzhydrylamine resin[14] via the preformed symmetrical anhydride method[15] or to the hydroxymethylated polystyrene–divinylbenzene copolymer,[16] according to Wang.[17] All subsequent amino acid derivatives, including Boc-Gln-OH, were added to the peptide via the preformed symmetrical anhydride coupling method; DMF–CH_2Cl_2 (2:10) was used as a solvent for most derivatives, except Boc-Gln and Boc-Arg(Tos), for which pure DMF was used. Side-chain protection for trifunctional Boc-substituted amino acids used in this work was the following: Arg(Tos)-OH and Lys(2-ClZ)-OH; the L- or D-Trp side chain was left unprotected. DL-Methionine (1%, w/v) was added to the deprotection reagent (F_3AcOH–CH_2Cl_2, 4:6, v/v), which prevented most of the oxidation and alkylation of the sulfur atom of methionine and of the indole moiety when tryptophan was incorporated in the peptide.[18] HF cleavage of the completed peptides were carried out for 0.5 h at −20 °C and for an additional 0.5 h at 0 °C in a mixture of HF distilled over CoF_3–anisole (20:80, v/v) and DL-methionine (1%, w/v). After extraction from the resin and lyophilization, the analogues were purified by gel filtration on Bio-Gel P4, followed by ion-exchange chromatography on Sephadex C-25. In certain cases (tryptophan-containing peptides and [D-Ala[8],Phe[10]]SP) a third step of purification on Whatman CM-23 resin (fibrous form) was needed. All peptides were found homogeneous by TLC, paper electrophoresis, and amino acid and elemental analysis (Table I).

Structure–Activity Studies. The analogues of substance P described above have been tested in four pharmacological preparations, an in vivo assay, the rat blood pressure, and three in vitro smooth-muscle preparations: the dog carotid artery,[28] the rabbit mesenteric vein,[26] and the currently used guinea pig ileum.[29,30]

The results of the biological assays are summarized in Table II, by showing the relative (substance P being 100%)

(1) Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commision on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1971)]. Other abbreviations used are: SP, substance P; F_3AcOH, trifluoroacetic acid; DEA, diisopropylethylamine; DMF, dimethylformamide; 2-ClZ, 2-chloro-carbobenzoxy; Cha, cyclohexylalanine.

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potency of the 21 analogues as hypotensive agents on the blood pressure of the anesthetized rat and as inhibitors of the dog carotid artery contracted with noradrenaline or as stimulants of the rabbit mesenteric vein and of the guinea pig ileum. In the three in vitro preparations, both the maximum effect (intrinsic activity, indicated by α^E) and the apparent affinity (expressed in terms of pD_2 values) have been measured.

As shown in Table II, the replacement of the natural aromatic residue Phe[7] is accompanied by a variable loss of apparent affinities with little (rabbit mesenteric vein) or no change (dog carotid artery, guinea pig ileum) in the intrinsic activity. A fairly good affinity, superior to 20% in vivo and to 5% in the in vitro preparations, is found for analogues in which Phe is replaced with aromatic [Tyr(Me), Trp] or nonaromatic (Cha) residues, while the affinity is drastically reduced when Leu or D-amino acids are used. No correlations can be made concerning those results on the basis of the hydrophobicity or size of the side chain of the residue at position 7. However, the length of the side chain seems important for an optimum peptide–receptor interaction. Moreover, the absolute configuration of the residue in position 7 appears to be critical, because the replacement of Phe[7] with D residue drastically reduces the peptide activity. This effect might be the result of a change in the spacial arrangement of the side chain or in the conformation of the whole peptide.

This last conclusion is also applicable to position 8, whereby D residues are also not tolerated, while Phe[8] can be replaced by aromatic [Tyr(Me)] or nonaromatic (Cha) residues and by an amino acid containing a neutral branched side chain (Ile) without loss of affinity. The elimination of the two aromatic residues (as in [Leu[7],Leu[8]]SP) is accompanied by a very important decrease of affinity. A few double substitutions have been made in positions 8 and 10 or 8 and 9 in order to explore the effect of shifting the aromatic group of Phe[8] toward the C terminal. The effect has been a drastic reduction of affinity. The same results have been obtained with the use of D residues to replace Gly. This effect is, however, to be seen only with D-amino acids, since [Ala[9]]SP[11] and [Pro[9]]SP[6] retain a good activity.

Substitutions in position 11 have been made in the four last compounds (18–21 of Table II) to elucidate the importance of the C-terminal residue. The spacial orientation of Met[11] is important, since [D-Met[11]]SP is a weak agonist. The size of the C-terminal side chain is critical,[11] and the sulfur atom is not essential, since [Nle[11]]SP retains a good affinity. Finally, the presence of a C-terminal free carboxyl is not tolerated, amidation or methylation being required to maintain affinity.

In summary, our results with SP-COOH confirm the findings of Mroz and Leeman[6] that the C-terminal carboxyl of SP has to be amidated; those with [Nle[11]]SP support the conclusions of other workers that Met[11] can be replaced with the isosteric amino acid Nle without[3,31] or with very little (present results) reduction of activity. The decrease in affinity of analogues in which Phe[7] was replaced is more important than that of similar analogues in which the substitution was made in position 8. This confirms the conclusions of the study by Yamaguchi et al.[12] with D-Phe analogues. Results obtained in the present study with [Ile[8]]SP confirm previous finding by Rackur et al.,[13] and those obtained with [D-Leu[8]]SP are in agreement with the data reported by Yamaguchi.[12] [D-Leu[9]]SP and [D-Leu[8],D-Phe[9]]SP were synthesized and tested in order to determine if the substitution of Gly[9] by a D-amino acid confers to the peptide antagonistic property in a similar way as it has been observed with LH-RH.[12] Moreover, [D-Leu[8],D-Phe[9]]SP has been found to act as an antagonist of SP in the guinea pig ileum by Leban et al.[32]

In the present study, both of these peptides were carefully tested as antagonists; they were administered a few minutes or just before adding to the bath an average concentration of substance P, in order to avoid the metabolic degradation of these peptides. Both peptides were found to be inactive as antagonists. In a similar way, practically all the analogues of substance P described in Tables I and II did not exert any antagonism against substance P in several pharmacological tests, namely, the rabbit jugular vein and the guinea pig ileum. Finally, results obtained in the present study with [D-Phe[7]]SP confirm the recent findings by Growcott and Petter[33] that this compound is not an antagonist but a full agonist which probably desensitizes the tissue and therefore reduces the activity of Substance P.

The results presented in this paper indicate that the replacement of Phe[7], Phe[8], Gly[9], or Met[11] with a D residue produces variable changes (generally decrease) of affinity but does not yield any antagonist of substance P.

Experimental Section

Reagents and Solvents. Boc-protected amino acids were purchased from Bachem Fine Chemicals Inc., and their purity was carefully checked before use. The benzhydrylamine resin (0.27 mequiv/g) and the hydroxymethylated resin (copolystyrene–divinylbenzene 1%, 0.5 mequiv/g) were also obtained from Bachem. Reagent grade solvents were bought from Fisher Scientific and Anachemia and purified prior to use: methylene chloride was distilled from sodium carbonate, dioxane from sodium, and DMF from ninhydrin, in vacuo, after shaking with KOH pellets. Methylene chloride was stored over Linde type 4A molecular sieves and dioxane over alumina. F_3AcOH (Halocarbons) was distilled from calcium sulfate and DEA (Aldrich) from ninhydrin. DCC was obtained from Chemical Dynamics and used without further purifications, after testing its solubility in ethyl ether.

Urea was purchased from Matheson, Coleman and Bell, and Bio-Gel P4 resin was obtained from Bio-Rad Laboratories. Ion-exchange resins were obtained from Pharmacia Fine Chemicals (Sephadex C-25) and BDH Chemicals (Whatman CM-23).

Peptide Synthesis. A. Protected Peptide-Resin. C-terminal Boc-protected amino acid was coupled to the benzhydrylamine resin[14] (0.27 mequiv/g) via the preformed symmetrical anhydride procedure[15] after neutralization of the resin with 25% triethylamine–CH_2Cl_2. Boc-protected methionine was also esterified to hydroxymethylated resin[16] (0.50 mequiv/g) according to Wang's procedure,[17] and the extent of attachment (0.36 mequiv/g) was measured by the picric acid method.[19] The excess hydroxyl groups were acetylated to avoid further esterification during coupling.

B. Solid-Phase Peptide Synthesis. Peptide syntheses were carried out with a Beckman Model 990B peptide synthesizer in the automatic mode. *N*-α-Boc protection was used for all amino acids, and the side chains of Arg and Lys were respectively protected by tosyl (Tos) and 2-chlorobenzyloxycarbonyl (2-ClZ). Solutions of symmetrical anhydrides were prepared as previously described[20] and poured into their respective reservoirs on the synthesizer. DMF (20%; v/v) was added, and the Boc-protected amino acid symmetrical anhydride solutions were kept at −20 °C by means of a Lauda circulating bath Model RC-3.

The 0.5-mmol scale syntheses (1.9 g of benhydrylamine resin; 1.4 g of hydroxymethylated resin) were generally performed and the operations were carried out as previously described.[10] Every coupling step was checked using the ninhydrin test.[21] After the

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Table I. Characterization of the Analogues of SP by TLC, Paper Electrophoresis, Amino Acid Analysis, and Elemental Analysis

no.	compd	yield,[a] %	electrophoresis[b] R_f(Arg)	TLC (R_f) BPAW[c]	MPAW[d]	EPAW[e]	amino acid analysis[f] Arg	Pro	Lys	Glu	Phe	Gly	Leu	Met	Cha	Ile	Ala	Nle	Trp	Tyr	formula[g]	H_2O	M_r
	SP	30	0.81	0.57	0.64	0.54	0.98	2.13	0.97	2.16	2.09	1.00	1.11	1.04							$C_{69}H_{110}O_{19}N_{18}S$	7	1526
1	[D-Phe^7]SP	23	0.81	0.57	0.64	0.53	1.16	2.12	1.02	1.83	1.76	1.00	1.11	1.00							$C_{69}H_{110}O_{19}N_{18}S$	8	1526
2	[Tyr(Me)^7]SP	21	0.79	0.59	0.64	0.57	0.98	2.07	0.99	1.97	1.04	1.00	0.96	1.04						0.92	$C_{70}H_{112}O_{20}N_{18}S$	8	1556
3	[Trp^7]SP	10	0.77	0.57	0.66	0.54	*h*														$C_{71}H_{111}O_{19}N_{19}S$	2.5	1567
4	[D-Trp^7]SP	11	0.77	0.58	0.66	0.56	*h*														$C_{71}H_{111}O_{19}N_{19}S$	2.5	1567
5	[Cha^7]SP	22	0.73	0.58	0.66	0.55	1.10	2.00	1.02	1.74	1.06	1.00	0.95	1.04	0.99						$C_{69}H_{116}O_{19}N_{18}S$	7	1534
6	[Leu^7]SP	25	0.74	0.58	0.58	0.50	1.12	1.91	1.02	1.89	1.10	1.00	1.81	0.95							$C_{66}H_{112}O_{19}N_{18}S$	8	1494
7	[D-Phe^8]SP	26	0.80	0.57	0.64	0.55	1.07	2.03	0.84	2.09	2.24	1.00	0.89	0.86							$C_{69}H_{110}O_{19}N_{18}S$	6	1526
8	[Tyr(Me)^8]SP	28	0.78	0.58	0.65	0.57	0.95	1.95	1.03	2.08	1.08	1.00	1.01	0.90							$C_{70}H_{112}O_{20}N_{18}S$	7	1556
9	[D-Trp^8]SP	19	0.78	0.57	0.67	0.53	*h*														$C_{71}H_{111}O_{19}N_{19}S$	3	1567
10	[Cha^8]SP	27	0.74	0.58	0.65	0.55	0.92	2.00	1.09	1.95	1.10	1.00	0.94	1.05	0.97						$C_{69}H_{116}O_{19}N_{18}S$	8	1534
11	[Leu^8]SP	30	0.74	0.57	0.64	0.51	1.11	2.08	0.96	1.93	1.02	1.00	1.96	0.94							$C_{66}H_{112}O_{19}N_{18}S$	8	1494
12	[Ile^8]SP	22	0.80	0.55	0.63	0.47	1.14	2.08	0.83	2.09	1.12	1.00	1.00	0.82		0.98					$C_{66}H_{112}O_{19}N_{18}S$	4	1494
13	[Leu^7,Leu^8]SP	26	0.68	0.56	0.60	0.51	0.94	1.93	1.07	2.08		1.00	3.07	0.93							$C_{63}H_{114}O_{19}N_{18}S$	3	1460
14	[Gly^8,Phe^{10}]SP	30	0.78	0.52	0.52	0.45	0.99	1.75	0.89	2.03	2.15	2.00		1.14							$C_{65}H_{102}O_{19}N_{18}S$	6	1472
15	[D-Ala^8,Phe^{10}]SP	20	0.80	0.54	0.42	0.48	0.89	2.14	0.95	2.06	1.74	1.00		1.09			1.18				$C_{66}H_{104}O_{19}N_{18}S$	4	1486
16	[D-Leu^8,D-Phe^9]SP	28	0.83	0.60	0.65	0.57	1.05	1.92	1.00	1.92	2.09		1.96	1.03							$C_{75}H_{118}O_{19}N_{18}S$	6	1608
17	[D-Leu^9]SP	32	0.83	0.61	0.68	0.62	0.99	2.40	0.98	2.10	2.14		2.00	0.82							$C_{73}H_{118}O_{19}N_{18}S$	3	1583
18	[D-Met^{11}]SP	23	0.81	0.57	0.65	0.54	1.02	2.00	0.98	1.97	2.04	1.00	1.02	0.95							$C_{69}H_{110}O_{19}N_{18}S$	7	1526
19	[Nle^{11}]SP	25	0.81	0.60	0.65	0.58	0.95	2.09	1.02	1.98	1.94	1.00	1.05	-				1.00			$C_{70}H_{112}O_{19}N_{18}S$	10	1510
20	SP-COOMe	25	0.86	0.58	0.69	0.56	1.04	1.92	1.03	1.94	1.97	1.00	1.07	1.03							$C_{70}H_{111}O_{20}N_{17}S$	8	1543
21	SP-COOH	21	0.75	0.46	0.57	0.47	0.99	2.02	0.95	2.04	1.99	1.00	0.96	1.10							$C_{69}H_{109}O_{20}N_{17}S$	5	1529

[a] Yield of final product obtained after chromatography, based on the total quantity of C-terminal amino acid attached on the resin before the synthesis. [b] Paper electrophoresis in acetic–formic acid solution (pH 2.1) using Arg·HCl as a standard. [c] 1-Butanol–pyridine–acetic acid–water (30:24:6:20, v/v). [d] Methyl ethyl ketone–pyridine–acetic acid–water (30:15:5:15, v/v). [e] Ethyl acetate–pyridine–acetic acid–water (5:5:1:3). [f] Amino acid analysis from acid hydrolysates (distilled 6 N HCl from Pierce Chemical Co. at 110 °C in evacuated sealed tubes for 20 h). [g] All peptides are triacetate salts. [h] Not determined.

Table II. Primary Structures and Biological Activities of Analogues of Substance P (SP)[a]

no.	compd	primary structure 1 2 3 4 5 6 7 8 9 10 11	rat blood pressure RP	*n*	dog carotid artery α^E	pD_2	RA	*n*	rabbit mesenteric vein α^E	pD_2	RA	*n*	guinea pig ileum α^E	pD_2	RA	*n*
	SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- Phe- Gly- Leu-Met- NH_2	100.0	50	1.0	10.00	100.0	21	1.0	7.55	100.0	36	1.0	8.78	100.0	60
1	[D-Phe^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-D-*Phe*- Phe- Gly- Leu-Met- NH_2	9.7	12	1.0	8.93	8.6	10	0.9	6.14	3.9	12	1.0	7.68	8.0	15
2	[Tyr(Me)^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-*Tyr-Me*-Phe- Gly- Leu-Met- NH_2	31.0	8					0.8	6.87	20.5	10	1.0	8.27	31.0	17
3	[L-Trp^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-L-*Trp*- Phe- Gly- Leu-Met- NH_2	51.0	7	1.0	9.56	37.1	12		6.55	10.0	6	1.0	8.38	39.7	6
4	[D-Trp^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-D-*Trp*- Phe- Gly- Leu-Met- NH_2	0.2	7	1.0	7.33	0.2	10			<0.1	6	1.0	5.90	0.2	12
5	[Cha^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-*Cha*- Phe- Gly- Leu-Met- NH_2	21.0	8	1.0	8.80	6.4	10	0.8	6.66	13.2	11	1.0	8.08	20.0	10
6	[Leu^7]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-*Leu*- Phe- Gly- Leu-Met- NH_2	0.8	6	1.0	7.64	0.4	9		5.66	1.3	11	1.0	7.15	2.3	12
7	[D-Phe^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- D-*Phe*- Gly- Leu-Met- NH_2	2.0	8						5.17	0.4	10	1.0	7.15	2.3	11
8	[Tyr(Me)^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- *Tyr-OMe*-Gly- Leu-Met- NH_2	100.0	8	1.0	9.98	95.0	9	1.0	7.55	100.0	10	1.0	8.78	100.0	6
9	[D-Trp^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- D-*Trp*- Gly- Leu-Met- NH_2	0.8	6	1.0	7.12	0.2	9		4.92	0.3	7	1.0	6.68	0.8	8
10	[Cha^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- *Cha*- Gly- Leu-Met- NH_2	100.0	6					1.0	7.55	100.0	10	1.0	8.78	100.0	14
11	[Leu^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe- *Leu*- Gly- Leu-Met- NH_2	70.0	13	1.0	9.81	64.0	9	0.8	6.92	23.6	10	1.0	8.61	68.0	6
12	[Ile^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe *Ile*- Gly- Leu-Met- NH_2	100.0	8					1.0	7.55	100.0	8	1.0	8.78	100.0	8
13	[Leu^7,Leu^8]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Leu- *Leu*- Gly- Leu-Met- NH_2	0.2	8	1.0	6.38	<0.1	8			<0.01	8		5.29	<0.1	4

14	[Gly^8,Phe^{10}]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	*Gly-*	Gly-	*Phe*-Met-	NH_2	0.07	8	1.0	7.33	0.2	10			<0.1	4		5.31	<0.1	8
15	[D-Ala^8,Phe^{10}]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	D-*Ala*-	Gly-	*Phe*-Met-	NH_2	0.09	8	1.0	8.15	1.4	10			<0.1	4		4.73	<0.01	7
16	[D-Leu^8,D-Phe^9]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	D-*Leu*-	D-*Phe*-Leu-Met-		NH_2	1.3	4	1.0	7.89	0.8	10			<0.01	4		6.83	1.2	10
17	[D-Leu^9]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	*Phe*-	D-*Leu*-Leu-Met-		NH_2	0.7	8	1.0	7.49	0.3	8	5.11		0.4	6	1.0	7.21	2.7	8
18	[D-Met^{11}]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	Phe-	Gly-	Leu-D-*Met*-	NH_2	9.0	8	1.0	8.23	1.7	10	6.48	0.7	8.5	10	1.0	7.30	3.3	7
19	[Nle^{11}]SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	Phe-	Gly-	Leu-*Nle*-	NH_2	63.0	8	1.0	9.76	57.3	11	7.09	1.0	34.6	16	1.0	8.49	50.0	8
20	SP-COOMe	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	Phe-	Gly-	Leu-Met-	*COOMe*	69.0	6	1.0	9.19	15.5	9	6.95	1.0	25.1	9	1.0	8.47	49.0	7
21	SP-COOH	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-	Phe-	Gly-	Leu-Met-	*COOH*	0.7	5					5.35	1.0	0.6	6	1.0	6.69	0.8	5

[a] α^E = intrinsic activity; pD_2 = −log of the concentration of peptide producing 50% of the maximum effect; RP = relative potency, substance P = 100; RA = relative affinity; n = number of determinations.

resin is properly washed by the alternate use of CH_2Cl_2 and 2-propanol, the synthesizer is ready to proceed automatically to the coupling of the next amino acid.

C. Cleavage of the Peptides from the Support. After the last amino acid was introduced, the deprotection program[10] was executed and the synthesizer stopped. The free N-terminal peptide resin was dried overnight in vacuo and placed in the reaction vessel of a liquid HF apparatus (Protein Research Foundation). For a 2-g scale cleavage, 12 mL of HF distilled from CoF_3 was added to the vessel already containing anisole (3 mL) and DL-methionine (0.2 g). The cleavage reaction proceeded for 30 min at −20 °C and at 0 °C for another 30 min. HF was rapidly evaporated in vacuo and the resin was washed with ether. The peptide was extracted with 30% acetic acid (ca. 200 mL), and the solution was lyophilized. Identical cleavage procedure was used for SP on hydroxymethylated resin; protected SP-COOMe was obtained from the same resin by alcoholysis as described by Stewart and Young.[22] We have modified the technique by adding 30% of CH_2Cl_2 to the transesterification mixture in order to ensure proper swelling of the resin.

D. Purification of the Peptides. All peptides were purified as described earlier[10] by successive use of gel filtration on Bio-Gel P4 resin equilibrated with 6 M urea–10% acetic acid and Sephadex C-25 carboxymethyl ion-exchange resin in ammonium acetate buffers. When a third step of purification was necessary (for instance, Trp-containing peptides and [D-Ala^8,Phe^{10}]SP), the weakly substituted ion-exchange resin Whatman CM-23 (1.5 × 8 cm) was used with a linear gradient of ammonium acetate (0.005 to 0.10 M) at pH 5.0. The flow rate was maintained at 700–1000 mL/h, and the peptide was eluted as a symmetrical peak (conductivity = 6.5 mmhos). This peak was collected and lyophilized several times to remove volatile salts. Overall yields for the analogues of SP, based on the quantity of C-terminal amino acid originally attached to the resin, varied between 10 and 32%.

Characterization of the Peptides. Homogeneity of the peptides was demonstrated by TLC on 0.25-mm-thick Merck chromatogram plates (F-254 with fluorescent indicator) in the solvent systems BPAW (1-butanol–pyridine–acetic acid–water, 30:24:6:20), EPAW (ethyl acetate–pyridine–acetic acid–water, 5:5:1:3), and MPAW (methyl ethyl ketone–pyridine–acetic acid–water, 30:15:5:15). The spots were revealed by spraying with a solution of ninhydrin in ethanol (1%) or with the Reindel–Hoppe reagent.[23] Paper electrophoresis were carried out with a Beckman Model R apparatus, using Whatman 3MM paper sheets at 450 V for 45 min at ambient temperature, in acetic–formic acids solution (pH 2.1); Arg·HCl was used as a standard. Amino acid analyses were performed with peptide hydrolysates using a Beckman automatic amino acid analyser, and elemental analyses (C, N, and H) were carried out by Galbraith Laboratories, (Knoxville, TN). All characterization data are given in Table I.

Determination of Biological Activity. The biological experiments were performed using female Wistar rats purchased from Canadian Breeding Farms (St. Constant, Quebec), and rabbits, guinea pigs, and dogs were obtained from local breeders. Rabbits and guinea pigs were killed by stunning and exsanguination; dogs were first anesthetized with sodium pentobarbital (50 mg/kg, ip) and then killed by exsanguination. The in vivo experiments were carried out on rats weighing 200–250 g. The animals were anesthetized with urethane (1.4 g/kg, sc), and the peptides were injected into the jugular vein. Blood pressure was recorded directly through a cannula inserted into the right carotid artery and with a mercury manometer on a smoked drum driven by a Palmer kymograph, as described earlier.[24]

Helicoidal strips[25,26] of the anterior mesenteric vein of the rabbit (1.5–2 kg, both sexes) or of the dog common carotid artery (dogs 9–15 kg, both sexes) and longitudinal strips[27] of the ileum of guinea

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pigs (200–400 g, both sexes) were suspended in 10-mL organ baths containing oxygenated (95% O_2, 5% CO_2) warm (37 °C) Krebs solution. For the bioassays with the dog carotid artery, EDTA (59.5 μM) and indomethacin (2.8 μM) were added to the Krebs' solution, and the preparation was contracted with noradrenaline (4.4×10^{-6} M) in order to enable the measurement of the relaxant effect of substance P.[28]

The tissues were stretched with 0.5 g (vein and ileum strips) and with 2 g (carotid artery strips), and the changes of tension were recorded isometrically with Grass (FT .03C) force transducers connected to a Grass polygraph (Model 7D). Concentration–response curves to SP and its analogues were measured by consecutive additions of drug solutions (0.05–0.1 ml) to the bath fluid after an initial equilibration period of 60 min for the ileum and the vein and 120 min for the carotid artery. The arteries were contracted (1.0-g tension) with noradrenaline and cumulative dose–response curves were measured for SP and its analogues.

For more details concerning some of the pharmacological preparations, the reader is referred to recent publications.[26,28]

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2,3-Dihydro and Carbocyclic Analogues of Tryptamines: Interaction with Serotonin Receptors

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Several dihydro and carbocyclic analogues of tryptamine were evaluated in order to determine the role of the heterocyclic portion of the indole nucleus on the interaction of indolealkylamines with the serotonin receptors of the rat fundus. Reduction of the C_2–C_3 double bond or replacement of the indole nitrogen with an sp^3-hybridized carbon atom results in a 50% decrease in receptor affinity. Complete removal of the five-membered ring of *N,N*-dimethyltryptamine reduces affinity by an order of magnitude. It appears that an intact indole nucleus, though not entirely necessary, results in an optimal receptor interaction for the indolealkylamines examined.

Recent reports from this laboratory have outlined our findings with respect to the affinity of indolealkylamines for the serotonin (5-hydroxytryptamine, 5-HT) receptors of the rat fundus preparation.[1,2] Not only have structure–activity relationship been formulated,[2] but the interaction has been demonstrated to be a stereoselective event.[3] Phenylalkylamines also interact with these 5-HT receptors, and certain phenyl-substituted derivatives possess an affinity greater than that of some indolealkylamines.[1,4] Thus, it appears that the presence of a heterocyclic ring is not an essential feature for these drug–5-HT receptor interactions. The aim of this present study was to examine the influence of molecular modification in the heterocyclic portion of the indole nucleus and thereby determine the extent to which this portion of the ring system contributes to indolealkylamine–5-HT receptor interactions.

Chemistry. 2,3-Dihydro-*N',N'*-dimethyltryptamine (**3a**) was synthesized by reduction of 3-indole-*N',N'*-dimethylglyoxamide with BH_3·THF, essentially according to the method of Littell and Allen.[5] The 5-methoxy derivative **3b** was prepared by an adaptation of the above method; the 5-methoxy glyoxamide was reduced to the indole analogue with BH_3·THF, and further reduction to the indoline was effected in situ by the addition of CF_3C-OOH, after the method of Maryanoff and McComsey.[6] The tetrahydroquinoline derivatives **9** and **10** were prepared by acyloxyborohydride reduction of the corresponding aminoquinoline by the method of Gribble and Heald.[7]

Results and Discussion

The apparent 5-HT receptor affinity of the compounds in Table I are reported as pA_2 values. Using the equation $pA_x = -\log K_2 - \log (x - 1)$, where x is the dose ratio, pA_2 is equivalent to $-\log K_2$.[20] Thus, when $\log (x - 1)$ is plotted against pA_x, a straight line results with a slope of $-n$. This line intersects the pA_x axis at a point corresponding to pA_2, and the interaction is assumed to be competitive when $-n$ is approximately 1.[20]

Based on the slopes of their Schild plots, compounds **1a–d**, **2a**, **3a,b**, **4a–d**, and **5–8** interact with the 5-HT receptors of the rat fundus in a competitive manner. Comparing the affinity (pA_2) of *N',N'*-dimethyltryptamine (DMT, **1c**) or its 5-methoxy derivative (5-OMe-DMT, **1d**) with that of the corresponding 2,3-dihydro derivatives **3a** and **3b**, respectively, reveals that reduction of the "pyrrolic" or 2,3 bond halves affinity. We have previously reported that replacement of the indole nitrogen atom of DMT with an sp^3-hybridized carbon atom (i.e., comparing **1c** with **2a**) results in a 2-fold decrease in affinity;[2] the

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