morphine-induced circling behavior in rats with monolateral lesion of nigrostriatal tract as described by Ungerstedt.[22] Male Sprague–Dawley rats weighing 140–180 g at the beginning of the experiments were anesthetized with sodium pentobarbital (40 mg/kg ip) and placed in a David Kopf stereotaxic frame. Chemical denervation was induced by slow infusion of 6-OH-DOPA (8 μM/5 μL/5 min) directly into substantia nigra. Coordinates were taken from ref 29. Ten days after surgery all the animals were checked for their sensitivity to dopaminergic stimulation by measuring the number of revolution/60 min occurring after the subcutaneous injection of 2 mg/kg apomorphine. For further circling studies, only those responding with, at least, 200 turns/60 min after apomorphine injection were used. Approximately 40% of the operated animals failed at this criterion and were discarded. Rotational behavior was evaluated by a rotamer apparatus. The circling behavior was measured as the number of turns performed by the animal every 5 min after apomorphine injection and the extent of circling was recorded for 60 min. Each compound was administered subcutaneously 10 min before apomorphine injection. The results are expressed as the inhibitory dose that produces 50% of inhibition of rotational behavior induced by apomorphine for 60 min.

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Effect of Reductive Alkylation of Lysine in Positions 6 and/or 8 on the Histamine-Releasing Activity of Luteinizing Hormone-Releasing Hormone Antagonists[1]

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The solid-phase reductive alkylation of the Lys side chain in positions 6 (D) and 8 (L) and position 8 alone of the LH-RH antagonist [*N*-Ac-D-Nal[1],D-Ph[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH was investigated in an attempt to reduce the histamine-releasing activity inherent to most potent antagonists while retaining high antiovulatory activity. The protected parent analogues were prepared by conventional solid-phase peptide synthesis. After selective removal of the Lys Fmoc side chain protection, the resin-bound peptides were readily and conveniently alkylated at the ε amino groups with various aldehydes and ketones in the presence of $NaBH_3CN$. The analogues were then cleaved from the resin with simultaneous deprotection by anhydrous hydrogen fluoride and purified to homogeneity in two stages: gel permeation followed by preparative reversed-phase liquid chromatography. The analogues were assayed in standard rat antiovulatory and in vitro histamine-release assays.

Two aims for the synthesis of antagonists of luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Tyr-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,[2] are the control of fertility by the blockade of ovulation and the control of hormone-dependent tumors. Toward these goals, over 1000 analogues have been synthesized, internationally, in the last several years in the search for ever more potent antagonists. Currently, the most active analogues are characterized by distinct hydrophobic and hydrophilic regions and are typified by the antagonist [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH.[3] Recently, however, the antagonists, when injected subcutaneously into rats at 50–100 times the effective antiovulatory dose, have been shown to cause transient edema of the face and extremities.[4] Additionally, many compounds are mast cell secretagogues, release histamine, and are able to induce a cutaneous anaphylactoid-like response in rats, causing a dose-related wheal reaction.[5] Other peptides, most notable those containing several closely spaced basic residues, are also known to cause the release of histamine including substance P, somatostatin, and neurotensin, and the phenomenon is clearly linked to the presence of highly basic Lys and Arg residues.[6–8] In this study of the structure–activity relationship of the histamine-releasing activity of the LH-RH antagonists, we decided to investigate the effects of changing the hydrophobicity and basicity of the basic side chains at positions 8 and 6, and position 8 alone.

Results and Discussion

Chemistry. Since only a limited number of naturally occurring basic amino acids are available commercially, we

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature and Symbols as described in the following: *Eur. J. Biochem.* **1972**, *27*, 201. *J. Biol. Chem.* **1975**, *250*, 3215. Glp, pyroglutamic acid; D-Nal, 3-(2-naphthyl)-D-alanine.

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Table I. Synthesis of N^{ϵ}-Alkylated Lys Derivatives

peptide	amino acid	carbonyl compd	alkylation condtns[a]
	Position 8 Analogues		
II	N^{ϵ}-neopentyl-Lys	trimethylacetaldehyde	1 h
III	N^{ϵ}-isopropyl-Lys	acetone	2 × 1 h
IV	N^{ϵ}-(1-propylbutyl)-Lys	heptan-4-one	2 × 1 h at ~50 °C
V	N^{ϵ}-cyclopentyl-Lys	cyclopentanone	1 h, 16 h
VI	N^{ϵ}-benzyl-Lys	benzaldehyde	4 × 16 h
VII	N^{ϵ}-(4-methylbenzyl)-Lys	4-tolualdehyde	3 × 16 h
VIII	N^{ϵ}-(2,4,6-trimethylbenzyl)-Lys	2,4,6-trimethylbenzaldehyde	2 × 16 h
	Position 6 and 8 Analogues		
X	N^{ϵ}-methyl-Lys	formaldehyde[b]	4 × 1 h
XI	N^{ϵ}-isopropyl-Lys	acetone	1 h
XII	N^{ϵ}-(1-propylbutyl)-Lys	heptan-4-one	2 × 1, 16 h
XIII	N^{ϵ}-(1-butylpentyl)-Lys	nonan-5-one	1 h at ~50 °C
XIV	N^{ϵ}-cyclopentyl-Lys	cyclopentanone	3 × 1 h
XV	N^{ϵ}-cyclohexyl-Lys	cyclohexanone	1 h

[a] Reaction time required to give a negative, or almost negative, Kaiser test. [b] Aqueous formaldehyde solution (37%).

developed a rapid in situ solid-phase procedure in which the exposed side-chain amino group of a dibasic amino acid is reductively alkylated by a carbonyl compound in the presence of sodium cyanoborohydride.[9] By suitable choice of aldehyde or ketone, a range of analogues containing amino acids of varying hydrophobicities and basicities can be produced. We chose to use Lys for the LH-RH analogues since it has a side chain of similar size to Arg and previous studies have shown that shorter basic side chains in position 6 are poorly tolerated.[10] This facile method is an extension of the work of Roeske et al.,[11] in which authentic, side-chain protected N^{ϵ}-isopropyl-Lys was synthesized and incorporated into analogues by conventional solid-phase peptide synthesis.

Lys was incorporated as Boc-Lys(Fmoc) during the solid-phase peptide syntheses on 4-methylbenzhydrylamine functionalized, 1% cross-linked polystyrene resin.[12] Other bifunctional amino acids were masked as follows: Arg(Tos), Tyr(2-BrZ), and Ser(Bzl). After the protected peptide was assembled, the side-chain amino group of Lys was selectively deblocked by treatment with 50% piperidine in dimethylformamide (DMF) for 16 h. This is considerably longer than is required to cleave the N^{α}-Fmoc group, which is much more labile to base.[13] The deblocked, resin-bound peptides were suspended in DMF containing 1% acetic acid, and the exposed ϵ amino group was then subjected to reductive alkylation with a variety of ketones and aldehydes, listed in Table I. An excess of the carbonyl compound was added to form an intermediate Schiff base, which was reduced to the desired N^{ϵ}-alkylated Lys derivative by the addition of $NaBH_3CN$.

The alkyl aldehydes and the smaller ketones reacted readily with Lys in position 8 alone and gave very weak rose colored Kaiser ninhydrin tests.[15] The larger ketones were less reactive and required gentle heating or extended reaction times to achieve weak Kaiser tests. The aryl aldehydes were much less reactive since the Schiff bases are acid sensitive and required reaction in neat DMF for extended periods. This lability is reduced by the presence of electron-donating groups such that 2,4,6-trimethylbenzaldehyde reacted in the presence of 1% acetic acid.

Similarly, with the double substituted peptide containing Lys in position 8 and D-Lys in position 6, the smaller carbonyl compounds reacted readily, with the larger ketones requiring gentle heating. However, many bulky carbonyl compounds gave positive Kaiser tests or heterogeneous products even with extended reaction times or elevated temperatures, including trimethylacetaldehyde, dicyclopropyl ketone, tridecan-7-one, nonadecan-10-one, and (*N*,*N*-diethylamino)butan-3-one.

The alkylated peptides were cleaved from the support, with simultaneous side-chain deprotection, by acidolysis in anhydrous hydrogen fluoride. The crude materials were then desalted by gel filtration. Most peptides were purified to homogeneity, as judged by thin-layer chromatography (TLC) and analytical reversed-phase high-performance liquid chromatography (RP-HPLC) (see Table II), by a single elution from a preparative C_{18}, reversed-phase column. The exceptions were peptides IV, X, XII, and XIII, which required two elutions to achieve homogeneity. All peptides gave acceptable amino acid analyses, within ±10% of the theoretical values for all the amino acids (see Table III). The alkylated amino acids gave rise to single, nonstandard peaks and were calculated with the Leu color value. The exceptions were N^{ϵ}-neopentyl-Lys and N^{ϵ}-methyl-Lys, which coeluted with NH_3 and Nal, respectively, and N^{ϵ}(4-methylbenzyl)Lys and N^{ϵ}-(1-butylpentyl)-Lys, which did not appear to elute even during column regeneration.

Biology. The antagonist [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH was chosen for this study of the effect of histamine-releasing activity and antiovulatory activity (AOA) of the basic side chains in positions 6 and 8 and position 8 alone. This analogue had previously been used by this group in an initial investigation of position 6,[9] had high antiovulatory activity (56 blockade at 0.5 μg), and could be produced in good yield.[3] It was hoped that subtle modifications to the hydrophobicity and basicity of the side chains in positions 6 and 8 or position 8 alone would permit the retention of the desirable high antiovulatory activity while diminishing the histamine-releasing activity of the analogues.

Position 8 Analogues. The parent analogue (I), containing Lys[8], had an ED_{50} of 0.38, a value typical of many current antagonists,[16] and blocked ovulation in 29% of rats

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Table II. Peptide Chromatographic and Purity Data

peptide	HPLC		TLC				
	t_R, min	purity, %	R_f 1	R_f 2	R_f 3	R_f 4	R_f 5
I	14.1	98.3	0.20	0.68	0.58	0.22	0.51
II	15.3	97.8	0.30	0.80	0.65	0.37	0.60
III	14.1	96.2	0.22	0.70	0.58	0.19	0.49
IV	17.9	97.8	0.36	0.82	0.72	0.43	0.65
V	15.7	97.3	0.28	0.75	0.63	0.30	0.57
VI	17.7	97.3	0.48	0.89	0.71	0.43	0.64
VII	19.1	96.3	0.49	0.89	0.75	0.53	0.67
VIII	17.7	95.5	0.36	0.82	0.72	0.44	0.65
IX	16.9	99.3	0.17	0.64	0.58	0.20	0.52
X	14.1	97.3	0.54	0.46	0.41	0.07	0.16
XI	15.0	99.2	0.22	0.72	0.56	0.18	0.44
XII	20.3	98.2	0.54	0.93	0.77	0.55	0.73
XIII	23.5	96.5	0.71	0.97	0.84	0.65	0.76
XIV	15.9	96.8	0.32	0.79	0.65	0.33	0.57
XV	17.4	96.6	0.39	0.86	0.68	0.42	0.61

Table III. Amino Acid Analyses

peptide	Ser	Pro	Ala	Tyr	Phe	Nal	Lys	Arg	X[a]	t_R[b]
I	0.92	1.01	1.00	0.99	2.93	1.03	0.99	0.97		
II	0.93	1.09	1.00	1.00	3.01	1.02		1.03	2.51[c]	63.9
III	0.91	1.01	1.00	0.95	2.72	1.04		1.08	0.98	63.3
IV	0.90	0.99	1.00	1.01	3.01	1.09		0.99	1.07	73.7
V	0.89	1.05	1.00	0.97	2.79	1.07		1.05	1.02	69.0
VI	0.93	0.97	1.00	1.04	2.97	1.01		1.00	1.04	71.0
VII	0.95	1.06	1.00	0.98	2.86	1.10		0.95	*d*	
VIII	0.93	1.00	1.00	1.02	2.96	0.90		0.99	0.91	74.1
IX	0.93	0.94	1.00	0.99	2.88	1.00	1.85			
X	0.90	1.02	1.00	0.94	2.73	3.06[e]			*e*	
XI	0.97	1.07	1.00	0.98	2.72	0.91			1.89	63.3
XII	0.91	1.01	1.00	0.97	3.00	1.00			1.95	71.8
XIII	0.98	0.94	1.00	1.06	3.12	1.09			*d*	
XIV	0.97	1.01	1.00	0.92	2.60	1.06			1.93	68.1
XV	0.96	0.92	1.00	0.96	2.99	1.07			1.91	71.9

[a] All alkylated Lys derivatives calculated as Leu. [b] Retention time in minutes on analyzer. [c] N^{ϵ}-Neopentyl-Lys and ammonia coeluted. [d] Not eluted under conditions of analysis. [e] N^{ϵ}-Methyl-Lys and Nal coeluted.

Table IV. Antiovulatory and Histamine-Releasing Activities of Analogues with the General Formula [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],X[8],D-Ala[10]]LH-RH

peptide	X	antiov act.[a]	in vitro histamine release: ED_{50}[b]
I	Lys	29 at 3 (7)	0.38
II	N^{ϵ}-noepentyl-Lys	89 at 3 (9) 50 at 1 (10)	4.68 ± 0.32
III	N^{ϵ}-isopropyl-Lys	43 at 3 (7)	8.15 ± 1.42
IV	N^{ϵ}-(1-propylbutyl)-Lys	0 at 3 (8)	4.60 ± 0.37
V	N^{ϵ}-cyclopentyl-Lys	50 at 3 (10)	3.74 ± 0.33
VI	N^{ϵ}-benzyl-Lys	5 at 3 (20)	14.17 ± 1.19
VII	N^{ϵ}-(4-methylbenzyl)-Lys	10 at 3 (10)	33.00 ± 2.07
VIII	N^{ϵ}-(2,4,6-trimethylbenzyl)-Lys	10 at 3 (10)	4.73 ± 0.30

[a] Expressed as the percentage of (*n*) rats blocked at a dose of *x* μg. [b] Expressed as the mean ED_{50} ± standard error in units of micrograms/milliliter.

Table V. Antiovulatory and Histamine-Releasing Activities of Analogues with the General Formula [*N*-Ac-D-Nal[1],D-Phe[2,3],D-X[6],Phe[7],X[8],D-Ala[10]]LH-RH

peptide	X	antiov act.[a]	in vitro histamine release: ED_{50}[b]
IX	Lys	60 at 3 (10)	0.30 ± 0.03
X	N^{ϵ}-methyl-Lys	17 at 3 (10)	1.32 ± 0.08
XI	N^{ϵ}-isopropyl-Lys	89 at 3 (9)	6.48 ± 0.15
XII	N^{ϵ}-(1-propylbutyl)-Lys	0 at 3 (10) 56 at 6 (9)	21.92 ± 1.00
XIII	N^{ϵ}-(1-butylpentyl)-Lys	0 at 6 (9)	>300
XIV	N^{ϵ}-cyclopentyl-Lys	70 at 3 (10)	6.10 ± 0.46
XV	N^{ϵ}-cyclohexyl-Lys	38 at 3 (10)	5.86 ± 0.27

[a] Expressed as the percentage of (*n*) rats blocked at a dose of *x* μg. [b] Expressed as the mean ED_{50} ± standard error in units of micrograms/milliliter.

at a dose of 3 μg (see Table IV). The introduction of N^{ϵ}-neopentyl-Lys[8] (II) decreased the histamine-releasing activity 10-fold (ED_{50} = 4.68) and increased the antiovulatory activity slightly (89% at 3 μg). Replacement with N^{ϵ}-isopropyl-Lys caused a further 2-fold increase in ED_{50} and a slight loss in AOA (III, ED_{50} = 8.15, 43% AOA at 3 μg). N^{ϵ}-(1-Propylbutyl)-Lys[8] caused a loss of antiovulatory activity at the 3-μg dose and also had a reduced ED_{50} (IV, ED_{50} = 4.60, 0% at 3 μg). Substitution with N^{ϵ}-cyclopentyl-Lys[8] produced a similar ED_{50} value, but also resulted in a slightly improved AOA (V, ED_{50} = 3.74, 50% AOA at 3 μg). The introduction of N^{ϵ}-benzyl-Lys[8] (VI) was accompanied by a greater than 30-fold increase in ED_{50} relative to peptide I, but the antiovulatory activity was almost abolished (VI, ED_{50} = 14.17, 5% at 3 μg). Para substitution of the aromatic ring with an electron-donating methyl group (VII) caused a doubling of the ED_{50} relative to peptide VI despite an increase in the apparent basicity of the residue. However, the introduction of two additional methyl groups into the aromatic ring caused a drop in the ED_{50} to that of the alkylated derivatives (VII, ED_{50} = 33.00, 10% AOA at 3 μg and VIII, ED_{50} = 4.73, 10% at 3 μg, respectively).

Position 6 and 8 Analogues. The parent analogue IX containing two Lys residues had an AOA and ED_{50} very similar to that of the Lys[8] peptide (IX, ED_{50} = 0.30, 60% at 3 μg, and I, ED_{50} = 0.38, 29% at 3 μg, respectively; see Table V). Substitution with N^{ϵ}-methyl-Lys caused a small

decrease in histamine-releasing activity and a reduction in AOA (X, ED_{50} = 1.32, 17% AOA at 3 μg). The introduction of N^{ϵ}-isopropyl-Lys in positions 6 and 8 (D and L, respectively) restored the antiovulatory activity and also increased the ED_{50} ~20-fold (XI, ED_{50} = 6.48, 89% at 3 μg). Substitution with the higher, branched homologues N^{ϵ}-(1-propylbutyl)-Lys (XII) and N^{ϵ}-(1-butylpentyl)-Lys (XIII) also continued the trend to decreased histamine-releasing activity (XII, ED_{50} = 21.92, 56% AOA at 6 μg; XIII, ED_{50} > 300, 0% at 6 μg). Indeed, the ED_{50} for peptide XIII was estimated as being greater than 1000-fold larger than that for the unalkylated Lys analogue (IX), a remarkable result, considering that LH-RH and the potent agonist [D-Trp^6]LH-RH have ED_{50} = 328 ± 62 and 46 ± 7, respectively. However, this increase in ED_{50} was accompanied by a reduction in the antiovulatory activity and was abolished at the 6-μg dose for peptide XIII. The introduction of the cycloalkylated derivatives (peptides XIV and XV) produced ED_{50}'s closer to that of the N^{ϵ}-isopropyl-Lys analogue (XI) rather than the acyclic analogue of similar chain length (XII). Additionally, the antiovulatory activities were in the same range (XIV, ED_{50} = 6.10, 70% AOA at 3 μg; XV, ED_{50} = 5.86, 38% at 3 μg).

The monosubstitution of Lys derivatives in position 8 and the disubstitutions in 6 and 8 together produced quite different results from the similar, previously reported substitutions in position 6 alone.[9] In each series, the incorporation of underivatized Lys produced analogues with low ED_{50}'s, typical of many present-generation antagonists. The substitution of alkylated Lys derivatives in position 6 had not produced any significant changes in the histamine-releasing activity of the analogues whereas similar substituents in position 8 caused a greater than 10-fold change in ED_{50} (peptides II–V, Table IV). Additionally, the inclusion of arylated Lys substituents caused the greatest increase in ED_{50} in both series; however, this trend was also associated with a reduction in antiovulatory activity. The greatest reduction in histamine-releasing activity occurred in the disubstituted compounds (peptides IX–XV, Table V), with the disubstitution of N^{ϵ}-(1-butylpentyl)-Lys producing an ED_{50} > 300. Unfortunately, these analogues also had the lowest antiovulatory activities of the series, although a final assessment of effectiveness must await time-course studies on inhibition of gonadotropin release.

From these observations one may conclude that the hydrophobicity of the alkylated Lys derivative is of less importance than the flexibility of the alkyl group and thus its ability to shield the charged basic site in the moiety. For analogues of apparently equal side-chain basicities, the presence of flexible alkyl groups confers a lower histamine-releasing activity and antiovulatory activity than constrained alkyl groups of similar size (compare peptides XII and XV). Additionally, the lowest histamine-releasing activities can be obtained, together with the greatest retention of antiovulatory activity, by modifications in position 8 alone or positions 8 and 6 together rather than by changes in position 6 alone.

Experimental Section

Materials. 4-Methylbenzhydrylamine hydrochloride resin[12] (ca. 0.7 mequiv g^{-1}) was obtained from Vega Biotechnologies Inc. Most *tert*-butoxycarbonyl (Boc) protected amino acids were purchased from Bachem Inc. The reactive side chains of the amino acids were masked as follows: Arg, N^{g}-tosyl; Lys, N^{ϵ}-(fluorenylmethoxy)carbonyl; Ser, *O*-benzyl; Tyr, *O*-2-bromobenzyloxycarbonyl. Boc-3-(2-naphthyl)-D-alanine was provided by the Southwest Foundation for Research and Education, San Antonio, TX, through the courtesy of Dr. Marvin Karten, Center for Population Research, Contraceptive Development Branch, National Institutes of Health, Bethesda, MD. All reagents and solvents were ACS grade or better and used without further purification.

Peptide Synthesis. The parent protected peptides [*N*-Ac-D-Nal[1], D-Phe[2,3],Ser(Bzl)[4],Tyr(2-BrZ)[5],D-Arg(Tos)[6],Phe[7],Lys(Fmoc)[5],Pro[9],D-Ala[10]]LH-RH and [*N*-Ac-D-Nal[1],D-Phe[2,3],Ser(Bzl)[4],Tyr(2-BrZ)[5],D-Lys(Fmoc)[6],Phe[7],Lys(Fmoc)[8],Pro[9],D-Ala[10]]-LH-RH were assembled on 4-methylbenzhydrylamine functionalized (0.7 mequiv g^{-1}) 1% cross-linked polystyrene resin. The peptides were synthesized on a 3-mmol scale by utilizing a Vega Model 50 synthesizer and using a modified solid-phase procedure[16,17] to give the resin-bound, protected, acetylated parent peptides.

Side-Chain Modification. The Fmoc side-chain protection was removed from Lys by treatment with 50% piperidine in DMF for 16 h followed by thorough washing and drying under nitrogen. The deprotected resins were split into aliquots (0.2 mmol), and the exposed Lys amino groups were reductively alkylated with an aldehyde or ketone (10 mmol) in the presence of $NaCNBH_3$ (2 mmol) in DMF (25 mL) containing 1% acetic acid at ambient temperature.

Peptide Cleavage. The decapeptides were cleaved from the resin support, with simultaneous side-chain deprotection, by acidolysis using anhydrous hydrogen fluoride containing anisole (~15% v/v) and dithiothreitol (~0.3% w/v) as scavengers for 1 h at 0 °C.

Purification. The crude peptides were subjected initially to gel permeation chromatography on Sephadex G25 (2.5 × 100 cm) with 50% acetic acid eluent. Final purification was effected by preparative RP-HPLC on C_{18} bonded silica gel (Vydac C_{18}, 10–15 μm, 1.0 × 45 cm) eluted with a linear acetonitrile gradient with a constant concentration of trifluoroacetic acid (0.1% v/v). The linear gradient was generated by using a Chromat-a-Trol Model II (Eldex Laboratories Inc.) gradient maker. The separations were monitored at 280 nm, by TLC on silica gel plates (Merck F60) and by analytical RP-HPLC. The fractions containing the product were pooled, concentrated in vacuo, and subjected to filtration. Each peptide was obtained as a fluffy white powder of constant weight by lyophilization from aqueous acetic acid. The purity of the final peptides was assessed by RP-HPLC and TLC in five solvent systems, and the results are given in Table II. Analytical RP-HPLCs were recorded by using a Vydac C_{18} support (4.6 × 250 mm, 5 μm, 30-nm pore size, Liquid Separations Group). Buffer A, 0.1 M triethylammonium phosphate, pH 3, containing 5% acetonitrile; buffer B, 20% buffer A in acetonitrile. A linear gradient of 20–80% B over 30 min was employed for all the analyses at a flow rate of 1.5 mL min^{-1}. Column eluent was monitored at 215 nm. The retention time and purity of each peptide were assessed by an LKB 2220 recording integrator. Each peptide produced only one spot, at a loading of ~10 μg, in each of the following solvent systems when visualized by UV or chlorine/starch–iodide:[16] 1, ethyl acetate–pyridine–acetic acid–water, 10:5:1:3; 2, ethyl acetate–pyridine–acetic acid–water, 5:5:1:3; 3, butan-1-ol–acetic acid–water–ethyl acetate, 1:1:1:1; 4, butan-1-ol–acetic acid–water, 4:1:1; and , propan-2-ol–1 M acetic acid, 2:1.

Amino Acid Analysis. The peptides were hydrolyzed in vacuo (110 °C, 20 h) in 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole[19] (Pierce). Amino acid analyses were performed on the hydrolysates by using an LKB 4150 analyzer, equipped with an Ultropac 11 column (6 × 215 mm) and a Shimadzu C-R3A recording integrator with in-house software. The buffer sequence pH 3.20 (13.5 min), pH 4.25 (27 min), pH 10.00 (borate; 33 min) and temperature sequence 50 °C (5 min), 55 °C (5 min), 58 °C (30.5 min), 65 °C (7 min), 80 °C (26 min) were used. Standard retention times were as follows: His, 54.9; Nal, 59.3; Lys, 60.3; NH_3, 63.5; Arg, 66.0 min respectively. The unknown amino acids were calculated as Leu, and acceptable values were obtained for all residues. The results are given in Table III.

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Biological Assays. The antiovulatory activity of each analogue was determined in Sprague–Dawley rats in a standard assay[20] using a 40% propane-1,2-diol/0.9% saline vehicle. The results (given in Table IV) are expressed as the percentage of (n) rats that did not ovulate at a dose of x μg of analogue. The in vitro histamine-releasing activity of each analogue was determined by using peritoneal cells from male Sprague–Dawley rats in a standard assay,[15] and the results are given as the ED_{50} values expressed in micrograms/milliliter (standard compound 48/80 has an ED_{50} of 0.58 in this assay system). The results are given Table IV.

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Effect of β-Alkyl Substitution on D-1 Dopamine Agonist Activity: Absolute Configuration of β-Methyldopamine

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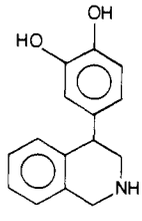
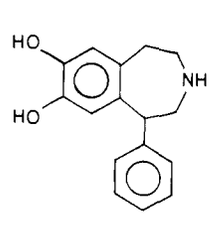
β-Methyldopamine and its enantiomers and racemic β-phenyldopamine were synthesized and evaluated for dopamine D-1 agonist activity. In the dopamine-sensitive adenylate cyclase assay, β-phenyldopamine had about one-sixth the activity of dopamine. Racemic β-methyldopamine was less potent. The absolute configuration of β-methyldopamine was determined to be *R*-(+) and *S*-(−). Evaluation of (*R*)-(+)- and (*S*)-(−)-β-methyldopamine revealed no enantioselectivity for stimulation of adenylate cyclase.

4-(3,4-Dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**1**) has been found to be a selective dopamine D-1 and DA_1 agonist.[1,2] A related compound, SKF 38393 (**2**), was also found to be a selective dopamine D-1 and DA_1 agonist.[3] Both **1** and **2** incorporate a β-phenyldopamine fragment, and it has been postulated that this fragment may be responsible for the selective dopamine agonist properties of **1** and **2**.[4]



1



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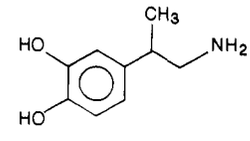

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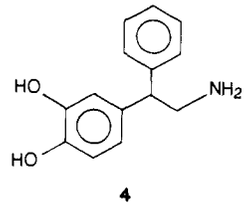

4

In 1978, Goldberg and Kohli[5] stated that no α- or β-substituted dopamine analogue were known that were active in the canine renal artery assay, a model of the vascular DA_1 receptor. Indeed, α-methyldopamine is an extremely weak dopamine agonist. This can probably be attributed to a lack of tolerance by the receptor for steric bulk attached to the α side chain carbon.[6] However, no dopamine agonist data have been reported for simple β-alkyl-substituted dopamines.[7] Therefore, it was decided to investigate the dopamine agonist properties of racemic β-methyldopamine (**3**), its resolved enantiomers, and β-phenyldopamine (**4**).

β-Methyldopamine was previously synthesized by Chavdarian et al.[8] These workers studied the oxidation potential and pressor effects of **3**. In that study, it was found that β-methyldopamine had about half of the pressor effect of dopamine itself. It should be noted that their data did not distinguish between dopaminergic and adrenergic activity.

β-Phenyldopamine (**4**) can be viewed as a partial structure of **1** and **2**. The X-ray crystal structure of

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