in a water bath at 60 °C and the contents analyzed as in method A.

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Registry No. **3b**, 110271-24-4; **4a**, 75475-91-1; **4b**, 110271-25-5; **5a**, 110271-26-6; **5b**, 110271-27-7; **6a**, 110271-28-8; **6b**, 110271-29-9; **7a**, 110271-30-2; **8a**, 110271-31-3; **8b**, 110271-32-4; **9a**, 110271-33-5; **10a**, 107124-28-7; **10b**, 107124-27-6; 11, 110271-34-6; **12a**, 107124-34-5; **12b**, 107124-32-3; **13a**, 110271-35-7; **15**, 110271-36-8; **16**, 110271-37-9; **17**, 110271-38-0; thiazolidine-4-carboxylic acid, 444-27-9; 3,4-dichlorobenzoyl chloride, 3024-72-4; dimethyl acetylenedicarboxylate, 762-42-5; N-formylproline, 13200-83-4; dimethyl 2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate, 62563-06-8; 6,7-bis(hydroxymethyl)-5-methyl-2,3-dihydro-1H-pyrrolizine, 62523-01-7; 4-(4-nitrobenzyl)pyridine, 1083-48-3.

Congener Derivatives and Conjugates of Histamine: Synthesis and Tissue and Receptor Selectivity of the Derivatives†

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A series of 19 congener derivatives and conjugates of histamine was synthesized and tested to determine whether the ligands would alter the conventional histamine activity in various tissues. The derivatives, which contained either branched or unbranched aliphatic groups, aromatic amide groups, or dipeptides, exhibited affinities for histamine type 1 and/or type 2 receptors that were widely different from the progenitor. The p-trifluoromethyl derivative of histamine with an intermediate chain length of four methylenes (compound 13) was the most potent lymphocytes H_2 receptor agonist but was inactive on guinea pig myocardium H_2 receptors. The deletion of a single methylene chain (compound 12) from this compound resulted in total loss of its H_2 activity on lymphocytes and its H_1 activity on aorta. Compound 12 became an exclusive H_1 agonist on lymphocytes H_1 receptors. The dipeptide conjugate (compound 17) and the aliphatic congener derivative (compound 18), both with four methylenes, retained some of the activity on guinea pig myocardium H_2 receptors, but lost their activity on lymphocytes H_2 receptors. Therefore, histamine can be modified at sites that are at a distance from the imidazole moiety, resulting in tissue selective histamine receptor agonists.

If histamine could be derivatized so that its effects became tissue and effect specific, the derivatives would be useful in probing the structure of the receptor microenvironment and of the receptor itself. If the selective effects were maintained in vivo, the derivatives could be used to explore their immune cardiovascular modulatory roles with a view toward developing them as therapeutic agents.

We have previously reported the effects of two parallel series of derivatives of a β-adrenergic agonist and two β-antagonists.[1-8] In each series, the synthesis utilized the amine end of the molecule even though this moiety was not considered responsible for recognition of the receptor. The effects of these derivatives included alterations of potency and effect and tissue specificity in each series.[1-8] What was most striking was that generally the analogous derivatives of the agonist and antagonists had an analogous skew of their pharmacologic effects. That is, the most potent or selective drugs were the closely related derivatives of each series.[1-8] Since the derivatization process did not manipulate the receptor recognition moiety (imidazole group) of the progenitor drugs, we reasoned that the ligands attached to some microenvironment of the β receptor. If such were the case, histamine might be made effect specific by similar derivatization. We took advantage of the side-chain terminal amino group on histamine to make a third series of derivatives.

This paper describes the synthesis and summarizes the comparative pharmacology of our new histamine derivatives with an emphasis on their effects on peripheral vasculature—the effects for which this amine is predominantly known. A limited report of the effects of 13 con-

Scheme I. General Methods of Histamine Modifications

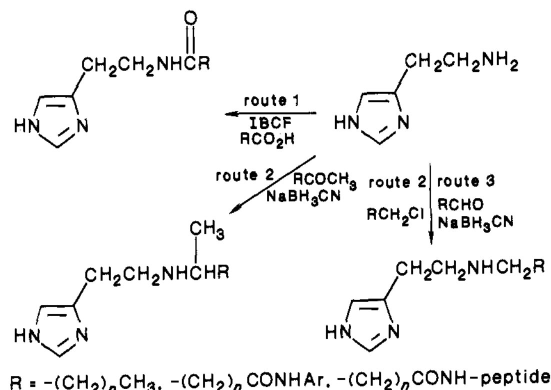

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Table I. Yields and Microchemical Analyses of Histamine Derivatives

compound	X	yield, %	mp, °C	purification		elemental analysis (C, H, N)
				method	solvents	
1	$(CH_2)_5CH_3$	16	229–231	recrystallization	EtOH	$C_{11}H_{23}N_3Cl_2$
2	$(CH_2)_4CONHC_6H_4CH_3$	35	225–227	recrystallization	$EtOH/Et_2O$	$C_{17}H_{26}N_4OCl_2$
3	$C(O)(CH_2)_4CH_3$	47	128–129	recrystallization	EtOH	$C_{11}H_{19}N_3O$
4	$C(O)(CH_2)_3CONHC_6H_4CH_3$	53	175–176.5	precipitation	H_2O	$C_{17}H_{22}N_4O_2 \cdot 1.5H_2O$
5 (n = 1)	$CH(CH_3)(CH_2)_nCONH$-$C_6H_4CH_3$	40	hygroscopic	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 70:30:10	$C_{16}H_{24}N_4OCl_2 \cdot H_2O$
6 (n = 2)		22	>80 dec	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 6:3:1	$C_{17}H_{26}N_4OCl_2$
7 (n = 3)		27	hygroscopic	recrystallization	$CHCl_3/Et_2O$	$C_{18}H_{28}N_4OCl_2 \cdot H_2O$
8 (n = 4)		10	152–155	recrystallization	$EtOAc/Et_2O$	$C_{19}H_{30}N_4OCl_2 \cdot H_2O$
9 (n = 5)		50	144 dec	recrystallization	$EtOH/Et_2O$	$C_{20}H_{32}N_4OCl_2$
10 (n = 1)	$CH(CH_3)(CH_2)_nCONH$-$C_6H_4CF_3$	32	hygroscopic	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 70:10:3	$C_{16}H_{21}N_4OCl_2F_3 \cdot H_2O$
11 (n = 2)		26	105 dec	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 6:3:1	$C_{17}H_{23}N_4OCl_2F_3 \cdot 0.5H_2O$
12 (n = 3)		20	hygroscopic	recrystallization	$CHCl_3/Et_2O$	$C_{18}H_{25}N_4OCl_2F_3 \cdot 5H_2O$
13 (n = 4)		37	hygroscopic	recrystallization	$EtOH/Et_2O$	$C_{19}H_{27}N_4OCl_2F_3 \cdot H_2O$
14	$CH(CH_3)(CH_2)_4CONH$-2-CF_3-C_6H_4	47	195–199	recrystallization	$EtOH/Et_2O$	$C_{19}H_{27}N_4OCl_2F_3 \cdot 0.5H_2O$
15 (n = 2)	$CH(CH_3)(CH_2)_nCONH$(Phe-(Boc)-Gly-$NHCH_3$)	9	>80 dec	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 6:3:1	$C_{27}H_{43}N_7O_5Cl_2 \cdot 5H_2O$
16 (n = 3)		9	>85 dec	flash chromatography	$CH_2Cl_2/MeOH/AcOH$, 6:3:1	$C_{28}H_{45}N_7O_5Cl_2 \cdot 6H_2O$
17 (n = 4)		30	hygroscopic	Whatman Partisil Magnum 10/25 ODS-3, 1 cm × 25 cm flow rate 1.0 mL/min retention time 15 min	52% MeOH/0.01 M NH_4HCO_3, pH 8.5	
18	$CH(CH_3)(CH_2)_4CONH(CH_2)_3$-$CH_3$	51	hygroscopic	recrystallization	$EtOH/Et_2O$	$C_{16}H_{32}N_4OCl_2$
19	$CH(CH_3)(CH_2)_4CH_3$	66	194–195.5	recrystallization	$EtOH/Et_2O$	$C_{12}H_{25}N_3Cl_2$

geners and conjugates of histamine on lymphocytes has been published elsewhere.

Synthesis of Congeners and Model Derivatives

The attachment of drug modifiers to histamine was explored by acylation and alkylation of the side chain primary amine. Scheme I illustrates the general methods used to make these modifications. Initially, several alkyl analogues were prepared in order to study the effect of the mode of attachment on histaminergic activity. Subsequently, aromatic amide groups derivatizing the β-antagonist series were used to modify histamine (Table I).

The acyl derivatives of histamine (compounds 3 and 4) were synthesized from histamine and the appropriate acid by the mixed-anhydride method (Scheme I, route 1). For the unbranched derivatives (compounds 1 and 2), the alkylation of the primary amino group on histamine was achieved by reductive amination with the appropriate aldehyde (Scheme I, route 3) or by S_N2 displacement of chlorine from the appropriate chloro compound (Scheme I, route 2). The most efficient route for the synthesis for compound 1 was found to be the reductive amination of hexanal and histamine with sodium cyanoborohydride as the reducing agent. Other routes such as reductive amination via hydrogenation with platinum dioxide or displacement reaction with 1-chlorohexane resulted in low yields. In the case of nonbranched toluide derivative (compound **2**), the displacement reaction gave an acceptable yield.

The syntheses of all the branched histamine derivatives (compounds **5–19**) were accomplished by reductive amination (Scheme I, route 3). These reactions never proceeded to completion even though the presence of the initial reactants could be verified by thin-layer chromatography and excess reducing agent was still present. Attempts to force the reaction to completion by the use of molecular sieves to remove water formed in the course of the reaction or by increasing the reaction temperature were unsuccessful.

Crude purifications to remove side products and starting materials were carried out by a series of extractions at pH ca. 2 and 9 with $CHCl_3$. In most cases, the product was isolated as the HCl salt, where final purifications usually involved recrystallization from EtOH/ether or flash chromatography with $CH_2Cl_2/MeOH/AcOH$ as eluent. For the isolation of the histamine dipeptide (compound **15**), semipreparative HPLC (52% methanol/0.01 M NH_4HCO_3, pH 8.5) was found to be the most effective method.

Pharmacologic Results

Nineteen congener derivatives and conjugates of histamine were tested in various in vitro models to determine whether our synthetic strategy has rendered tissue/effect specificity: (1) histamine type 1 receptor mediated contraction of the isolated rabbit aorta;[9] (2) accumulation of

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Table II. Effects of the Congener Derivatives and Conjugates of Histamine on Peripheral Vasculature, Natural Suppressor Cells, and Guinea Pig Myocardium

compound	% change in contractility (H_1)[a]	rel potency in NS (H_2)[b]	rel potency in GPM (H_2)[b]
histamine	284 ± 38 ($P < 0.05$)	1	1
1	93 ± 23 ($P > 0.05$)	5.0	0
2	0	2.0	0.09
3	0	0	0
4	0	4.4	0
5	0	0	NT
6	0	0	NT
7	90 ± 29 ($P < 0.05$)	4000.00	0.01
8	0	3.0	0.07
9	0	2.0	0.02
10	0	0	NT
11	0	0	NT
12	0	0	0
13	?	40000	0
14	0	0	0
15	0	0	0
16	0	0	0
17	0	0	0.0007
18	0	0	0.01
19	0	0	0

[a] Average ± SEM was calculated from at least three independent experiments. [b] Biological activity was measured by cAMP assay in natural suppressor cells (NS) and in guinea pig myocardium (GPM) by adenylate cyclase stimulation assay. Relative potency is expressed as the ratio of EC50 for histamine to EC50 for the derivatives. Inactive compounds were given a relative potency of 0. Inactive compounds did not cause any accumulation cAMP or the stimulation of adenylate cyclase. The relative potencies were calculated from EC50 values, which were average of at least three different determinations. The coefficient of variability was <10%. *The EC50 values of some of these congeners on natural suppressor cells and on guinea pig myocardium are published elsewhere.*[10]

intracellular cAMP in murine lymphocytes for the H_2 response;[10] (3) measurement of enhanced suppressor activity of natural suppressor cells mediated via H_1 receptors;[10] and (4) H_2-mediated adenylate cyclase stimulation of the guinea pig myocardium.[11]

To determine the effects of 19 congener derivatives and conjugates of histamine on peripheral vasculature, the derivatives were tested for their vasoconstrictive ability of the rabbit aorta. Histamine-induced vasoconstriction of the aorta is mediated by histamine type 1 receptors.

In Table III the effects of the derivatives on rabbit aorta are summarized. A representative experiment is shown in Figure 1. With the exception of congeners **1** and **7** and the progenitor drug, all the congener derivatives and conjugates of histamine lost their H_1 activity on the aorta. The most interesting congener derivative was number **12** of the series. This trifluoromethyl toluidide derivative of histamine (methylene chain length = 3) lost most of the receptor-mediated effects on the blood vessel, but retained H_1 activity on lymphoid cells.[10]

For the lymphocyte H_2 response, the activity of each derivative on natural suppressor cells was measured as the EC50 (concentration of a drug for half maximal activity) in molarity units and an Σ_{max} (maximum response or efficacy) in units of femtomoles of cAMP measured in 10^6 cells. Each compound with H_2 activity was competitively blocked by a histamine type 2 receptor antagonist cimetidine. Seven of the 19 congener derivatives of histamine not only retained their H_2 activity on natural suppressor cells, but also became several-fold more potent than the progenitor drug. These derivatives retained their H_2 activity despite the derivatization of the structure far removed from the pharmacophore that recognizes the receptor. In general, the potency was dependent upon the length of the methylene chain. As the methylene chain lengthened or shortened from three, the potency fell. Substituents on the aromatic ring also were key determinants of the activity of the toluide derivatives. When the *p*-methyl group was replaced by the *p*-trifluoromethyl

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Table III. Summary of the Effects of the Derivatives of Histamine on H_1 and H_2 Receptors in Peripheral Vasculature, Myocardium, and Lymphocytes[a]

	aorta (H_1)	natural suppressor (H_1)	lymphocytes (H_2)	myocardium (H_2)
histamine	+	+	+	+
1	+	−	+	−
2	−	+	+	+
3	−	−	−	−
4	−	−	+	−
5	−	+	−	NT
6	−	+	−	NT
7	+	+	++	+
8	−	−	+	+
9	−	+	+	+
10	−	+	−	NT
11	−	+	−	NT
12	−	+++	−	−
13	?	+++	+++	−
14	−	−	−	−
15	−	+	−	?
16	−	+	−	−
17	−	−	−	+
18	−	−	−	+
19	−	−	−	−

[a] + = histamine-like pharmacological effect was retained; − = no response; NT = not tested.

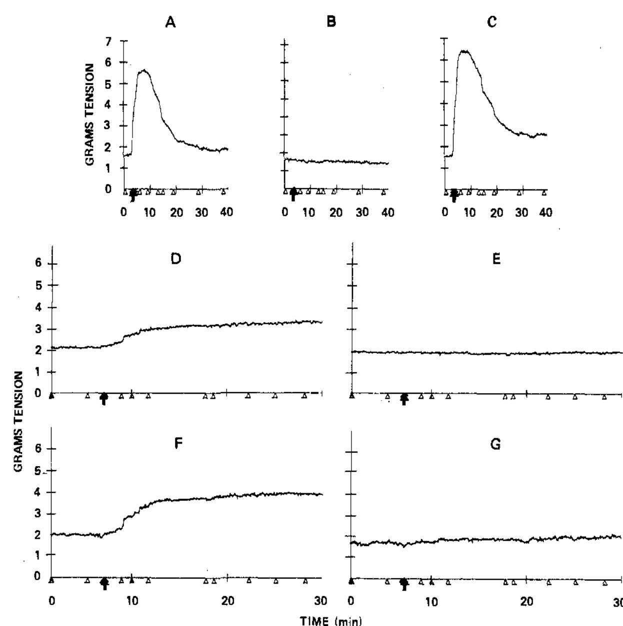

Figure 1. (i) The effect of histamine (10^{-4} M) (A) on the contractility of rabbit aorta and its blockade by pyrilamine (10^{-6} M) (B) and cimetidine (10^{-5} M) (C). (ii) The effect of congener **1** (10^{-4} M) (D) on the contractility of rabbit aorta and its blockade by pyrilamine (10^{-6} M) (E) and cimetidine (10^{-5} M) (F). The graph shows that congener **12** at 10^{-4} M (G) did not change the contractile response of rabbit aorta. The arrow shows the time when the drug was given.

group, there was a significant increase in the relative potency. A shift of the trifluoromethyl group from para to ortho position nullified all of the activity (Table II).

Selected compounds were tested for their capacity to stimulate adenylate cyclase in the guinea pig myocardium. This screening was performed to determine whether the congener derivatives of histamine exhibit similar profiles of H_2 activity on lymphocytes vs the myocardium. In the guinea pig myocardium, histamine caused a 3- to 5-fold stimulation of adenylate cyclase over basal levels. All of the derivatives of histamine tested were less potent than histamine when tested on the guinea pig myocardium (Table II). Of interest were compounds **1**, **4**, **13**, **17**, and **18**. Compounds **1**, **4**, and **13** were more potent than histamine on H_2 receptors of the natural suppressor cells but were completely inactive on the guinea pig myocardium, whereas compounds **17** and **18** were weak agonists of the H_2 receptor on the guinea pig myocardium but were impotent on natural suppressor cells. We had demonstrated selective action of the derivative tested in vitro for the guinea pig myocardium vs the murine lymphocytes.

To study the effects of congener derivatives of histamine on the histamine type 1 receptor mediated suppressor activity of natural suppressor cells, one established clone was incubated in 10% concanavalin rat spleenocyte supernatants, 10% fetal calf serum, and RPMI 1640 medium containing 10^{-4} M concentration of a given congener derivative of histamine. The exposure to histamine was brief and limited to the phase before washing the cells and adding them to responder and stimulator cells. Cocultured and stimulator cells were given 3300 rad in vitro just before incubation. We have previously reported that in this in vitro model, histamine enhanced the suppressive capacity of natural suppressor cells via an H_1-receptor effect.

Nine (congeners **1**, **2**, **7–9**, **11–13**, and **16**) of the 19 congener derivatives and conjugates of histamine had H_1 activity of the natural suppressor cells. They enhanced the suppressive capacity of the natural suppressor cells via H_1 receptor mediated mechanisms. Compounds **12**, **13**, and **17** were of special interest. Compound **12** retained both H_1 and H_2 activity; however, deletion of one methylene group nullified all H_2 activity and the compound became a pure H_1 agonist. Compound **16**, a dipeptide conjugate of histamine with three methylenes, was a potent H_1 agonist and did not possess H_2 activity. The addition of one methylene to the dipeptide conjugate resulted in total loss of H_1 and H_2 activity on the natural suppressor cells.

Blocking Histamine Effects in Natural Suppressor Cells. This assay was carried out in the same fashion as the agonist assay except that a concentration of test compound was chosen that produced the maximum accumulation of intracellular cAMP. All congener model compounds were used at a concentration of 10^{-4} M with the exception of compound **9**, where a concentration of 10^{-7} M was the most efficacious. Cimetidine was added at the same time in concentrations ranging from 10^{-4} to 10^{-7} M, and mepyramine was used at 10^{-6} to 10^{-8} M.

Conclusions

Our findings strengthen our proposal that by substituting relatively small functional groups on a parent compound at a point far removed from what is commonly thought to be the biological recognition site (active portion) of the molecule, new desired drugs might be made with altered potency and receptor or tissue specificity.

The derivatives and conjugates of histamine lacked the classical H_1 effects on the peripheral vasculature and H_2 effects on the heart but were effect selective for H_1 or H_2 receptors of lymphocytes. The differences in the activity of these derivatives cannot be explained by any factor other than the pharmacologically inert ligand. These ligands do not interact with H_1 or H_2 receptors in all of the four tissues tested nor do they prevent the imidazole from recognizing the receptors. The unusual effects seem attributable to the effects of the inert ligand that may be attracted to some undefined microenvironment.

We conclude from our data that the heterogeneity of the microenvironment of histamine receptors in different tissues may have been responsible for the novel spectrum of activity among the derivatives of histamine. The mechanisms for such discrimination by these derivatives are not clear, but those effects may be biologically and perhaps therapeutically useful. Hopefully, additional data may provide some insight as to what is chemically common to the microenvironment of histamine and β-adrenergic receptors.

Experimental Section

Melting points were determined in open glass capillaries with a Thomas-Hoover melting point apparatus and are uncorrected. High-resolution NMR spectra were obtained on a 360-MHz NMR spectrometer in the Fourier transform mode, which was built in-house.[12] IR spectra were recorded on a Perkin-Elmer 180 spectrophotometer. High-pressure liquid chromatographic purification of the drug derivatives was carried out on a system consisting of a Waters M-6000 pump, a Waters Model 660 solvent programmer, a Hewlett-Packard 3390A integrator, and a Schoeffel GM 770 detector monitoring at 254 nm. Elemental analyses were performed by Galbraith Laboratories, Knowville, TN, and Desert Analytics, Tucson, AZ.

Solvents were of reagent grade. *N,N*-Dimethylformamide was dried over KOH, distilled from ninhydrin, and then stored over 4A molecular sieves. Histamine was purchased from Sigma. Reactions were routinely monitored by thin-layer chromatography with aluminum-backed EM silica gel 60 F_{254} plates (Merck). Products were visualized by UV and ninhydrin spray (0.5% solution in ethanol).

The procedure used for the reductive amination of histamine and methyl ketones is illustrated by the synthesis of compound **8** below. Yields, elemental analyses, and purification conditions for the other branched derivatives are given in Table I.

1-[[2-(4-Imidazolyl)ethyl]amino]hexane Dihydrochloride (1). Histamine dihydrochloride (0.50 g, 2.7 mmo) and *n*-hexanal (0.33 mL, 2.7 mmol) were dissolved in 10 mL of MeOH in a flask that had been flushed with nitrogen. Several 3A molecular sieves were added, and the mixture was stirred at room temperature for 40 min before addition of sodium cyanoborohydride (0.17 g, 2.7 mmol). After 10 h, the MeOH was removed under reduced pressure, and the residue was dissolved in 75 mL of 0.1 N HCl and extracted with $CHCl_3$ to remove unreacted aldehyde and the alcohol side product. The aqueous phase was made basic with saturated $NaHCO_3$ and was extracted with *n*-BuOH. The BuOH extracts were combined and washed with brine, and the BuOH was removed in vacuo. The residual material was triturated with 2-propanol to separate the product from contaminating NaCl. The supernatant was concentrated under reduced pressure to an oil, which was dissolved in $CHCl_3$, dried over Na_2CO_3, and acidified by the addition of 4 N HCl in dioxane. The solvents were removed under reduced pressure to give 0.11 g (16%) of white solid. Recrystallization from EtOH gave a first crop of 31 mg of white crystals, which were shown to be homogeneous by thin-layer chromatography (BuOH/pyridine/AcOH/H_2O, 30:10:3:12, R_f 0.49), mp 229–231 °C. Anal. ($C_{11}H_{23}N_3Cl_2$) C, H, N.

5-[[2-(4-Imidazolyl)ethyl]amino]pentanoic Acid *p*-Toluidide Dihydrochloride (2). The reactant 5-chlorovalery chloride (7.75 g, 0.05 mol) was dissolved in 50 mL of dry THF, and the solution was cooled in an ice bath. *p*-Toluidine (5.35 g, 0.05 mol) and triethylamine (7.7 mL, 0.06 mol) were dissolved in 50 mL of dry THF, and the mixture was added dropwise to

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the solution of acid chloride over a period of 1 h. After the addition was complete, the solution was warmed to room temperature and stirred for an additional 3 h. The THF was then removed under reduced pressure, and the residue was dissolved in 300 mL of EtOAc. The EtOAc solution extracted with H_2O, 0.5 N HCl, 2.5% NaOH, and brine was then dried over Na_2SO_4. After filtration and concentration of the filtrate under reduced pressure, hexane was added to induce crystallization. The product was isolated as white platelets (9.52 g, 78%) and was shown to be homogeneous by thin-layer chromatography ($CHCl_3$/MeOH/AcOH, 95:5:3, R_f = 0.62, mp 92–93 °C.

A 10-mL round-bottom flask equipped with condenser and nitrogen bubbler was flushed with nitrogen. 5-Chlorovaleric acid *p*-toluidide (0.44 g, 1.9 mmol) and histamine free base (0.50 g, 4.5 mmol) were dissolved in 2 mL of dry 1-propanol. The mixture was heated to 100 °C in an oil bath for 5 h. After cooling to room temperature, the reaction mixture was dissolved isn 100 mL of 0.1 N HCl and was extracted with $CHCl_3$. The aqueous layer was saturated with solid $NaHCO_3$, the pH was brought to 9 by the addition of 1 N NaOH, and the solution was extracted with $CHCl_3$ and *n*-BuOH. The combined BuOH fractions were back-extracted with brine, and the BuOH was removed in vacuo. The white solid residue was triturated with BuOH to isolate the product from contaminating NaCl, and the supernatant was concentrated in vacuo to an oil. Lyophilization of the oil from H_2O gave 0.20 g (35%) of compound **2** judged to be pure by thin-layer chromatography (BuOH/pyridine/AcOH/H_2O, 30:10:3:12, R_f 0.45). The product was converted to the hydrochloride salt by treatment of a $CHCl_3$ solution of the product with 4 N HCl in dioxane. Removal of the solvents under reduced pressure followed by crystallization of the residue from EtOH/ether gave material shown to be pure by thin-layer chromatography (same system and R_f is given above), mp 225–227 °C. Anal. ($C_1{,}7H_{26}N_4OCl_2$) C, H, N.

4-(2-Hexanamidoethyl)imidazole (3). Caproic acid (0.11 g, 1.0 mmol) was dissolved in 5 mL of dry THF, and the solution was cooled in an ice bath. *N*-Methylmorpholine (0.33 mL, 3.0 mmol) was added followed by isobutyl chloroformate (0.13 mL, 1.0 mmol), and the solution was stirred for 10 min. Histamine dihydrochloride (0.18 g, 1.0 mmol) was dissolved in 1.0 mL of DMF and added to the above solution. After the reaction mixture was allowed to warm to room temperature and to stir overnight, the solvent was removed in vacuo, and the residue was dissolved in 0.1 N HCl (930 mL). The acidic solution was extracted with $CHCl_3$ and then saturated with solid $NaHCO_c$. The basic aqueous phase was then extracted with $CHCl_3$. The $CHCl_3$ phases were combined, washed with brine, and dried over Na_2SO_4. Filtration to remove the drying agent followed by evaporation of the solvent under reduced pressure gave a solid, which was triturated with EtOH and was dried to give 0.10 g (47%) of compound **3**. The product was shown as a single spot on thin-layer chromatography (BuOH/pyridine/AcOH/H_2O, 30:10:3:12, R_f 0.59), mp 128–129 °C. Anal. ($C_{11}H_{19}N_3o$) C, H, N.

***N*-[5-[[2-(4-Imidazolyl)ethyl]amino]glutaryl]-*p*-toluidine (4).** Glutaric anhydride (5.0 g, 44 mmol) was added to a solution of *p*-toluidine (9.39 g, 88 mmol) in dry THF. The clear brown solution immediately became warm, and white crystals were precipitated from the solution. The mixture was stirred at room temperature overnight followed by evaporation of the solvent under reduced pressure. The solid residue was dissolved in 120 mL of 0.5 N NaOH and was extracted with $CHCl_3$. Upon acidification of the aqueous layer with 3 N HCl (to pH 1), copious amounts of precipitate were formed. The solid was isolated by filtration, washed extensively with H_2O, EtOAc, and ether, and was dried in vacuo to give 7.6 g of product. An additional 2 g of material was isolated by extraction of the initial acidic filtrate with EtOAc. The two crops of product were combined and recrystallized from MeOH/EtOAc to give 8.4 g (87%) of colorless needles shown to be homogeneous by thin-layer chromatography ($CHCl_3$/MeOH/AcOH, 95:5:3, R_f 0.23), mp 176–177.5 °C.

The product (0.22 g, 1.0 mmol) and *N*-methylmorpholine (0.11 mL, 1.0 mmol) were dissolved in 5 mL of dry DMF, the solution was cooled to 0 °C, and isobutyl chloroformate (0.13 mL, 1.0 mmol) was added. After 10 min, a solution of histamine dihydrochloride (0.20 g, 1.1 mmol) and *N*-methylmorpholine (0.24 mL, 2.2 mmol) in 2 mL of DMF was added. The reaction mixture was warmed to room temperature and was stirred overnight. After removal of DMF in vacuo, the residue was dissolved in 40 mL of 0.1 N HCl and was extracted with $CHCl_3$ to remove unreacted starting acid. The aqueous phase was made basic by the addition of solid $NaHCO_4$, which induced the product to crystallize from the solution. The product was isolated by filtration, washed with H_2O, $CHCl_3$, and ether, and dried in vacuo to give 0.185 g (53%) of compound **4**, which was shown to be homogeneous by thin-layer chromatography (BuOH/pyridine/AcOH/H_2O, 30:10:3:12, R_f 0.55), 175–176.5 °C. Anal. ($C_{17}H_{22}N_4O_2{\cdot}1.5H_2O$) C, H, N.

Methyl-Branched Histamine Congener Derivatives and Conjugates. The methyl-branched N-alkylated histamine conjugates were synthesized via reductive amination from histamine dihydrochloride and the appropriate methyl ketone. This procedure is illustrated by the synthesis of 6-[[2-(4-imidazolyl)-ethyl]amino]heptanoic acid *p*-toluidide (**8**). The synthesis of the methyl ketones has been described previously.[2,6] Table I lists the yields, elemental analyses, and purification conditions for the other members of this series.

6-[[2-(4-Imidazolyl)ethyl]amino]heptanoic Acid *p*-Toluidide (8). Histamine dihydrochloride (0.18 g, 1.0 mmol), 6-oxoheptanoic acid *p*-toluidide (0.23 g, 1.0 mmol), and sodium cyanoborohydride (0.06 g, 1.0 mmol) were dissolved in 5 mL of MeOH in a vial that had been flushed with nitrogen. The reaction mixture was heated overnight at 55 °C. To quench unreacted borohydride, 3 N HCl was added to pH 1–2 (pH paper). After the evolution of gas had subsided, the reaction mixture was added to 50 mL of 0.1 N HCl, and the solution was extracted with $CHCl_3$. The aqueous phase was made basic by the addition of 20 mL of 1 N NaOH and was extracted with $CHCl_3$. The combined $CHCl_3$ fractions were back-extracted with brine and were dried over K_2CO_3. Filtration to remove the drying agent and evaporation of the filtrate under reduced pressure gave the product as a clear glass. To convert the material to the hydrochloride salt, the residue was redissolved in a small amount of $CHCl_3$, and 0.5 mL of 4 N HCl in dioxane was added dropwise. Evaporation of the solvents under reduced pressure followed by precipitation of the product from EtOH/ether gave 41 mg (10%) of compound **8** shown to be pure by thin-layer chromatography (BuOH/pyridine/AcOH/H_2O, 30:10:3:12, R_f 0.35), mp 152–155 °C. Anal. ($C_{19}H_{30}N_4OCl_2{\cdot}H_2O$) C, H, N, (Table I).

Vascular Effects on Rabbit Aorta. The effects of the congener derivatives and conjugates of histamine were determined in rabbit aorta. White New Zealand rabbits of either sex weighing 2.5 (±0.5) kg were sacrificed by a blow to the head and cervical dislocation. Upon excision and removal of the aorta, sectioned 5-mm ring segments were placed in a multichambered tissue bath. Ring segments were attached by two metal pins (0.018 in.) and were then attached to Gould (UC-3) force-displacement transducer. Normal physiological solution (modified Tyrode's) consisted of the following composition in mM: NaCl 118, KCl 4, $MgSO_4$ 1.2, $CaCl_2$ 2, dextrose 5, $NaHCO_3$ 24, NaH_2PO_4 1.2. The pH was kept constant at 7.40–7.45, and each bath was aerated with 95% O_2 and 5% CO_2. The aorta segments were equilibrated for 60–90 min. Approximately 1.2 g of resting tension was applied to each ring segment. All congener derivatives of histamine at concentrations (10^{-4} to 10^{-7} M) were added to the bath in the presence and absence of H_1 receptor antagonist (pyrilamine 10^{-6} M). Total tension was recorded as the absolute tension minus the base line or resting tension. Significance of data was analyzed by Student's *t* test. A p value < 0.5 was considered statistically significant.[9]

Natural Suppressor Cells and the Assay of Their cAMP. Natural suppressor cells were a gift of Dr. Samuel Strober (Stanford University School of Medicine). They were obtained and cultured as follows: 4–6-month-old Balb/c mice were anesthetized with pentobarbital, and all major lymphoid organs, including all major lymph nodes, the spleen, and the thymus, were irradiated. The skull, lungs, tail, and hind legs were shielded with lead. The mice were given 200 rads/day, 5 times/week, to a total dose of 3400 rads. Irradiation was delivered from a single 250 kV (15 A) source (Philips Medical Systems, Inc., Shelton, CT). The mice were killed between 5 and 15 days after completion of total lymphoid irradiation. Spleens were removed aseptically, and single cell suspensions were prepared by gently pressing the spleen fragments through a nylon fiber mesh (Tetko, Inc.,

Elmsford, NY). These spleen cells were fed daily with tissue culture medium containing RPMI 1640, fetal calf serum, glutamine, Con A soup, and 2-mercaptoethanol.[13]

Natural suppressor cells were centrifuged and resuspended at 10^6 cells/mL in phosphate buffered saline (PBS) and incubated at 37 °C for 10 min with 10 mM isobutylmethylxanthine (IBMX) at a final concentration of 20 μM.

The cells were incubated with and without histamine derivatives for 1 min at 37 °C with continuous shaking. Maximum intracellular accumulation of cAMP occurred in 1 min. Cold TCA (100%) was added to a final concentration of 8%, and the tubes were stored in ice. Samples were extracted three times with three volumes of ether. The ether was evaporated at 40 °C in a water bath for approximately 45 min. Acetylation of both experimental samples and cAMP standards was performed by adding of 10 μL of triethylamine and acetic anhydride (2:1). Acetylation improved the sensitivity of the assay about 50–100 fold. The radioimmunoassay was performed as reported previously.[13]

Guinea Pig Myocardial Adenylate Cyclase Assay. Male, partly albino, guinea pigs (600 g average weight) were killed by a blow to the head. The hearts were excised and immediately immersed in ice-cold oxygenated Tyrode's solution. Both ventricles were dissected free and placed in 250 mM sucrose, 5 mM Tris, and 1 mM EGTA, pH 7.45. The tissue was minced with a screen and was then homogenized with three consecutive 5-s bursts of a Polytron (Brinkman Instruments, Inc., Westbury, NY) at a setting of 11. The homogenate was then centrifuged at 1085*g* for 20 min. The pellet was resuspended and recentrifuged twice. The final suspension was filtered through four layers of gauze. Adenylate cyclase was assayed by the method of Bristow et al.[11] Enzyme protein (75–250 μg) was added to a reaction mixture that consisted of 0.1 mM MgATP, 0.5 mM $MgCl_2$, 10 mM phosphocreatine, 14.5 μg of creatine kinase (1381 units/mg), 100 mM Tris·HCl (pH 7.45), 10^{-5} M guanylimidophosphate (GPP(NH)P), and variable concentrations of histamine. ^{3}H-labeled cyclic AMP (10000–12000 cpm/assay) was added prior to incubation for determination of recovery. The final reaction volume before addition of [α-^{32}P]ATP was 225 μL. Reaction tubes were stored in cryogenic racks (Kryorack, Isolab, Inc., Akron, OH) at 0 °C.

The reaction mixture was prewarmed in a shaking water bath at 30 °C for 5 min after which 25 μL (1.25–2.5 Ci) of [α-^{32}P]ATP (250–500 Ci/mM) was added to label the ATP pool. The assay time was 20 min, the time needed to obtain maximum stimulation of adenylate cyclase. The ^{32}P reaction was stopped by the addition of 750 μL of 1% sodium dodecyl sulfate. ^{32}P-labeled cyclic AMP was then isolated by dual Dowex-alumina chromatography. [α-^{32}P]ATP (New England Nuclear Corp., Boston, MA) that gave reagent blanks of 50 cpm was purified on Dowex columns as described by Salomon et al.[14] as modified by Bristow et al.[11]. Recovery of cAMP ranged from 70–90%. Reagent blanks exhibited 0.005% of the activity of the added [α-^{32}P]ATP and were in all cases <10% of basal activity. All assays were performed in duplicate, and activity was linear with respect to added enzyme protein and to time over a period of 5–30 min. The coefficient of variability of the duplicates was <5%.

Mixed Leukocyte Reaction (MLR) Suppressor Assay. Responder (BALB/c) and stimulator (C57 BL/6) spleen cells (5 × 10^5 each) were incubated with graded numbers of natural suppressor cells in 0.3 mL/well in 96-well flat-bottomed microculture plates (Costar, Data Packaging, Cambridge, MA). The culture medium was supplemented with 100 units/mL penicillin, 100 μg/mL of streptomycin (both GIBCO, Grand Island, NY), and 10% pooled human serum (VSP human serum, Biocell Laboratories, Carson, CA). Cocultured cells and stimulator cells were given 3300 rads before incubation. Cultures were maintained at 37 °C in 5% CO_2 for 5 or 6 days. Eighteen hours before termination, 1 μCi of [^{3}H]thymidine (specific activity 6.7 Ci/mM) (New England Nuclear, Boston, MA) was added to each culture. Cells were harvested as described above. The data were expressed as the arithmetic mean of triplicate cultures. Suppression was calculated as follows: percent suppression = 1 − (CPM with cocultured cells)/(CPM without cocultured cells) × 100. To test the activation capability of congener derivatives of histamine, the natural suppressor cells were incubated with agonist (10^{-4} M) for 4 h at 37 °C and washed three times before the cells were cocultured in an MLR. To block the effects of agonists, 10^{-5} M cimetidine was used as an H_2 antagonist and 10^{-6} M mepyramine was used as an H_1 antagonist.[13]

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Registry No. 1, 103827-09-4; 1·2HCl, 51720-60-6; **2**, 103827-13-0; **2**·2HCl, 110224-11-8; **3**, 103827-10-7; **4**, 103827-12-9; **5**, 110224-04-9; **5**·2HCl, 110224-12-9; **6**, 110224-05-0; **6**·2HCl, 110224-13-0; **7**, 103827-14-1; **7**·2HCl, 110224-14-1; **8**, 103827-15-2; **8**·2HCl, 110224-15-2; **9**, 103839-69-6; **9**·2HCl, 110224-16-3; **10**, 110224-06-1; **10**·2HCl, 110224-17-4; 11, 110224-07-2; 11·2HCl, 110224-18-5; **12**, 110224-08-3; **12**·2HCl, 110224-19-6; **13**, 103827-16-3; **13**·2HCl, 110224-20-9; **14**, 103827-17-4; **14**·2HCl, 110224-21-0; **15**, 110224-09-4; **15**·2HCl, 110224-22-1; **16**, 110241-93-5; **16**·2HCl, 110224-23-2; **17**, 110224-10-7; **18**, 103827-18-5; **18**·2HCl, 110224-24-3; **19**, 103827-11-8; **19**·2HCl, 58620-53-4; $CH_3C(O)CH(CH_3)$-$CH_2C(O)NH$-*p*-C_6H_4Me, 110224-26-5; $CH_3C(O)CH(CH_3)$-$CH_2)_2C(O)NH$-p-C_6H_4Me, 110224-27-6; $CH_3C(O)CH(CH_3)$-$(CH_2)_3C(/)NH$-*p*-C_6H_4Me, 110224-28-7; $CH_3C(O)CH(CH_3$-$(CH_2)_4C(O)NH$-*p*-C_6H_4Me, 110224-29-8; $CH_3C(O)CH(CH_3$-$(CH_2)_5C(O)NH$-*p*-C_6H_4Me, 110224-30-1; $CH_3C(O)CH(CH_3)$-$CH_2C(O)NH$-*p*-$C_6H_4CF_3$, 110224-31-2; $CH_3C(O)CH(CH_3)$-$(CH_2)_2C(O)NH$-*p*-$C_6H_4CF_3$, 110224-32-3; $CH_3C(O)CH(CH_3)$-$(CH_2)_3C(O)NH$-*p*-$C_6H_4CF_3$, 110224-33-4; $CH_3C(O)CH(CH_3)$-$(CH_2)_4C(O)NH$-*p*-$C_6H_4CF_3$, 110224-34-5; $CH_3C(O)CH(CH_3)$-$(CH_2)_4C(O)NH$-o-$C_6H_4CF_3$, 110224-35-6; $CH_3C(O)CH(CH_3)$-$(CH_2C(O)NH$-*p*-(*N*-Boc-Phe-Gly-$NHCH_3$), 110224-36-7; CH_3C-$(O)CH(CH_3)(CH_2)_3C(O)NH$-*p*-(*N*-Boc-Phe-Gly-$NHCH_3$), 110224-37-8; $CH_3C(O)CH(CH_3)(CH_2)_4C(O)NH$-*p*-(*N*-Boc-Phe-Gly-$NHCH_3$), 110224-38-9; $CH_3C(O)CH(CH_3)(CH_2)_4C(O)NHBu$, 110224-39-0; $CH_3C(O)CH(CH_3)(CH_2)_4CH_3$, 6137-08-2; histamine, 51-45-6; histamine hydrochloride, 56-92-8; *n*-hexanal, 66-25-1; 5-chlorovaleryl chloride, 1575-61-7; 5-chlorovaleric acid *p*-toluidide, 110224-25-4; caproic acid, 142-62-1; glutaric anhydride, 108-55-4; *N*-(*p*-methylphenyl)glutarimide, 81305-69-3; *p*-toluidine, 106-49-0.

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