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Histamine receptor activation by unsaturated (allyl and propargyl) homologs of histamine

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Abstract

The spectrum of agonist activity for three new homologs of histamine (cis- and trans-imidazolylallylamine and imidazolylpropargylamine) was evaluated in the isolated guinea pig ileum and right atrium. The homologs were about three log units less potent than histamine in stimulating contractions of the longitudinal muscles of the ileum, but they were histamine-like, pharmacologically, because they were sensitive to blockade by pyrilamine and resistant to blockade by atropine. In the right atrium, these weak agonists were partially sensitive to blockade by cimetidine. The agonist activity of the cis-isomer in particular was completely blocked by a combination of cimetidine and propranolol, but resistant to reserpine treatment (neuronal catecholamine depletion). Therefore, these homologs of histamine have the ability to stimulate H_1- and H_2-histamine receptors and *beta*-adrenoreceptors *in vitro*.

Introduction

The structural and electronic properties of histamine which are required for activity at the H_1 and H_2 receptors have been the subject of extensive investigation over the years [1–9]. Methods employed have included cyclopropane-type rigid analogs [1], NMR studies [2,3], and molecular orbital calculations of the most stable conformations [3, 4, 6–9]. The most recent conclusions [8, 9], including a consideration of the selective H_2-agonist dimaprit [10], were that the H_1-active conformation for histamine was likely to be a transoid cationic side chain with an uncharged imidazole able to rotate, and that the H_2-active conformation of histamine was a cationic side chain of unspecified conformation attached to an uncharged imidazole that was capable of acting as a proton transfer agent through ring tautomerization.

We have synthesized several unsaturated histamine homologs as potential inhibitors of diamine oxidase (DAO) [11, 12], and thought it would be of interest to measure histamine receptor activity for some of these homologs since such compounds have not previously been reported in the literature. In the work presented here we determined the extent of direct H_1- and H_2-histamine receptor interaction for three new unsaturated histamine homologs: cis and trans-imidazolylallylamine (cis- and trans-IAA, respectively) and imidazolylpropargylamine (IPPA) (Fig. 1). The major emphasis of our experiments was focused on the pharmacodynamics of cis-IAA because preliminary data [11] showed that the cis-isomer had a relatively low K_i (1.7×10^{-7} M) for inhibition of DAO [13]. For comparison, the activity of the saturated homolog, imidazolylpropylamine

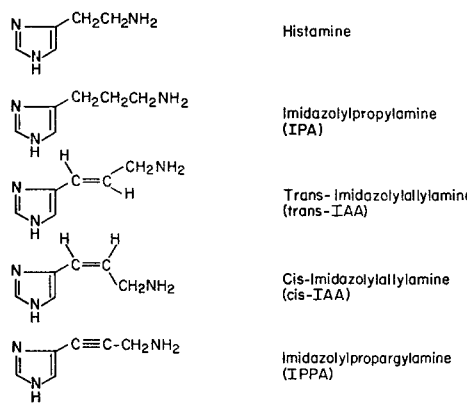


Figure 1
Chemical structures of histamine and the histamine homologs.

(IPA), on histamine receptors was also studied. IPA had been reported as inactive [14]. However, we wished to test the hypothesis that shortening the propyl side chain length by the introduction of unsaturated (double or triple) bonds between carbon atoms and the concomitant introduction of conformational rigidity into the chemical structures might impart histamine-like activity to one or more of these compounds. In the study of H_1 receptor mediated actions, we measured the dose-related increase in the isotonic contractions of longitudinal muscles in the isolated guinea pig ileum. For H_2 receptor mediated actions of these agents, we studied their ability to increase the rate of spontaneous contractions in the guinea pig right atrium *in vitro*. A preliminary report on this research has appeared in Federation Proceedings [15].

Material and Methods

General Considerations. Isolated tissues from freshly killed animals were suspended in 10 ml jacketed tissue baths containing a physiological salt solution (PSS) maintained at 36 C. The composition of the solution was (mM): NaCl, 118; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 0.54; $CaCl_2 \cdot 2H_2O$, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 25 and glucose, 11. These chemicals were dissolved in double-distilled, demineralized water (DDDW). A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled through the solution.

Isolated guinea pig atria. Albino guinea pigs (Camm Research Institute, Inc.) weighing from 300 to 600 g were killed by a sharp blow to the head. The right atrium with the sinus node was removed from the animal and suspended in a 10 ml jacketed tissue bath. Spontaneous atrial contractions were recorded via a Grass isometric force-displacement transducer (FT03C) connected to a Grass 4-channel polygraph (Model 79D). Simultaneously, a calibrated tachograph output (Model 7P4F) was used to convert the atrial rate into a linear recording. The resting atrial tension was 0.5 g. The tissue was allowed at least 1 hr equilibration before a cumulative dose-response curve for an agonist was constructed. For those atria subjected to antagonists, the appropriate dose of the antagonist was added to the PSS 1 hr before the initiation of the agonist dose-response curve. The chronotropic effect was recorded as the change in rate from the resting rate and expressed as a percentage of the maximal change in atrial rate (contractions/min) produced by histamine.

Isolated guinea pig ileum. After the right atrium was removed from the animal, a 2 cm segment of the terminal portion of the ileum was removed and suspended in a 10 ml tissue bath. Contractions of the longitudinal ileal muscle were recorded via an E and M isotonic myograph transducer connected to a Narco 4-channel physiograph. The resting ileal muscle tension was 0.5 g. The tissue was allowed at least 1 hr equilibration in the tissue bath before a cumulative dose-response curve for an agonist was constructed. For the ileal preparations subjected to antagonists, the appropriate dose of the antagonist was added to the PSS 1 hr before the initiation of the agonist dose-response curve. Ileal contractions were expressed as a percentage of the maximal contractile response to histamine.

Drugs. All drug solutions were freshly prepared with DDDW. Serial dilutions of the solutions were made with DDDW. The following substances were used: histamine 2HCl and tyramine HCl (Regis Chemical Co.); pyrilamine maleate, cimetidine and dl-propranolol (Sigma Chemical Co.); atropine sulfate monohydrate, (Aldrich Chemical Co.); reserpine (Ciba Pharmaceutical Co.). The histamine homologs cis- and trans-IAA, IPPA (unsaturated homologs) and IPA (saturated homolog) were synthesized in the medicinal chemistry laboratories of the University of Michigan College of Pharmacy.

Statistical Evaluation. The log dose-response relationships were expressed in tabular or graphical form as the mean ± S.E.M. Where appropriate, a statistically significant difference between groups of data was established with the aid of Student's *t*-test, and $p < 0.05$ (two-tailed test) was accepted as statistically significant.

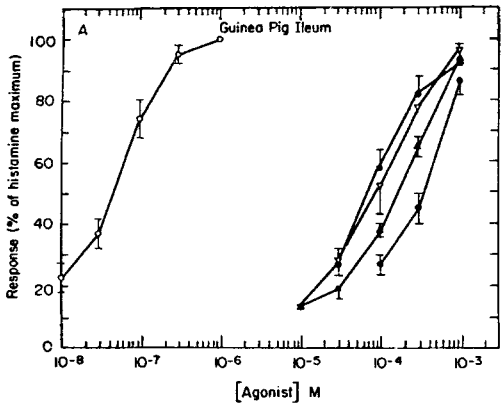
For those atria or ilea exposed to antagonists, the dissociation constant (K_B) of the receptor-antagonist complex was calculated by the method described by Furchgott [16]:

$$K_B = [\text{Antagonist}]/\text{Dose-ratio} - 1.$$

Dose-ratio is defined as the ratio of EC_{50} concentrations of the agonist in the presence and absence of the antagonist. The concentration of antagonist is expressed in moles/liter. The slopes of the log dose-response relationships for an agonist in the absence or presence of an antagonist were subjected to least squares linear regression analysis within the EC_{20} to EC_{80} dose range. The nul hypothesis that the linear regression lines were parallel (no statistically significant difference in slopes) was accepted or rejected with the aid of the Pharmacological Calculation System (Pharm/PCS Version 3.0, 1984) obtained from MicroComputer Specialists (Elkins Park, PA). The computations were performed on an International Business Machines Personal Computer.

Results and Discussion

Comparison of effects of histamine and histamine homologs on isolated ilea and atria. All of the homologs exhibited agonist activity in the guinea pig ileum or atrium, but they were much weaker agonists than histamine (Fig. 2). IPA and cis-IAA had a relatively greater affinity for ileal or atrial receptors than did IPPA (Table 1). The cis- and trans-isomers of IAA were not stereoselective for receptors in either tissue. Pretreatment of atrial or ileal tissue preparations for 1 hr with a supramaximal dose of aminoguanidine (0.1 mM), a noncompetitive DAO inhibitor [17], did not potentiate (i.e. did not produce a leftward shift of) the dose-response relationship for cis-IAA on either ileal or atrial tissues (data not shown). **Identification of receptors mediating homolog activity by blockade with selective receptor antagonists.**



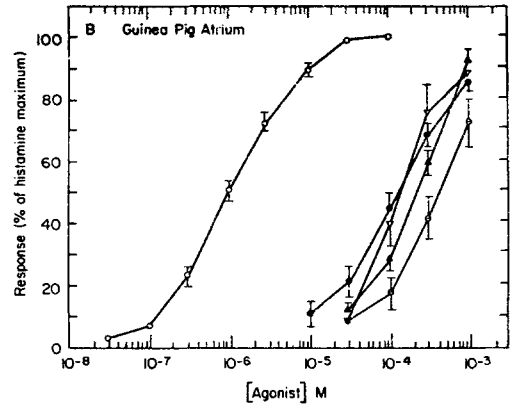


Figure 2
Cumulative dose-response relationships of histamine (○) and the histamine homologs: IPA (●); CIS-IAA (▽), trans-IAA (▲) and IPPA (○). Cumulative responses to histamine were obtained after complete washout of a particular homolog which was assumed to coincide with the return to pre-homolog baseline conditions. A. Isotonic contraction induced in longitudinal smooth muscles of the isolated terminal portion of guinea pig ileum (n = 4-10). B. Stimulatory effects on atrial contraction rate (ACR) of the isolated guinea pig right atrium (n = 4-7). Mean baseline ACR prior to homolog dose-response curves in these experiments has 179 ± 4 contractions/min. Symbols with attached vertical bars in this figure (and all subsequently appearing figures) represent the mean (± SEM) response.

Table 1
Comparison of -log EC_{50} values for histamine and histamine homologs[a] on isolated ilea and right atria from guinea pigs

	-log EC_{50} [M]	
	ILEA	R. ATRIA
Histamine	7.33 ± 0.01[b] (6)	6.01 ± 0.06 (6)
IPA	4.08 ± 0.12 (7)	3.94 ± 0.12 (8)
cis-IAA	3.98 ± 0.07 (10)	3.86 ± 0.12 (4)
trans-IAA	3.60 ± 0.11 (4)	3.61 ± 0.05 (4)
IPPA	3.35 ± 0.12[c] (7)	3.36 ± 0.12[c] (8)

[a]-Log EC_{50} values for homologs calculated at the level of a one-half maximal response to histamine.
[b]Values represent the mean ± S.E.M. Numbers in parentheses are number of animals used.
[c]The affinity (-logEC_{50}) of IPPA for ileal or atrial receptors was significantly less than that for IPA or cis-IAA ($p < 0.05$, t-test).

H_1 histamine receptor blockade

Pyrilamine maleate (PAM), a selective H_1 histamine receptor blocker, was used to determine if the stimulation of ileal contraction by the homologs was mediated by H_1 receptors. In preliminary experiments, PAM (5 nM) treatment for 1 hr produced an 11.5-fold parallel shift to the right in the dose-response relationship for histamine. The -log K_B (9.32, Table 2) calculated from this shift was in good agreement with published values of about 9.3 [18]. Therefore, PAM produced classical competitive antagonism of the response to histamine in the guinea pig ileum.

PAM produced dose-dependent decreases in apparent potency of cis- and trans-IAA, IPPA and IPA on ileal muscles (Fig. 3 and Table 2). Complete dose-response curves for the unsaturated homologs in the presence of PAM could not be produced in all experiments because of the relatively low affinity of the ileal receptors for these homologs. In spite of that limitation, the rightward shift (dose-ratio) in the dose-response relationships of cis- or trans-IAA and IPPA in the presence of PAM (1 or 5 nM) could be interpreted as classical competitive antagonism (Table 2 and Figs 3A, B, C). At higher concentrations PAM (10 or 100 nM) completely blocked the ileal responses to these homologs. The decrease in maximal effect which was quite apparent with PAM (5 nM) blockade of the cis-IAA response may be related to the fact that cis-IAA was a weak agonist in comparison to histamine (Fig. 2). Blockade of the response to a weak agonist by a strong antagonist is known to produce a decrease in the maximal effect in addition to the expected shift in the apparent potency of the weak agonist [19].

Table 2
Evidence for H_1-histamine receptor activation by unsaturated histamine homologs

DRUG	n[b]	% EFFECT	-LOG EC[a] [M] CONTROL	PYRILAMINE	LOG(DR[c]-1)	-LOG K_B[d]
Histamine	6	50	7.33 ± 0.01	6.27 ± 0.04 [5][e]	1.02	9.32
cis-IAA	4	50	4.04 ± 0.07	3.45 ± 0.05 [1]	0.46[f]	9.45
trans-IAA	4	30	4.22 ± 0.11	3.30 ± 0.22 [5]	0.86	9.15
IPA	4	40	3.69 ± 0.12	2.52 ± 0.15 [5]	1.14	9.45

[a] EC = Effective Concentration: concentration that produced the response (% of histamine maximum) used to calculate the dose ratio (DR) for each agonist.
[b] n = number of experiments.
[c] DR (Dose Ratio): the effective concentration (EC) at which a particular response occurred in the presence of an antagonist divided by the EC at which the response occurred in the absence of the antagonist.
[d] K_B is the dissociation constant of the antagonist-receptor complex which is defined as the concentration of antagonist that causes a 2-fold shift to the right in the dose-response curve for an antagonist.
[e] Concentration of pyrilamine used to obtain the Dose-Ratio (DR).
[f] Calculated with pyrilamine (1 nM); at 5 nM the dose-response curve for cis-IAA was depressed by pyrilamine (see fig. 3).

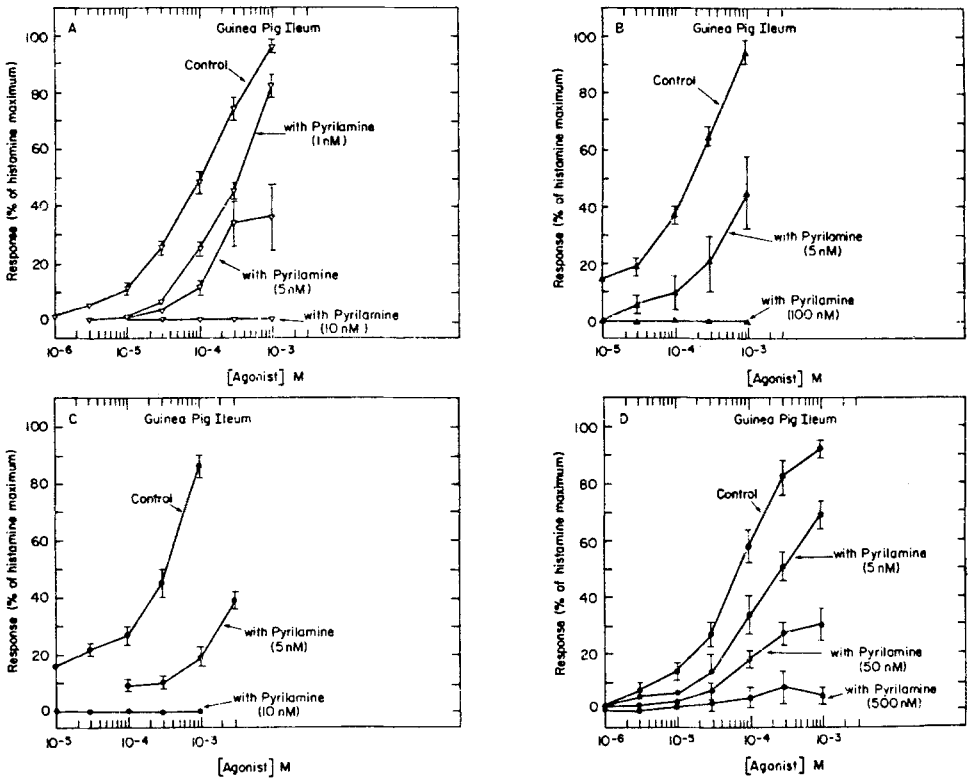


Figure 3
Inhibition of homolog-induced ileal contractions by pyrilamine maleate (PAM). 3A, B and C: PAM (1 or 5 nM, 1 hr) produced a shift to the right in the cumulative dose-response relationship for cis- and trans-IAA and IPPA. The slopes of the curves in the absence or presence of PAM were not significantly different when compared by least squares regression analysis. 3D. IPA-induced ideal contractions were comparatively more resistant to blockade by PAM (n = 4-7 for all curves).

The ileal response to IPA was considerably more resistant to blockade by PAM (Fig. 3D). Pam (5–500 nM) suppressed in a dose-related manner but did not completely block the ileal contractions to IPA. The -log K_B for PAM calculated from the dose-ratio shift (4.28) with IPA in the absence or presence of PAM (5 nM) was 8.81. This was about 0.5 log unit less than the expected value of 9.3. This fact together with the above-mentioned resistance to PAM blockade may indicate that IPA was sensitive to both competitive and noncompetitive inhibition by PAM. The lower -log K_B value for PAM indicates that the affinity of PAM for this site of blockade was lower than for the competitive site (H_1-receptor).

Muscarinic cholinergic receptor blockade. Homolog-induced contractions of longitudinal ileal muscle were completely resistant to blockade by atropine sulfate (1 μM), a selective blocker of muscarinic cholinergic receptors (data not shown). Thus, no muscarinic cholinergic agonist activity was present in these histamine homologs.

H_2-histamine receptor blockade

Cimetidine (CD), a selective H_2-receptor blocker was used to determine if the homolog-induced stimulation of the atrial contraction rate (ACR) was mediated by histamine receptors. CD treatment did not affect the baseline ACR which was 175 ($\pm$6.4) contractions/min prior to CD and 182.5 ($\pm$9.4) for the same atria treated with CD (100 μM, 1 hr). In preliminary experiments, CD (10 μM) produced a 12.6-fold rightward shift in the dose-response curve for histamine (data not shown). The -log K_B calculated for CD was 6.1, which was in good agreement with previously published results [20].

The cis-IAA-induced increases in ACR were resistant to blockade by CD (10 μM) (Fig. 4). None of the homologs were blocked entirely by CD. IPA was the most sensitive of the four homologs to CD. However, a 10-fold increase in the concentration of CD (100 μM) produced no further decrease in the response to IPA. CD concentrations in excess of 100 μM were not used in these experiments because (i) in general, the use of an antagonist at a concentration greater than 100 times the K_B is almost certain to block receptors other than the specific receptor [20], and (ii) at high concentrations cimetidine noncompetitively blocks *beta*-adrenoceptors [21]. Since the homologs were partially sensitive to antagonism by CD, the mechanism of action for these agonists may be related to more than one receptor system.

Blockade of H_2-receptors and beta-receptors

Atria were treated with CD (100 μM) and propranolol (PRO), a *beta*-adrenoceptor blocker (1 μM, 1 hr) and then dose-response curves for the homologs were performed. The combined antagonism produced by CD and PRO completely blocked the increases in ACR produced by cis-IAA or IPA (Fig. 4A and B). Therefore, adrenoceptors and histamine receptors both mediate the increase in ACR produced by these homologs in the isolated guinea pig right atrium.

Blockade of adrenergic neurons

The following experiment was performed to determine whether cis-IAA was acting directly on the *beta*-adrenoceptors in the sinus node or as an indirect agonist by the release of catecholamine stores in adrenergic neurons that innervate the nodal tissue. One animal was treated with reserpine (3 mg/kg, ip); the other (control) received a saline injection (1 ml, ip). The right atria were removed from both animals 16 hr later and were placed in the same tissue bath (i.e., 'paired'). Cis-IAA produced quantitatively similar (-log EC_{50} = 3.85 and 3.55) dose-related increases in ACR in both control and reserpine treated atria (Fig. 5) that were comparable to the mean control value for the cis-isomer in atria (Table 1). After washout of the effect of the cis-isomer, both atria received tyramine HCl (10–300 μM), an indirect (catecholamine releasing) adrenergic agonist. The reserpine-treated atrium was unresponsive to tyramine (Fig. 5B). Reserpine had depleted the readily releasable catecholamine stores in the adrenergic nerve terminals but did not affect the atrial response to cis-IAA. Therefore, cis-IAA was a direct acting agonist on *beta*-adrenoceptors in the atrial sinus node tissue.

Conclusions

On the basis of the apparent affinities of the homologs for receptors in the ileum, it would be likely to assume that no selectivity for H_1 or H_2 receptors existed in these compounds. However, a comparison of sensitivity of these agonists to selective H_1 and H_2 blockers revealed some important differences in their mechanisms of

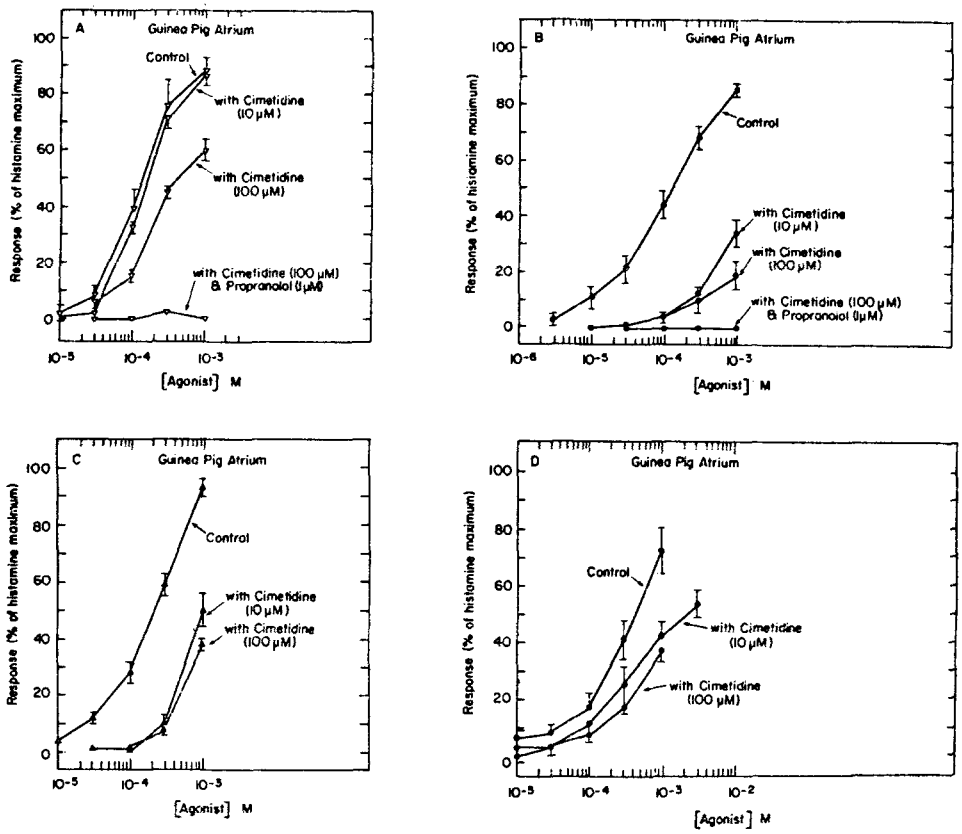


Figure 4
Effect of cimetidine and propranol on homolog-induced increases in atrial contraction rate. Varying degrees of resistance to blockade with cimetidine were evident. 4A and B. The combination of cimetidine with propranolol completely blocked atrial responses to cis-IAA or IPA (n = 4-8).

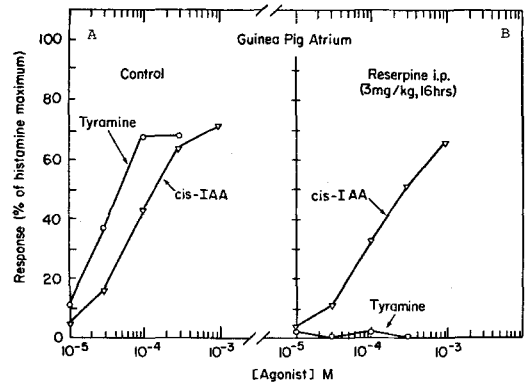


Figure 5
Evidence that rate-increasing effect of cis-IAA was not mediated by catecholamine release. Atrial responses to tyramine-induced release of neuro-transmitter were recorded after washout of cis-IAA. A. Control guinea pig received a 1 ml injection of saline (ip) 16 hrs prior to sacrifice. B. Reserpine treated guinea pig. Depletion of tyramine sensitive neuro-transmitter stores was indicated by lack of response to tyramine. Both atria were mounted in the same tissue bath in this experiment and, therefore, received identical exposure to these agonists.

action (Figs 3 and 4). For example, cis-IAA was relatively sensitive to H_1 receptor blockade but resistant to H_2 blockade. Thus, cis-IAA is structurally more compatible with H_1 receptors. In the atrium, the major receptor system contributing to the apparent affinity of cis-IAA for atrial chronotropic sites were the *beta*-adrenoceptors. In contrast, IPA, the saturated propyl

homolog, was the least sensitive to H_1 receptor blockade but the most sensitive to H_2 blockade. This structure-activity observation for IPA seems to correlate with the activity of the selective H_2 agonist dimaprit in which a fully saturated thio-propyl side chain separates the amino group from the tautomeric group.

If any advantage was to be gained by shortening the distance between the tautomeric imidazole group and the amino group in the side chain of the IPPA homolog, the advantage was readily offset by the rigid, linear nature to the side chain imparted by the introduction of the triple bond between the *beta* and *gamma* carbon atoms. This structural rigidity of IPPA may have accounted for its low ranking in terms of apparent affinity for receptors in either the ileum or atrium.

The average difference in apparent affinity of cis-IAA for receptors in the atrium or ileum (about 1.2×10^{-4} M) and the binding sites in the enzyme DAO (1.7×10^{-7} M) [11] is slightly over 700-fold. Cis-IAA can therefore be properly classified as a selective inhibitor of diamine oxidase. The apparent affinity of cis-IAA for DAO compares favorably with other inhibitors of this enzyme such as aminoguanidine which has an apparent affinity of 1.07×10^{-8} M [13].

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