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Histamine-Mediated Adenylate Cyclase Stimulation in Human Myocardium

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SUMMARY

We evaluated the mechanisms of histamine-mediated adenylate cyclase stimulation in 18 human hearts obtained from cardiac transplant recipients or prospective donors. Mg^{2+} and guanyl nucleotide were required for histamine stimulation. In the presence of 10^{-5} M GTP, histamine produced a maximal stimulation of 1.54 ± 0.06 times basal activity in left ventricle (39% of the isoproterenol maximum), which rose to 1.75 ± 0.14 times basal activity (68% of the isoproterenol maximum) in the presence of a 10^{-5} M concentration of the synthetic guanyl nucleotide 5′-guanylyl imidodiphosphate. Histamine stimulation of adenylate cyclase was antagonized by cimetidine ($K_B = 1.58 \times 10^{-6}$ M) but not by H_1-blocking doses of mepyramine or pyrrobutamine. The selective H_2 agonist dimaprit stimulated adenylate cyclase to approximately the same extent as histamine, whereas a selective H_1 agonist produced only minimal stimulation that was H_2-mediated. The selective H_2 agonist impromidine was a partial agonist and produced approximately 20–40% of maximal histamine stimulation at lower concentrations (10^{-7} and 10^{-6} M) and inhibition of histamine stimulation at higher concentrations (10^{-5} and 10^{-4} M). Histamine stimulated adenylate cyclase activity over the same dose range as that which produced a positive inotropic response in isolated papillary muscles. Under one set of assay conditions, contractile response and adenylate cyclase dose-response curves were essentially superimposable. We conclude that human myocardial adenylate cyclase is coupled to the H_2 receptor and linked to the contractile response, whereas the H_1 receptor does not mediate a biochemical or mechanical effect.

INTRODUCTION

Recent data from our laboratory suggest that histamine may be involved in cardiovascular disease processes (1–4). For example, histamine can cause coronary spasm in humans (2) and produce myocardial damage in animal systems (3, 4). Since histamine is found in abundance in heart (5) and may be released by a variety of mechanisms (1, 6), we have undertaken an investigation of the cardiovascular pharmacology of histamine in human heart.

We have previously shown that an H_2-receptor mechanism mediates the contractile response to histamine in human myocardium (7). Other investigators have described similar findings in guinea pig cardiac muscle (8–11). Although H_1-mediated (12, 13) and "non H_1-H_2" mediated (14) positive inotropic effects of histamine have been described in some species, we were unable to document a contractile response to histamine in isolated human myocardial tissue other than that mediated by H_2 receptors (7).

The contractile response of catecholamines and histamine is thought to be mediated through stimulation of adenylate cyclase and production of cyclic AMP (15–17). A previous report on histamine-mediated adenylate cyclase stimulation in human myocardium described blockade by diphenhydramine, an H_1 antagonist (17). A more recent study in developing human heart reported that cimetidine antagonized the response of adenylate cyclase to histamine stimulation (18), implying an H_2-receptor mechanism. Although animal studies utilizing guinea pig myocardium (8, 11, 19–21) have indicated H_2-receptor coupling to adenylate cyclase, studies in dog (22–24) and rabbit (12) tissue suggest that histamine may mediate a positive inotropic effect by a mechanism that does not include adenylate cyclase.

Because a uniform concept of myocardial histamine receptor subtypes has not emerged from previous animal or human studies, we decided to investigate the nature of human cardiac histamine receptor-adenylate cyclase coupling in more detail. The results indicate that in human heart histamine mediates its contractile response

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through an H_2 receptor coupled to adenylate cyclase, and that H_1 receptors do not mediate a mechanical or biochemical response.

METHODS

Human ventricular myocardium from transplant recipients ($n = 14$) or prospective transplant donors ($n = 4$) was used. Six cardiac transplant recipients had New York Heart Association Class IV symptomology related to congestive heart failure and idiopathic cardiomyopathy, and all demonstrated varying degrees of myocytic hypertrophy and fibrosis on light microscopic examination. Five patients were cardiac transplant recipients with end-stage coronary artery disease and Class IV heart failure; tissue was taken from noninfarcted areas. Morphological analysis of these areas revealed findings similar to those in the groups with idiopathic cardiomyopathy. One patient whose heart revealed hypertrophy and fibrosis under light microscopic examination was undergoing retransplantation for severe graft atherosclerosis. One patient was a heart-lung transplant recipient with normal left-ventricular morphology and moderate fibrosis in the right ventricle, and one patient was a cardiac transplant recipient with subendocardial fibrosis and a restrictive cardiomyopathy with normal left-ventricular morphology after removal of the subendocardial fibrosis. Finally, four hearts from prospective donors not utilized for transplantation because of technical reasons were studied, and these hearts had normal right and left ventricular morphology.

Hearts were immersed in ice-cold oxygenated Tyrode's solution (7) immediately after removal. Right- and left-ventricular papillary muscles were removed and mounted in a tissue bath according to previously described methods (7). Two grams of right- and left-ventricular myocardium were rapidly dissected and placed in 25 volumes of ice-cold sucrose (0.25 M)-Tris (5 mM)-EGTA[2] (1 mM) solution (pH 7.45). Tissue was obtained from the full thickness of the ventricular free walls, and areas of previous myocardial infarction were avoided. Aliquots of tissue were placed in 10% formalin and 2% glutaraldehyde and processed for light and electron microscopy.

Tissue was minced in 10 volumes of Tris-EGTA buffer and homogenized by three consecutive 5-sec bursts on a Polytron (Brinkmann Instruments, Westbury, N. Y.) at a setting of 11. The homogenate was then centrifuged at $1085 \times g$ for 20 min. The pellet was resuspended with an automatic mortar and pestle and then recentrifuged and resuspended twice.

After the final resuspension the homogenate was filtered through four layers of gauze to yield a final volume of 12 ml with a protein concentration of 5–12 mg/ml. Since in two initial experiments fresh and frozen preparations gave identical results, fresh preparations were not routinely evaluated, and 1.0-ml aliquots of homogenate were rapidly frozen in liquid nitrogen and stored at −70° for subsequent use.

Adenylate cyclase was assayed by the method of Salomon *et al.* (25) as modified by Johnson *et al.* (20). Enzyme protein (75–250 μg) was added to a reaction mixture that consisted of 0.1 mM Mg·ATP, 0.5 mM $MgCl_2$, 10 mM phosphocreatine, 14.5 μg of creatine kinase, 100 mM Tris-HCl (pH 7.45), and variable concentrations of guanyl nucleotides, agonists, and antagonists. ^{3}H-Labeled cyclic AMP (10,000–12,000 cpm/assay) was added prior to incubation for determination of recovery. Since the addition of 5 mM theophylline had no effect on isoproterenol or histamine-induced cyclic AMP production in four consecutive initial experiments, a phosphodiesterase inhibitor was not routinely added to the reaction mixture. The final reaction volume before addition of [α-^{32}P]ATP was 225 μl. Assay tubes were stored in cryogenic racks (Kryorack, Isolab Inc., Akron, Ohio) at 0°. The reaction mixture was prewarmed in a shaking water bath at 30° for 5 min, after which 25 μl (1.25–2.5 μCi) of [α-^{32}P]ATP (250–500 Ci/mM) were added to label the ATP pool. Assay time was 20 min; the reaction was stopped by the addition of 750 μl of 1% sodium dodecyl sulfate. ^{32}P-Labeled cyclic AMP was then isolated by the dual, Dowex-alumina column method of Salomon *et al.* (25). [α-^{32}P]ATP (New England Nuclear Corporation, Boston, Mass.) that gave reagent blanks of >50 cpm was purified on Dowex columns as described by Salomon (26). Recovery of cyclic AMP ranged from 70 to 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 triplicate, and activity was linear with respect to added enzyme protein and to time, over a period of 5–30 min.

^{3}H-Labeled cyclic AMP was obtained from New England Nuclear Corporation. Phosphocreatine, rabbit skeletal muscle creatine kinase, ATP, GTP, Gpp(NH)p, cyclic AMP, theophylline, histamine dihydrochloride, mepyramine, and (=)-isoproterenol HCl were obtained from Sigma Chemical Company (St. Louis, Mo.). Pyrrobutamine phosphate was a gift from Dr. Robert Hosley of Eli Lilly (Indianapolis, Ind.). Cimetidine was supplied by Smith Kline & French (Philadelphia, Pa.), and impromidine, 2,2T, and dimaprit were kindly supplied by Dr. Robin Ganellin of Smith Kline & French (Welwyn Garden City, Herts., England). (±)-Propranolol was supplied by Averst Laboratories (New York, N. Y.).

Proteins were assayed by the method of Lowry *et al.* (27). The dissociation constant (K_B) for cimetidine antagonism of histamine stimulation of adenylate cyclase was determined by the method of Furchgott (28), using results expressed as a percentage of maximal stimulation. For comparison of mean values between more than two groups, statistical significance was analyzed by the Newman-Keuls test of a one-way analysis of variance; a p value of $F \leq 0.05$ plus a mean difference greater than the 5% level of Student's range multiplied by the standard error of group mean was required for statistical significance (29). For differences between two groups the paired or unpaired Student's t-test was employed; $p \leq 0.05$ in the two-tailed distribution was considered statistically significant.

RESULTS

Kinetic Data and Choice of Assay Conditions

Mg:ATP and excess Mg (Mg^{2+}) kinetics was assessed in five initial experiments, and the results are expressed

[2] The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Gpp(NH)p, guanylyl imidodiphosphate; 2,2T, 2-(2-aminoethyl)thiazole.

in Table 1. In the presence of GTP, histamine did not significantly increase the V_{max} of Mg·ATP, as did 10^{-4} M (−)-isoproterenol and NaF. However, in the single experiment performed in the presence of Gpp(NH)p, histamine increased the Mg·ATP V_{max} to at least the same extent as isoproterenol, approximately 3 times the basal activity. In the presence of either GTP or Gpp(NH)p, both histamine and isoproterenol decreased the Mg^{2+} K_a without altering the Mg^{2+} V_{max}.

The ratio of maximal activity to basal activity did not increase beyond a Mg·ATP of 0.10 mM, and this substrate concentration was chosen as the standard assay condition. At this Mg·ATP concentration, stimulation relative to basal activity was found to be maximal at an excess Mg^{2+} of 0.5 mM for both histamine and isoproterenol, and this concentration of Mg^{2+} was chosen for standard assay conditions. The standard assay period was 20 min.

Effect of Guanyl Nucleotides

Histamine did not stimulate adenylate cyclase (data not shown) in the absence of added guanyl nucleotides. For GTP, 10^{-5} M proved to be the concentration that resulted in maximal enhancement of histamine and isoproterenol responses (data not shown). As shown in Fig. 1, 10^{-5} M Gpp(NH)p also proved to be the optimal concentration for producing maximal histamine stimulation. GTP did not increase basal activity, whereas Gpp(NH)p produced a dose-related increase in basal activity between 10^{-7} and 10^{-4} M, with a stimulation of basal activity at 10^{-5} M.

Dose-Response Characteristics of Histamine-Mediated Adenylate Cyclase Stimulation

Grouped left-ventricular data. In Fig. 2 are given grouped cumulative dose-response data in the 18 left-ventricular preparations. In the presence of 10^{-5} M GTP, histamine stimulation ranged from 1.21 to 2.06 times basal activity, with a mean of 1.54 ± 0.06. This mean maximal stimulation compared with 4.38 ± 0.45 times basal activity for isoproterenol in the same 18 preparations. As shown in Table 2, the presence of morphological damage did not affect either maximal histamine stimulation or the ED_{50}.

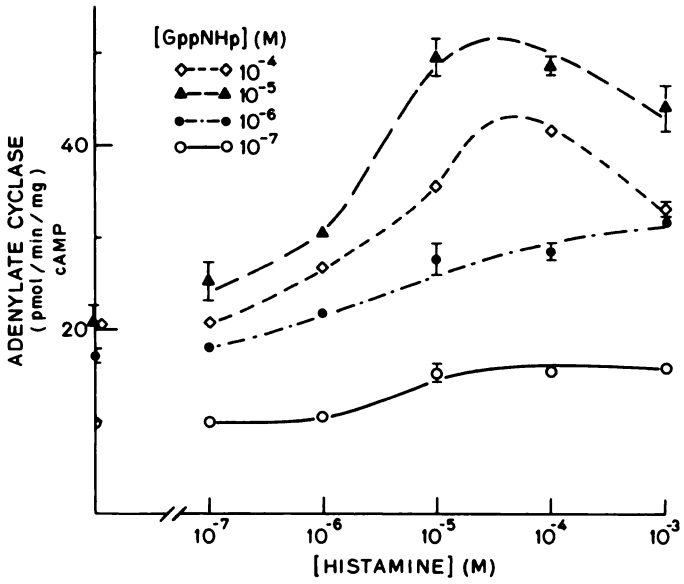

FIG. 1. *Effect of varying concentrations of Gpp(NH)p on histamine-stimulated adenylate cyclase activity in human (left-ventricular) myocardium*

Mg·ATP was 0.1 mM and Mg^{2+} was in excess of 0.5 mM. Bars represent standard error of the mean of triplicates; points with no bars represent mean values of duplicates. Points on the ordinate represent basal activities.

Dose-response curves derived in the presence of 10^{-5} M Gpp(NH)p in these same preparations are also given in Fig. 2. Maximal stimulation ranged from 1.19 to 3.20 times basal activity (mean 1.75 ± 0.14), and there was no effect of morphological damage on maximal stimulation or ED_{50} (Table 2). Isoproterenol produced a maximal effect 2.58 ± 0.23 times basal activity in these same 18 preparations.

As shown in Fig. 3 and Table 2, when histamine-GTP or Gpp(NH)p data were expressed as percentage of maximal stimulation, the ED_{50} for histamine-Gpp(NH)p was left-shifted relative to GTP. A Hill plot of the six most

TABLE 1

Effect on agonist stimulation on Mg·ATP and Mg^{2+} kinetics

Guanyl nucleotide, agonist	Mg·ATP			Mg^{2+}		
	K_m	V_{max}	Apparent Hill coefficient	K_a	V_{max}	Apparent Hill coefficient
	mM	*pmoles/min/mg cyclic AMP*		*mM*	*pmoles/min/mg cyclic AMP*	
GTP, 10^{-5} M[a]						
Basal	0.034±0.004	16.7±3.3	1.01±0.02	1.96±0.29	34.6±7.2	0.90±0.05
Histamine, 10^{-4} M	0.042±0.004	23.2±3.7	0.98±0.01	1.43±0.28[c]	36.2±6.6	0.87±0.04[b]
Isoproterenol, 10^{-4} M	0.059±0.004	46.7±8.0[c]	0.97±0.01	0.51±0.03[c]	44.4±7.8	1.02±0.05
NaF, 2 mM	0.064±0.012[c]	48.9±7.6[c]	0.93±0.03	3.64±0.17[c]	105.2±19.0[c]	0.99±0.01
Gpp(NH)p, 10^{-5} M[d]						
Basal	0.037	17.8	0.85	2.75	59.9	0.91
Histamine, 10^{-4} M	0.059	53.8	0.85	0.65	64.1	0.88
Isoproterenol, 10^{-4} M	0.032	42.2	1.12	0.77	64.5	0.87

[a] $n = 4$, mean ± standard error of the mean; two left-ventricular and two right-ventricular preparations.
[b] $p < 0.05$ compared with unity.
[c] $p(F) < 0.05$.
[d] Single experiment (right-ventricular preparation).

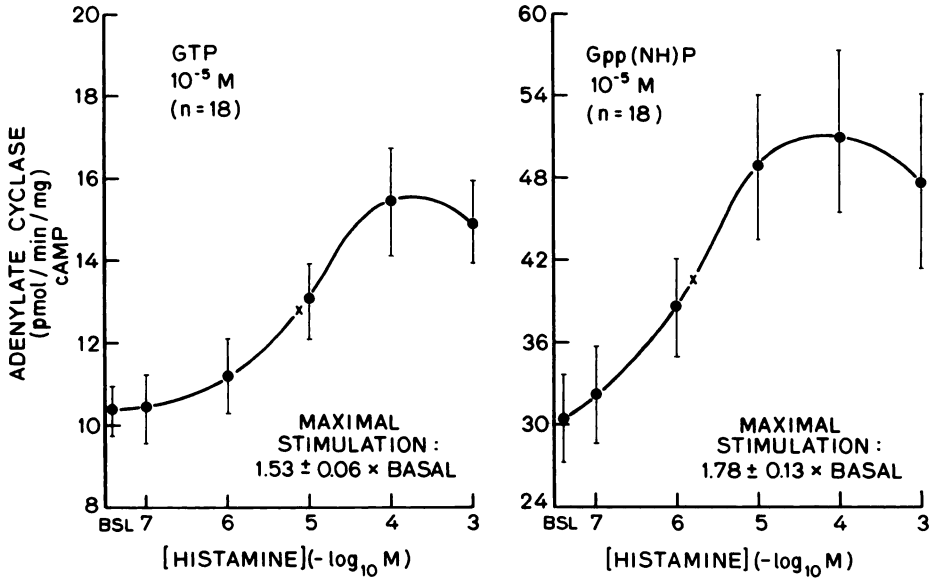

FIG. 2. *Grouped data from 18 human (left-ventricular) myocardial preparations: histamine-mediated adenylate cyclase stimulation in presence of 10^{-5} M Gpp(NH)p or GTP*

Mg·ATP was 0.1 mM and Mg^{2+} was in excess of 0.5 mM. Bars represent standard error of the mean. ×, ED_{50}.

active left-ventricular preparations yielded a K_{act} for histamine of 9.69×10^{-6} M in the presence of 10^{-5} M GTP and 1.39×10^{-6} M in the presence of 10^{-5} M Gpp(NH)p ($p < 0.05$), with respective apparent Hill coefficients of 0.90 and 0.74.

Right versus left ventricle: comparison with contractile response data. For the initial six patients full dose-response curves to histamine were determined in both ventricles. Compared with GTP, the synthetic, nonhydrolyzable guanyl nucleotide Gpp(NH)p increased basal activity and enhanced histamine stimulation in both ventricles. There was no statistically significant difference between left- and right-ventricular maximal histamine stimulation in the presence of either Gpp(NH)p or GTP: Gpp(NH)p, left ventricle = 2.24 ± 0.40, right ventricle = 1.98 ± 0.33; GTP, left ventricle = 1.57 ± 0.10, right ventricle = 1.58 ± 0.07 times basal activity.

Adenylate cyclase activity in right and left ventricle expressed as percentage of maximal stimulation is given in Fig. 3 and is compared with contraction dose-response curves expressed in the same manner. Dose-response curves for adenylate cyclase stimulation in the presence of Gpp(NH)p and contraction dose-response curves are comparable and are to the left of cyclase-GTP data (ED_{50} $pF < 0.05$ for both). Respective ED_{50} values (micromolar) obtained from data in Fig. 3 were as follows: left ventricle, GTP = 8.9 ± 0.9, Gpp(NH)p = 1.9 ± 0.4, contraction = 2.5 ± 0.6; right ventricle, GTP = 9.3 ± 1.8, Gpp(NH)p = 1.9 ± 0.5, contraction = 2.2 ± 0.4.

Effect of Beta-Adrenergic Blockade

Because histamine may release catecholamines in cardiac preparations (7, 22, 30, 31), we evaluated the effect of histamine on adenylate cyclase in the presence of 10^{-6} M propranolol (Fig. 4). Propranolol has a K_B value of 1.73 ± 0.38 × 10^{-9} M in this preparation, and 10^{-6} M propran-

TABLE 2

Summary of histamine-mediated adenylate cyclase stimulation in human left ventricular myocardium

Group	Basal activity	Histamine stimulation		Maximum/basal	ED_{50}
		Maximal[a]	Net[b]		
	pmoles/min/mg cyclic AMP	*pmoles/min/mg cyclic AMP*			*μM*
All preparations (n = 18)					
GTP, 10^{-5} M	9.71 ± 0.69	14.99 ± 1.19	5.25 ± 0.68	1.54 ± 0.06	10.04 ± 1.14
Gpp(NH)p, 10^{-5} M	30.43 ± 3.19	52.24 ± 6.04	21.81 ± 4.04	1.75 ± 0.14	2.05 ± 0.27
Normal morphology (n = 6)					
GTP, 10^{-5} M	9.83 ± 1.59	15.27 ± 2.50	5.44 ± 1.27	1.56 ± 0.11	11.03 ± 2.03
Gpp(NH)p, 10^{-5} M	35.91 ± 5.46	53.78 ± 7.77	17.87 ± 3.07	1.51 ± 0.07	2.03 ± 0.57
Abnormal morphology (n = 12)					
GTP, 10^{-5} M	9.65 ± 0.72	14.85 ± 1.37	5.20 ± 0.83	1.53 ± 0.07	9.55 ± 1.43
Gpp(NH)p, 10^{-5} M	27.69 ± 3.84	51.46 ± 8.40	23.77 ± 5.87	1.88 ± 0.19	2.06 ± 0.32

[a] Absolute maximal stimulation.

[b] Absolute maximal minus basal activity.

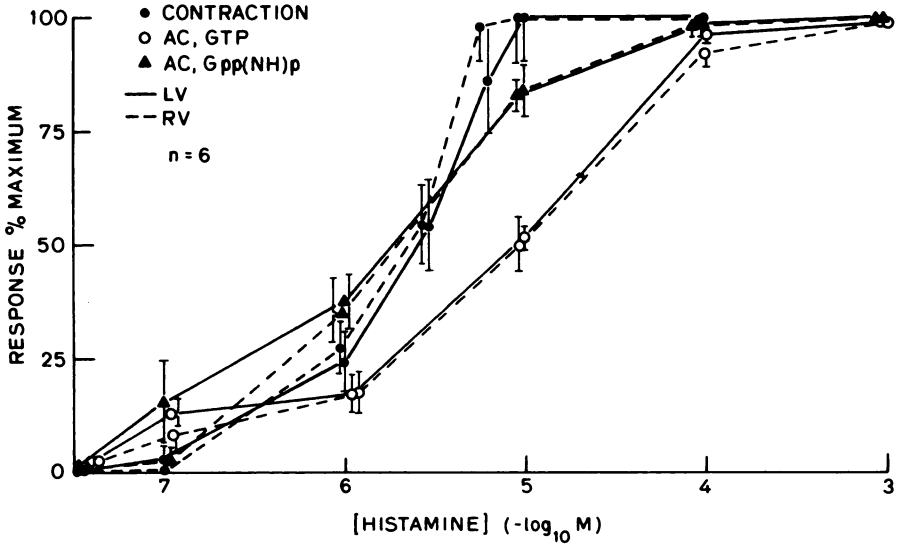

FIG. 3. *Comparison of histamine-mediated adenylate cyclase (AC) stimulation and positive inotropic effects in six left-ventricular and right-ventricular human myocardial preparations*

GTP and Gpp(NH)p were 10^{-5} M, Mg·ATP was 0.1 mM, and Mg^{2+} was 0.5 mM. Results are expressed as percentage of maximum, with 0 = basal activity (cyclase) or baseline tension and 100% = maximal effect obtained, defined as that dose beyond which no further increase in effect occurs. Bars represent standard error of the mean.

olol completely blocks the effect of isoproterenol in doses up to 10^{-5} M.[3] As shown in Fig. 4, in the presence of 10^{-5} M GTP there was no effect of propranolol on histamine stimulation. When 10^{-5} M Gpp(NH)p was employed as the guanyl nucleotide, propranolol decreased basal activity and maximal stimulation, but basal activity was decreased to a relatively greater extent. This led to an increase in "net" histamine stimulation in the presence of propranolol [35.79 ± 4.92 pmoles/min/mg of cyclic AMP versus 26.61 ± 6.34 in the absence of propranolol ($p < 0.05$)]. When dose-response data were expressed as percentage of maximal stimulation, propranolol did not affect the histamine ED_{50} (no propranolol, 2.87 ± 0.39 × 10^{-6} M; propranolol, 2.88 ± 0.38 × 10^{-6} M).

With 10^{-5} M Gpp(NH)p in the assay medium, isoproterenol produced a net stimulation of 46.89 ± 10.42 in these same five preparations ($p > 0.10$ versus histamine stimulation in the presence of propranolol). In the presence of propranolol, the histamine fold-stimulation (maximal activity/basal activity) was actually greater than that for isoproterenol stimulation in the absence of propranolol [2.49 ± 0.14 versus 2.01 ± 0.15 ($p < 0.05$).

Blockade by Selective H_1 and H_2 Antagonists

The increase in histamine sensitivity imparted by 10^{-5} M Gpp(NH)p allowed the determination of quantitative aspects of histamine-antagonist relationships. Dose-response curves for histamine were determined from 10^{-7} to 10^{-3} M in the presence and absence of increasing doses of the H_2 antagonist cimetidine ($n = 7$) or the potent H_1 antagonists mepyramine ($n = 4$) and pyrrobutamine ($n = 2$) (7).

Increasing doses of cimetidine produced a progressive rightward shift in the histamine dose-response curve, as shown in Fig. 5. At cimetidine doses above 10^{-6} M a small reduction in maximum was noted in all preparations, indicating a noncompetitive component of cimetidine antagonism. If competitive blockade by 10^{-6} M cimetidine is assumed, the mean K_B value in seven preparations (five left-ventricular and two right-ventricular) was 1.58 ± 0.56 × 10^{-6} M.

In the presence of Gpp(NH)p, mepyramine ($n = 4$) and pyrrobutamine ($n = 2$) at concentrations >10^{-6} M also antagonized histamine stimulation of adenylate cyclase (Fig. 5). However, doses of mepyramine or pyrrobutamine that produced a rightward shift in the histamine dose-response curves invariably produced a decrease in basal activity, usually accompanied by a decrease in slope. The addition of propranolol (10^{-6} M) did not alter the effect of mepyramine, pyrrobutamine, or cimetidine on histamine dose-response curves.

The effects of 10^{-7} M mepyramine and 10^{-5} M cimetidine on histamine-mediated adenylate cyclase stimulation in the presence of 10^{-5} M GTP were determined in six left-ventricular preparations exhibiting higher degrees of histamine stimulation; these data are presented in Fig. 6. The concentrations of both antagonists were approximately 10-fold higher than the dissociation constants (K_B values) determined for inhibition of histamine-mediated contraction in human coronary artery or rabbit aorta (mepyramine[3]), or inhibition of histamine-mediated stimulation of adenylate cyclase in the presence of 10^{-5} M Gpp(NH)p (cimetidine). These concentrations of mepyramine and cimetidine did not lower basal activity (data not shown), in contrast to higher concentrations. Cimetidine at 10^{-5} M lowered net histamine stimulation (histamine-stimulated activity minus basal activity) from 4.01 ± 0.57 to 0.64 ± 0.18 pmole/min/mg ($pF < 0.05$). Mepyramine did not significantly affect the histamine response (net stimulation = 2.96 ± 0.49 pmoles/min/mg, $pF > 0.05$).

[3] M. R. Bristow, R. Cubicciotti, R. Ginsburg, E. B. Stinson, and C. Johnson, unpublished data.

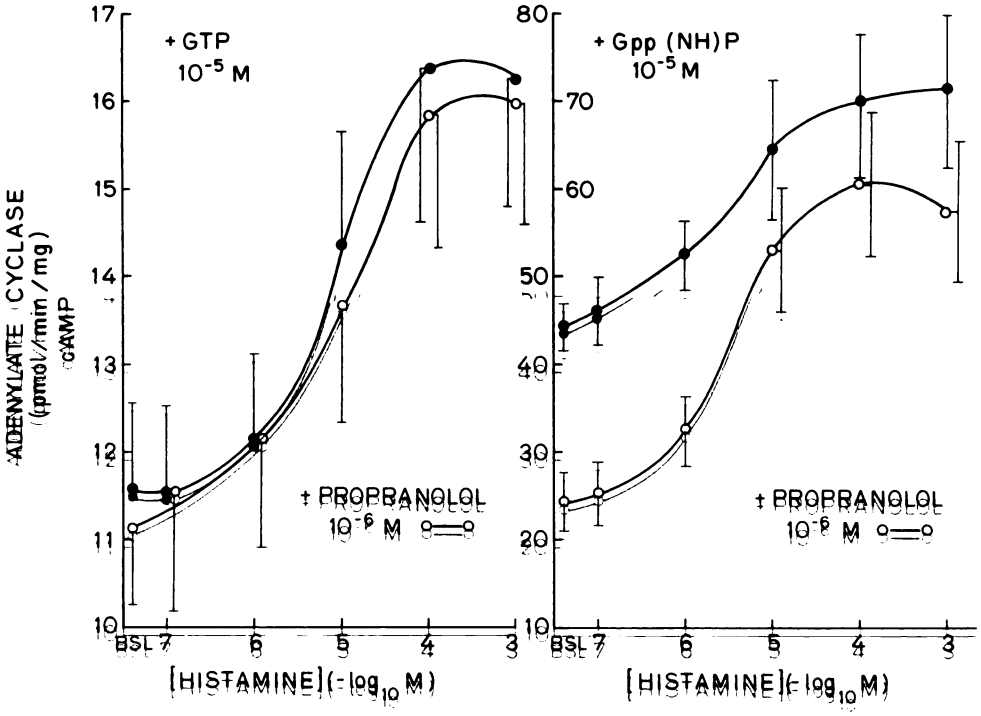

FIG. 4. *Effect of propranolol on histamine dose-response curves in five left-ventricular preparations*
Left, GTP 10^{-5} M: ●, histamine alone; ○, histamine + 10^{-6} M propranolol. Right, Gpp(NH)p 10^{-5} M: ●, histamine alone; ○, histamine + 10^{-6} M propranolol. See Fig. 2 for other assay conditions. Experimental points represent mean ± standard error of the mean.

Activation by Selective Agonists

The selective H_2 agonists impromidine (32) and dimaprit (33) and the selective H_1 agonist 2,2T (34) were evaluated for their effect on adenylate cyclase stimulation. In Fig. 7 is shown one of three experiments in which the activity of isoproterenol, histamine, impromidine, dimaprit, and 2,2T was assessed. Impromidine acted as a partial agonist with ≤50% of the activity of histamine. However, the affinity of impromidine was greater than that of histamine, as the ED_{50} for its effect was $\leq 10^{-2}$ M. That impromidine acts as a partial agonist for adenylate cyclase stimulation in human myocardium is further illustrated in Fig. 8, an individual experiment in which higher doses (10^{-5} and 10^{-4} M) of impromidine antagonized histamine stimulation.

Dimaprit was more potent than impromidine and produced 70–90% of the effect of histamine with a mean ED_{50} of 3.2×10^{-5} M. 2,2T produced a slight stimulation at concentrations $\geq 10^{-6}$ M; however, this activity was shown to occur through H_2 receptor stimulation, as in two experiments 10^{-5} M cimetidine but not 10^{-7} mepyramine blocked the 2,2T effect.

DISCUSSION

This investigation demonstrates that in human myocardium histamine is a relatively potent stimulant of adenylate cyclase activity, producing with one set of assay conditions a maximal stimulation that was at least as great as that of isoproterenol. Our data on the mechanism by which biogenic amines stimulate adenylate cyclase activity in human myocardium are in general agreement with previous work in other cardiac preparations. Under at least one set of assay conditions, histamine, (−)-isoproterenol, and fluoride ion increased the maximal reaction rate relative to available substrate, as has been reported for epinephrine in rabbit and guinea pig ventricular preparations (35). For histamine and isoproterenol at least part of the enhancement in reaction rate could be attributed to an increase in the affinity of Mg^{2+} for its catalytic binding site. The effect of histamine on Mg^{2+} was modulated by guanyl nucleotides, as shown by an additional lowering of the Mg^{2+} K_a in the presence of Gpp(NH)p. These data are in agreement with previous work in guinea pig cardiac tissue (36).

Human adenylate cyclase preparations exhibited guanyl nucleotide requirements similar to those described previously in guinea pig hearts (19). Guanyl nucleotides were necessary for histamine activation of adenylate cyclase, and relative to GTP the nonhydrolyzable compound Gpp(NH)p rendered the system more sensitive to histamine and increased the maximal stimulation. Assuming that receptor occupancy is proportional to response, the 4- to 5-fold decrease in the ED_{50} of histamine conferred by Gpp(NH)p could be due to an increase in agonist affinity, as indicated by the decrease in K_{act}. However, since data from *beta*-adrenergic systems indicate that the Gpp(NH)p-induced decrease in K_{act} is actually accompanied by a decrease in agonist affinity (37, 38), the effect of Gpp(NH)p observed in our preparations is most likely due to a direct action on the guanyl nucleotide regulatory subunit of adenylate cyclase, or an action beyond the actual combination of histamine with the H_2 receptor.

The effect of histamine on adenylate cyclase was clearly independent of catecholamine release (7, 22, 30, 31) or activation of the *beta*-adrenergic pathway, as propranolol (10^{-6} M) either had no effect or actually

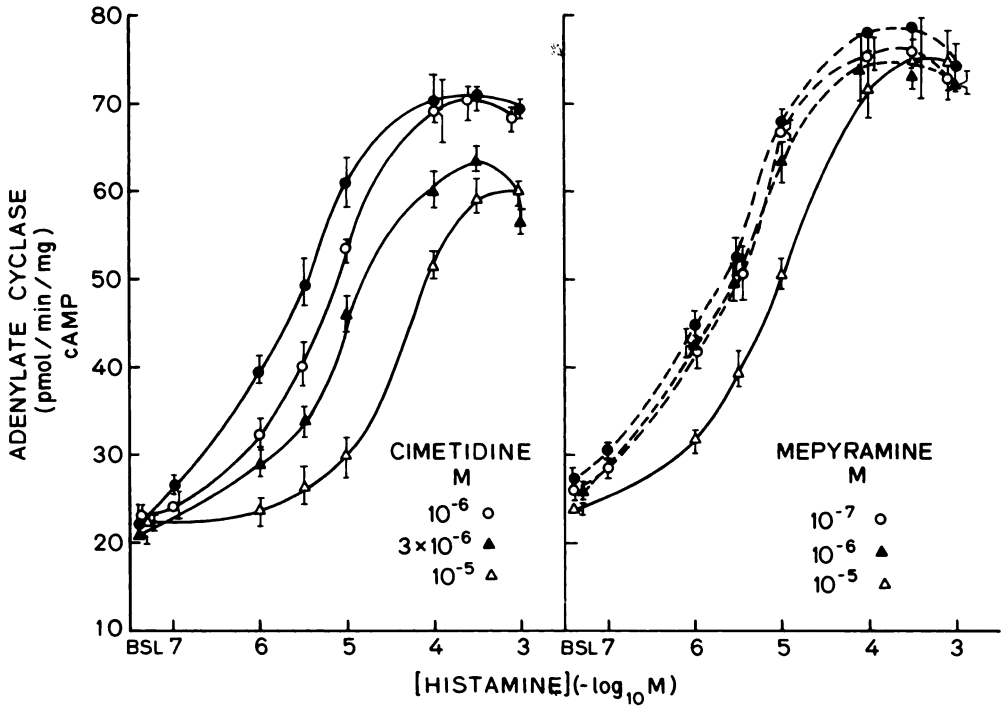

FIG. 5. *Effect of increasing doses of cimetidine or mepyramine on histamine-stimulated adenylate cyclase activity in left-ventricular preparations of human myocardium*

Left, ●, histamine alone; ○, histamine + 10^{-6} M cimetidine; ▲, histamine + 3×10^{-6} M cimetidine; △, histamine + 10^{-5} M cimetidine. Right, ●, histamine alone; ○, histamine + 10^{-7} M mepyramine; ▲, histamine + 10^{-6} M mepyramine; △, histamine + 10^{-5} M mepyramine. Assay conditions: 10^{-5} M (Gpp(NH)p and 10^{-6} M propranolol; 0.1 mM Mg·ATP and 0.5 mM Mg^{2+} in excess. Experimental points represent mean ± standard error of the mean of triplicates.

enhanced maximal histamine stimulation. The presence of myocardial disease did not alter the effect of histamine, as maximal responses and ED_{50} values were similar in morphologically normal hearts and hearts that had histological evidence of hypertrophy and fibrosis.

Our data are consistent with coupling of human cardiac adenylate cyclase to an H_2 receptor. Cimetidine antagonized the histamine response in both GTP- and Gpp(NH)p-incubated preparations. The antagonism of histamine by cimetidine appeared to have a noncompetitive component at higher ($>10^{-6}$ M) doses and thus the calculation of a dissociation constant from Schild plots (39) could not be accomplished. If antagonism by 10^{-6} M cimetidine was assumed to be competitive, the K_B for cimetidine was 1.58×10^{-6} M, which is similar to the value obtained in guinea pig hearts (20).

The response to selective agonists was also similar to that previously reported in guinea pig myocardium (19) and consistent with an H_2-receptor system. The selective H_2 agonists dimaprit and impromidine stimulated adenylate cyclase activity. Impromidine produced <50% of the maximal effect of histamine and at high doses antagonized histamine stimulation, thus demonstrating that impromidine is a partial agonist in human myocardium. Although the efficacy of impromidine was low (20–40% of histamine maximum) its affinity was quite high, with an ED_{50} less than that for histamine.

The selective H_1 agonist 2,2T [H_1/H_2 activity approximately 90:1 in experimental systems (34)] also stimulated adenylate cyclase activity, but the response was extremely small and occurred only at concentrations $\geq 10^{-5}$ M. That the effect was due to activation of the H_2 receptor rather than an H_1 response was demonstrated by blockade by cimetidine and not by mepyramine.

Although the potent H_1-selective antagonists mepyramine and pyrrobutamine inhibited histamine stimulation of adenylate cyclase slightly, they did so only at concentrations that would occupy >99% of H_1 receptor sites of the type that are found in human coronary artery (7) or rabbit aorta. This slight inhibition is therefore not consistent with occupation of the H_1 receptor known to be present in the human heart, as blockade of this receptor would be expected to produce a much greater degree of histamine antagonism. One explanation for inhibition of histamine-mediated adenylate cyclase by these drugs is "nonselective," low-affinity competitive blockade of the H_2 receptor, as described by Johnson *et al.* (20) in guinea pig heart. An alternative explanation for inhibition of histamine-mediated adenylate cyclase activity by ultrahigh doses of H_1 antagonists is "nonspecific" inhibitory effects, support for which comes from the decrease in basal activity produced by $>10^{-6}$ M mepyramine or pyrrobutamine. Such nonselective or nonspecific inhibition presumably explains the results of an earlier investigation that reported inhibition of human cardiac adenylate cyclase by a high dose of the H_1 antagonist diphenhydramine (17).

The results of this investigation are in general agreement with our previous work on histamine-mediated physiological responses in human heart (7) in that blockade by selective antagonists or activation by selective agonists is similar for adenylate cyclase and inotropic

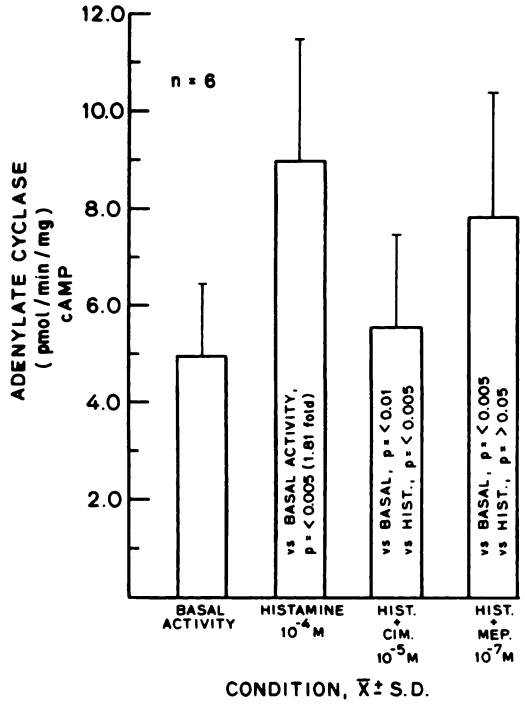

FIG. 6. *Effect of 10^{-7} M mepyramine (Mep) and 10^{-5} M cimetidine (Cim) on maximal histamine (Hist) stimulation in six consecutive left-ventricular preparations*

Values are means ± standard deviation. The guanyl nucleotide was GTP (10^{-5} M); other assay conditions are given in Fig. 2.

response stimulation. In the current investigation, enzymatic and mechanical dose-response relationships encompassed similar histamine concentration ranges when Gpp(NH)p was employed as the guanyl nucleotide. Although use of Gpp(NH)p is certainly not "physiological," it is unlikely that the conditions *in vivo* for receptor-adenylate cyclase-contractile response coupling can be

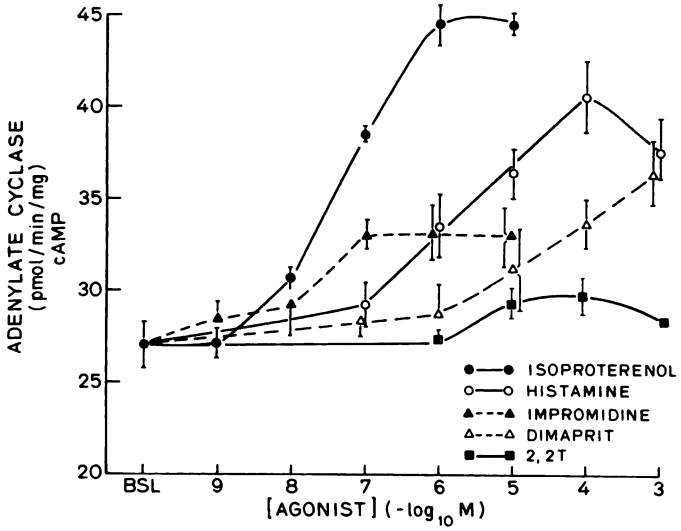

FIG. 7. *Effect of isoproterenol, histamine, impromidine, dimaprit, and 2,2T on adenylate cyclase activity in human (left-ventricular) myocardium*

See Fig. 5 for assay conditions. Bars represent standard error of the mean of triplicates.

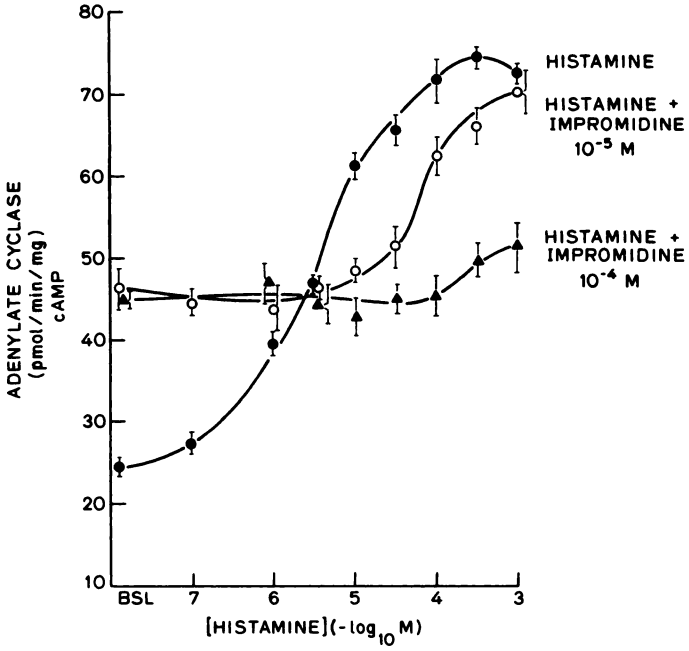

FIG. 8. *Effect of impromidine on histamine-stimulated adenylate cyclase activity in human left-ventricular myocardium*

●, Histamine alone; ○, histamine + 10^{-5} M impromidine; ▲, histamine + 10^{-4} M impromidine. *BSL*, basal activity (in absence of histamine). See Fig. 5 for assay conditions.

precisely duplicated by any set of assay conditions. The similarity of adenylate cyclase-Gpp(NH)p and contraction dose-response relationships merely demonstrates that under certain circumstances enzymatic and mechanical responses of histamine are quantitatively similar. Taken together, the results of this and our previous investigation (7) indicate that in human myocardium histamine increases the force of contraction by stimulating adenylate cyclase activity through combination with the H_2 receptor.

APPENDIX

List and Derivation of Terms and Abbreviations

K_m. Apparent Michaelis constant, or substrate concentration at one-half maximal reaction rate, determined from plot of $1/v$ versus $1/S$, where v = reaction velocity and S = substrate concentration. In such a plot, V_{max} (maximal reaction velocity) = $1/y$ intercept and K_m = slope × V_{max}.

K_a. Dissociation constant for catalytic (Mg^{2+}) activation = concentration required for one-half maximal activation, determined by double-reciprocal plot as for K_m.

K_B. Dissociation constant for competitive antagonist determined by "classical" methods involving shifts of maximal dose-response curves, where $K_B = B/x-1$ (28), x = dose ratio (or concentration of agonist necessary to produce a one-half maximal response in the presence of antagonist + concentration of agonist necessary to produce one-half maximal response in the absence of antagonist), and B = antagonist concentration.

K_{act}. Activation constant, or concentration of hormone agonist required to produce one-half maximal activity, as

determined by a Hill plot of log $v/V_{max} - v$ versus log S (hormone), where slope = apparent Hill coefficient and x intercept = K_{act}. If receptor occupancy is directly related to enzyme activity, $K_{act} = K_D$, or the dissociation constant of the agonist. If dose-response curves are expressed as percentage of maximum, then $K_{act} = ED_{50}$.

REFERENCES

1. Bristow, M. R., R. Ginsburg, and D. C. Harrison. Histamine and the human heart: the other receptor system. *Am. J. Cardiol.* **49**:249–251 (1982).
2. Ginsburg, R., M. R. Bristow, N. Kantrowitz, D. S. Baim, and D. C. Harrison. Provocation of coronary artery spasm by histamine: new insights into mechanisms. *Am. Heart J.* **102**:819–822 (1981).
3. Bristow, M. R., W. A. Minobe, M. E. Billingham, J. B. Marmor, C. A. Johnson, B. M. Ishimoto, W. S. Sageman, and J. R. Daniels. Anthracycline-associated cardiac and renal damage in rabbits: evidence for mediation by vasoactive substances. *Lab. Invest.* **45**:157–168 (1981).
4. Kantrowitz, N. E., W. A. Minobe, M. R. Bristow, and M. E. Billingham. Histamine-mediated myocardial necrosis. *Circulation* **64**:IV–282 (1981).
5. Mannaioni, P. F. Physiology and pharmacology of cardiac histamine. *Arch. Int. Pharmacodyn. Ther.* **196**:64–67 (1972).
6. Levi, R., and G. Allan. Histamine-mediated cardiac effects, in *Drug-Induced Heart Disease* (M. R. Bristow, ed.). Amsterdam, Biomedical Press, 377–395 (1980).
7. Ginsburg, R., M. R. Bristow, Sxinson, E. B., and D. C. Harrison. Histamine receptors in the human heart. *Life Sci.* **26**:2245–2249 (1980).
8. McNeill, J. H., and S. C. Verma. Blockade by burimamide of the effects of histamine and histamine analogs on cardiac contractility, phosphorylase activation and cyclic adenosine monophosphate. *J. Pharmacol. Exp. Ther.* **188**:180–188 (1973).
9. Levi, R., N. Capurro, and C. H. Lee. Pharmacological characteristics of cardiac histamine receptors: sensitivity to H_1- and H_2-receptor agonists and antagonists. *Eur. J. Pharmacol.* **30**:328–335 (1975).
10. Reinhardt, D., H. M. Wiemann, and H. J. Schuemann. Effects of the H_1-antagonist promethazine and the H_2-antagonist burimamide on chronotropic, inotropic and coronary vascular response to histamine in isolated perfused guinea-pig hearts. *Agents Actions* **6**:683–689 (1976).
11. Johnson, C. L., and H. Mizoguchi. The interaction of histamine and guanyl nucleotides with cardiac adenylate cyclase and its relationship to cardiac contractility. *J. Pharmacol. Exp. Ther.* **200**:174–186, (1977).
12. McNeill, J. H., and S. C. Verma. Histamine receptors in rabbit heart. *Proc. West. Pharmacol. Soc.* **21**:99–101 (1978).
13. Verma, S. C., and J. H. McNeill. Cardiac histamine receptors: differences between left and right atria and right ventricle. *J. Pharmacol. Exp. Ther.* **200**:352–362 (1979).
14. Wilson, C., and K. J. Broadley. A positive inotropic response of guinea pig isolated atria to histamine not mediated via H_1 or H_2 receptors. *Can. J. Physiol. Pharmacol.* **58**:167–173 (1981).
15. Sutherland, E. W., G. A. Robison, and R. W. Butcher. Some aspects of the biological role of adenosine 3′,5′-monophosphate (cyclic AMP). *Circulation* **37**:279–306 (1968).
16. Epstein, S. E., C. L. Skelton, G. S. Levey, and M. Entman. Adenyl cyclase and myocardial contractility. *Ann. Intern. Med.* **72**:561–578 (1970).
17. Klein, I., and G. S. Levey. Activation of myocardial adenyl cyclase by histamine in guinea pig, cat, and human heart. *J. Clin. Invest.* **50**:1012–1015 (1971).
18. Wollemann, M., and J. G. Papp. Blockade by cimetidine of the effects of histamine on adenylate cyclase activity, spontaneous rate and contractility in the developing prenatal heart. *Agents Actions* **9**:29–30 (1979).
19. Johnson, C. L., H. Weinstein, and J. P. Green. Studies on histamine H_2 receptors coupled to cardiac adenylate cyclase: effects of guanyl nucleotides and structural requirements for agonist activity. *Biochim. Biophys. Acta* **587**:155–168 (1979).
20. Johnson, C. L., H. Weinstein, and J. P. Green. Studies on histamine H_2 receptors coupled to cardiac adenylate cyclase: blockade by H_2 and H_1 receptor antagonists. *Mol. Pharmacol.* **16**:417–428 (1979).
21. Kanof, P. D., and P. Greengard. Pharmacological properties of histamine-sensitive adenylate cyclase from guinea pig cardiac ventricular muscle. *Mol. Pharmacol.* **15**:445–461 (1979).
22. Flacke, W., D. Atanackovic, R. A. Gillis, and M. H. Alper. The actions of histamine on the mammalian heart. *J. Pharmacol. Exp. Ther.* **155**:271–278 (1967).
23. Bristow, M. R., W. S. Sageman, R. H. Scott, M: E. Billingham, R. E. Bowden, R. S. Kernoff, G. H. Snidow, and J. R. Daniels. Acute and chronic cardiovascular effects of doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. *J. Cardiovasc. Pharmacol.* **2**:487–515 (1980).
24. Cros, G. H., S. Katz, and J. H. McNeill. The effect of histamine and epinephrine on adenylate cyclase prepared from dog ventricle. *Can. J. Physiol.* **58**:1124–1125 (1980).
25. Salomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
26. Salomon, Y. Adenylate cyclase assay. *Adv. Cyclic Nucleotide Res.* **10**:35–55 (1979).
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
28. Furchgott, R. F. The pharmacological differentiation of adrenergic receptors. *Ann. N. Y. Acad. Sci.* **139**:553–570 (1967).
29. Snedecor, G. W., and W. G. Cochran. *Statistical Methods*, Ed. 7. Iowa State University Press, Ames, Iowa (1980).
30. Planin, A., T. E. Tenner, Jr., and J. H. McNeill. The characterization of cardiac histaminergic receptors in the rabbit. *Can. J. Physiol. Pharmacol.* **59**:14–18 (1981).
31. Laher, I., and J. H. McNeill. Effects of histamine on isolated rat atria. *Can. J. Physiol. Pharmacol.* **58**:1114–1116 (1980).
32. Durant, G. J., W. A. M. Duncan, C. R. Ganellin, M. E. Parsons, R. C. Blakemore, and A. C. Rasmussen. Impromidine (SKF 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature* (*Lond.*) **276**:403–405, (1978).
33. Parsons, M. E., D. A. A. Owen, C. R. Ganellin, and G. J. Durant. Dimaprit ([S-[3-(N,N-dimethylamino)propyl]isothiorea]—a highly specific histamine H_2-receptor agonist. *Agents Actions* **7**:31–37 (1977).
34. Durant, G. J., C. R. Ganellin, and M. E. Parsons. Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. Med. Chem.* **18**:905–909 (1975).
35. Drummond, G. I., D. L. Severson, and L. Duncan. Adenyl cyclase: kinetic properties and nature of fluoride and hormone stimulation. *J. Biol. Chem.* **246**:4166–4173 (1971).
36. Alvarez, R., and J. J. Bruno. Activation of cardiac adenylate cyclase: hormonal modification of the magnesium ion requirement (3′:5′-cyclic AMP/epinephrine/histamine/fluoride ion/guanine nucleotides). *Proc. Natl. Acad. Sci. U. S. A.* **74**:92–95 (1977).
37. Lefkowitz, R. J., D. Millikin, and M. G. Caron. Regulation of β-adrenergic receptors by guanyl-5′-yl imidodiphosphate and other purine nucleotides. *J. Biol. Chem.* **251**:4686–4692 (1976).
38. Ross, E. M., M. E. Maguire, T. W. Sturgill, R. L. Biltonen, and A. G. Gilman. Relationship between the β-adrenergic receptor and adenylate cyclase. *J. Biol. Chem.* **252**:5761–5775 (1977).
39. Arunlakshana, O., and H. O. Schild. Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* **14**:48–58 (1959).

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