JCI The Journal of Clinical Investigation

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J Clin Invest. 1971;50(5):1012-1015. https://doi.org/10.1172/JCI106571.

Research Article

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Activation of Myocardial Adenyl Cyclase by Histamine in Guinea Pig, Cat, and Human Heart

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ABSTRACT Histamine has positive inotropic and chronotropic effects on the heart which are not abolished by beta adrenergic–blocking agents. Since the positive inotropic and chronotropic effects of other hormones on the heart are thought to be mediated by cyclic 3′,5′-AMP, we examined the effect of histamine on adenyl cyclase in particulate preparations of guinea pig, cat, and human myocardium. Histamine at the peak of its dose-response curve, 3×10^{-4}moles/liter, produced approximately a 300% increase in cyclic 3′,5′-AMP accumulation in the guinea pig, 60% in the cat, and 90% in the human heart particles. Half-maximal activity for the histamine mediated activation of adenyl cyclase in the guinea pig was 9×10^{-6}moles/liter, almost identical with that observed for norepinephrine in the same preparation. DL-Propranolol, 1×10^{-5}moles/liter, did not abolish the activation of adenyl cyclase produced by histamine but did abolish the activation produced by norepinephrine. In contrast, diphenhydramine hydrochloride, Benadryl, 8×10^{-5}moles/liter, abolished the activation of adenyl cyclase by histamine but not that produced by norepinephrine. These data suggest that there are at least two receptor sites in guinea pig heart mediating the activation of adenyl cyclase, one responsive to histamine, the other to norepinephrine. In addition, combined maximal doses of histamine and norepinephrine produced completely additive effects on the activation of adenyl cyclase, which suggests that at least two separate adenyl cyclase systems are present in the heart, each responsive to one of these hormones. However, definitive proof would require physical separation of the two enzymes.

INTRODUCTION

The inotropic and chronotropic effects of several hormones including the catecholamines, glucagon, and thyroid hormone are thought to be mediated by the adenyl cyclase-cyclic 3′,5′-AMP system (1–3). Histamine has also been shown to have positive inotropic and chronotropic effects (4–8). Two recent studies demonstrated that histamine activates adenyl cyclase in cerebellum (9) and gastric mucosa (10), and it is thought that the resultant increase in cyclic 3′,5′-AMP mediates the histamine response in these tissues. The purpose of the present investigation was to determine the effects of histamine on adenyl cyclase in particulate fractions of guinea pig hearts and the relationship to the catecholamine-mediated activation of adenyl cyclase in these same preparations. The effect of histamine on adenyl cyclase in particulate fractions of cat and human heart was also examined.

METHODS

Left ventricular muscle was obtained from normal guinea pigs, and a single guinea pig was used for each experiment. After the animals were anesthetized with pentobarbital, the heart was quickly excised. The left ventricle was dissected free of endocardium and epicardium, and approximately 220–250 mg of left ventricular muscle was homogenized in 4.5 ml of cold 0.25 M sucrose with a motor-driven homogenizer at 1°C. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant fluid was decanted; the particles were washed with cold 0.25 M sucrose, resuspended, and recentrifuged at 3000 rpm for 10 min. The washed particles were resuspended and homogenized in the cold 0.25 M sucrose. Adenyl cyclase was assayed by the method of Krishna, Weiss, and Brodie (11). The particulate fraction, containing 0.06–0.09 mg protein in a total volume of 0.06 ml, was incubated at 37°C for 4 min with the following: ATP, 1.6 mmoles/liter; ATP-α-^{32}P, 2.5–3.0 $\times 10^6$ cpm; theophylline, 8 mmoles/liter; MgCl, 2 mmoles/liter; Tris-Cl, 21 mmoles/liter (pH 7.7); human serum albumin, 0.8 mg/ml; and hormone at concentrations stated in the text. The incubations were started by adding the particulate fraction, which had been kept at 1°C, to the other components which were at 23°C. Hormone was added to the particles just before the incubations were initiated. DL-Propranolol and diphenhydramine, when present, were added immediately before the addition of hormone. The incubations were stopped by adding 0.1 ml of a solution con-

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Received for publication 27 October 1970.

TABLE I

Effect of Histamine on Adenyl Cyclase in Guinea Pig, Cat, and Human Heart Particles

	Cyclic 3′, 5′-AMP accumulated*	*P* value
	pmoles/4 min	
Guinea pig		
Control	98 ±16	—
Histamine (3×10^{-4} *moles/liter*	355 ±30	<0.01
Norepinephrine (5×10^{-5} *moles/liter*)	229 ±11	<0.01
Cat		
Control	104 ±3	—
Histamine (3×10^{-4} *moles/liter*)	165 ±7	<0.01
Norepinephrine (5×10^{-5} *moles/liter*)	297 ±26	<0.01
Human‡		
Control	90 ±3	—
Histamine (3×10^{-4} *moles/liter*	170 ±17	<0.05
Norepinephrine (5×10^{-5} *moles/liter*)	217 ±4	<0.01

* Each value represents the mean ±SE of 9–14 samples for the guinea pig, the mean ±SE of 12 samples for the cat, and the mean ±SE of 4 samples for the human. The rate of formation of cyclic 3′, 5′-AMP in response to histamine and norepinephrine in this preparation is linear for 5 min. Individual experiments are usually performed in triplicate or quadruplicate with standard errors averaging, in general, 3–12% of the mean.

‡ Right ventricular muscle was obtained from one patient during correction of a ventricular septal defect.

taining 4 μmoles of ATP, 1.25 μmoles of cyclic 3′,5′-AMP, and 0.15 μCi of cyclic 3′,5′-AMP-^{3}H, and boiled for 3 min. The cyclic 3′,5′-AMP-^{32}P accumulated was determined as previously described (3).

Histamine phosphate was a gift from Eli Lilly & Co., Indianapolis, Ind., DL-propranolol was from Ayerst Laboratories, New York, and diphenhydramine hydrochloride (Benadryl) was from Parke, Davis & Co., Detroit, Mich.

RESULTS

Effect of histamine on adenyl cyclase in guinea pig, cat, and human heart particles. Histamine, 3×10^{-4} moles/liter, increased cyclic 3′,5′-AMP accumulation approximately 300% in the guinea pig, 60% in the cat, and 90% in the human heart particles (Table I). The increase in cyclic 3′,5′-AMP was dose related over the concentration range 3×10^{-7} moles/liter to 3×10^{-4} moles/liter in the guinea pig heart particles (Fig. 1). Half-maximal activity was approximately 9×10^{-6} moles/liter almost identical with that observed for norepinephrine in the same preparation. Concentration response relationships were not examined in the cat or human hearts.

Since an accumulation of cyclic 3′,5′-AMP can occur as a result of phosphodiesterase inhibition as well as adenyl cyclase activation, we examined the effect of histamine on phosphodiesterase. In the presence of histamine, 3×10^{-4} moles/liter, 64 ±7 pmoles cyclic 3′,5′-AMP were hydrolyzed per 4 min as compared with a control of 55 ±3 pmoles cyclic 3′,5′-AMP hydrolyzed per 4 min (mean ±SE of three samples).

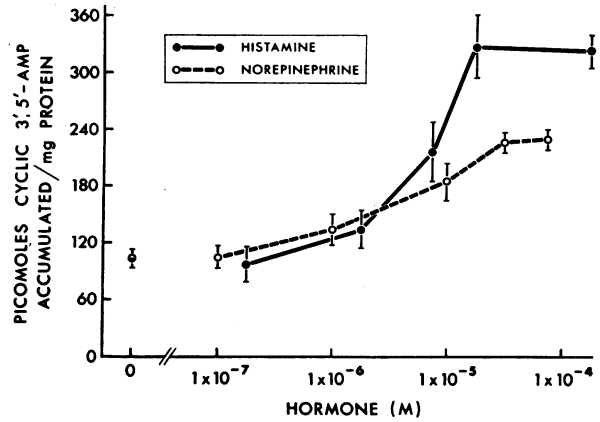

FIGURE 1 Concentration response curves to histamine and norepinephrine in guinea pig heart particles. Each point represents the mean ±SE of 8–14 samples obtained from five guinea pigs.

Effect of diphenhydramine on the histamine-mediated activation of adenyl cyclase. Antihistamines have been reported to antagonize the effects of histamine on guinea pig and rabbit heart (5, 8). Diphenhydramine hydrochloride at 8×10^{-5} moles/liter virtually abolished the activation of adenyl cyclase produced by histamine, 8×10^{-5} moles/liter (Fig. 2). The same concentration of diphenhydramine failed to abolish the norepinephrine-mediated activation.

Effect of DL-*propranolol on the histamine-mediated activation of adenyl cyclase.* The beta receptor–blocking agent, DL-propranolol, 1×10^{-5} moles/liter, abolished the activation of adenyl cyclase produced by norepinephrine, 5×10^{-5} moles/liter (Fig. 3). However, DL-propranolol did not abolish the histamine-mediated activation (Fig. 3).

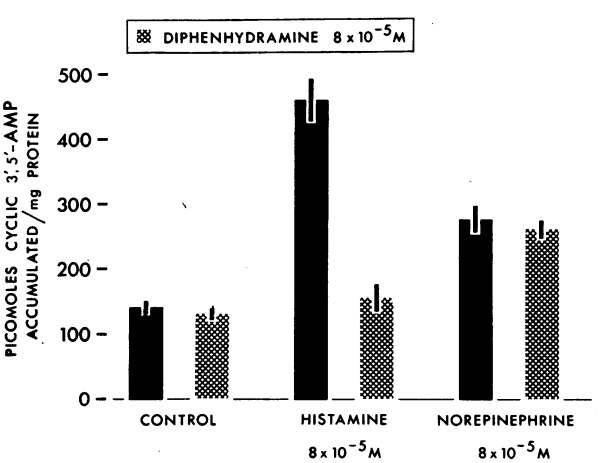

FIGURE 2 Effect of diphenhydramine on the histamine-mediated activation of adenyl cyclase. Each point represents the mean ±SE of eight samples from two guinea pigs.

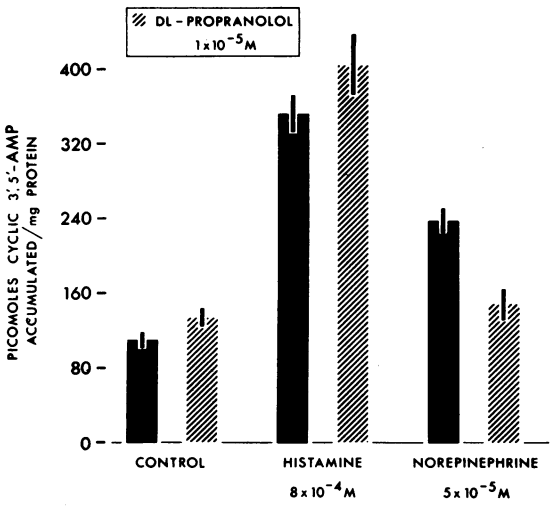

FIGURE 3 Effect of DL-propranolol on the histamine-mediated activation of adenyl cyclase. Each point represents the mean ±SE of seven samples from two guinea pigs.

Effect of combined maximal doses of histamine and norepinephrine on adenyl cyclase. An additive response was observed when combined maximal concentrations of histamine (3×10^{-4} moles/liter) and norepinephrine (5×10^{-5} moles/liter) were incubated together (Fig. 4). Histamine produced an increase of 199 ±24 pmoles and norepinephrine 132 ±5 pmoles above the control. The hormones in combination produced an increase of 384 ±23 pmoles. The additive increase was significantly higher than that produced by either hormone alone ($P < 0.02$).

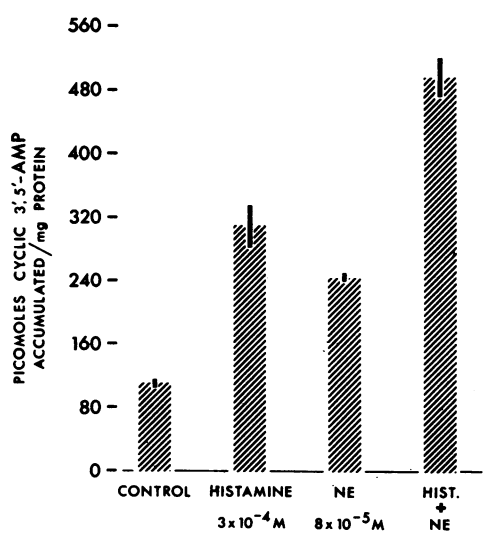

FIGURE 4 Effect of combined maximal concentrations of histamine and norepinephrine. Histamine was present at 3×10^{-4} moles/liter and norepinephrine at 5×10^{-5} moles/liter. Each point represents the mean ±SE of eight samples from three guinea pigs.

DISCUSSION

Histamine has been shown to have positive inotropic and chronotropic effects on the isolated hearts of guinea pigs, cats, and rabbits (4–9). Several lines of evidence indicate that these effects of histamine are independent of the adrenergic nervous system. First, reserpine pretreatment does not alter the responsiveness of the heart to histamine (4, 5). Second, beta adrenergic–blocking agents such as dichloroisoproterenol and DL-propranolol do not abolish the histamine-mediated augmentation of contractility and heart rate (4–7). Dean reported that propranolol and pronethalol produced a slight shift in the histamine dose response curve; however, the effect of the blockers was less pronounced as compared with the alteration produced on the norepinephrine dose-response curve, and he concluded that the antagonism was due more to a nonspecific local anesthetic effect than to a specific beta blockade.

Studies of the effects of antihistamines on the inotropic and chronotropic effects of histamine have produced conflicting results. Trendelenburg reported that pyrilamine and tripelennamine did not antagonize the responses of isolated cat and guinea pig atria to histamine (4). Bartlet found that mepyramine and diphenhydramine did not antagonize the action of histamine on the isolated guinea pig heart (6). On the other hand, Mannainoni reported that diphenhydramine abolished the histamine effect on isolated guinea pig atria (5), and Dean demonstrated that pyribenzamine antagonized the histamine-mediated increases in contractility and rate in rabbit atria while the norepinephrine-mediated responses were unimpaired (8). The reasons for the conflicting data are not clear.

Other hormones having positive inotropic and chronotropic effects on the heart including the catecholamines and glucagon, are thought to exert their effects by increasing the intracellular levels of cyclic 3′,5′-AMP resulting from activation of adenyl cyclase (12, 13). Phosphodiesterase inhibitors potentiate the inotropic and chronotropic responses of the catecholamines (14), an effect also observed with histamine (8). Therefore, it has been postulated by Pöch and Kukovetz (7) and Dean (8) that the cardiac actions of histamine are mediated by the adenyl cyclase–cyclic 3′,5′-AMP system.

The results of this investigation clearly indicate that histamine has the capacity to activate adenyl cyclase in particulate fractions of guinea pig, cat, and human heart homogenates. The histamine-mediated activation of adenyl cyclase is abolished by the antihistamine, diphenhydramine hydrochloride, but not by the beta adrenergic–blocking agent, DL-propranolol. In contrast, the nor-

epinephrine-mediated activation of adenyl cyclase is abolished by DL-propranolol but not by diphenhydramine hydrochloride. The data suggest the presence of separate receptors in the heart for histamine and norepinephrine.

Kakiuchi and Rall described the histamine-mediated increase in cyclic 3′,5′-AMP in rabbit cerebellum (9). They found that the histamine effect on cyclic 3′,5′-AMP production was blocked by diphenhydramine but not by beta-blocking agents. Furthermore, they reported that histamine and norepinephrine in combination produced additive effects on cyclic 3′,5′-AMP accumulation. We observed similar findings in the guinea pig heart; maximal concentrations of histamine and norepinephrine produced additive effects on the accumulation of cyclic 3′,5′-AMP. These results suggest that there are separate adenyl cyclase systems for histamine and norepinephrine. However, definitive proof would require physical separation of the two enzymes.

The physiologic role of histamine in relationship to the inotropic and chronotropic effects observed in experimental preparations has not been defined. However, histamine appears to be an important factor in anaphylaxis in guinea pigs, dogs, and man, and Bernauer and Hahn have postulated that histamine may be the mediator of the tachycardia seen in anaphylactic states (15). In any event, the data presented in this study provide evidence that the inotropic and chronotropic effects of histamine are mediated by cyclic 3′,5′-AMP.

ACKNOWLEDGMENTS

We are grateful to Dr. Stephen E. Epstein for initially suggesting the investigation and to Dr. Richard Rubinson for obtaining the surgical specimen of human heart.

REFERENCES

1. Murad, F., Y.-M. Chi, T. W. Rall, and E. W. Sutherland. 1962. Adenyl cyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3′,5′-phosphate by preparations from cardiac muscle and liver. *J. Biol. Chem.* **237**: 1233.
2. Levey, G. S., and S. E. Epstein. 1969. Activation of adenyl cyclase by glucagon in cat and human heart. *Circ. Res.* **24**: 151.
3. Levey, G. S., and S. E. Epstein. 1969. Myocardial adenyl cyclase: activation by thyroid hormones and evidence for two adenyl cyclase systems. *J. Clin. Invest.* **48**: 1663.
4. Trendelenburg, U. 1960. The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J. Pharmacol. Exp. Ther.* **130**: 450.
5. Mannaioni, P. F. 1960. Interaction between histamine and dichloroisoproterenol, hexamethonium, pempidine, and diphenhydramine, in normal and reserpine-treated heart preparations. *Brit. J. Pharmacol. Chemother.* **15**: 500.
6. Bartlet, A. L. 1963. The action of histamine on the isolated heart. *Brit. J. Pharmacol. Chemother.* **21**: 450.
7. Pöch, G., and W. R. Kukovetz. 1967. Drug-induced release and pharmacodynamic effects of histamine in the guinea-pig heart. *J. Pharmacol. Exp. Ther.* **156**: 522.
8. Dean, P. M. 1968. Investigation into the mode of action of histamine on the isolated rabbit heart. *Brit. J. Pharmacol. Chemother.* **32**: 65.
9. Kakiuchi, S., and T. W. Rall. 1968. The influence of chemical agents on the accumulation of adenosine 3′,5′-phosphate in slices of rabbit cerebellum. *Mol. Pharmacol.* **4**: 367.
10. Perrier, C. V., and L. Laster. 1969. Adenyl cyclase activity of guinea-pig gastric mucosa. *Clin. Res.* **17**: 596.
11. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple, sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exp. Ther.* **163**: 379.
12. Sutherland, E. W., G. A. Robison, and R. W. Butcher. 1968. Some aspects of the biological role of adenosine 3′,5′-monophosphate (cyclic AMP). *Circulation.* **37**: 279.
13. Epstein, S. E., C. L. Skelton, G. S. Levey, and M. Entman. 1970. Adenyl cyclase and myocardial contractility. *Ann. Intern. Med.* **72**: 561.
14. Rall, T. W., and T. C. West. 1963. The potentiation of cardiac inotropic responses to norepinephrine by theophylline. *J. Pharmacol. Exp. Ther.* **139**: 269.
15. Bernauer, W., and F. Hahn. 1969. Heart anaphylaxis: II. Effect of histamine and antigen on heart failure in heart-lung-preparation of guinea-pigs. *Arch. Int. Pharmacodyn. Ther.* **181**: 232