

ORIGINAL ARTICLE

HISTAMINE-INDUCED RELEASE OF ENDOGENOUS CATECHOLAMINES IN RAT ATRIA

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SUMMARY

The chronotropic and inotropic actions of histamine were recorded from rat atria. Histamine (5×10^{-4} to 10^{-3} M) produced only the positive chronotropic action whereas at higher concentrations ($>2.5 \times 10^{-3}$ M), a biphasic response characterized by a brief reduction in heart rate followed by a gradual increase in heart rate was observed in spontaneously beating right atria. Similar results were obtained when studying the inotropic actions of histamine on the paced left atria.

Propranolol (10^{-6} M) and reserpine abolished the positive chronotropic and inotropic but not the negative chronotropic and inotropic actions of histamine. Diphenhydramine at a concentration of 5×10^{-7} M, which failed to block the positive chronotropic action of norepinephrine, was effective in blocking the positive chronotropic action of histamine. Metiamide (5×10^{-5} M) failed to alter the cardiac actions of histamine. We suggest that the cardiac stimulant actions of histamine in rat atria are the result of released-endogenous catecholamines.

Histamine alters the electrical and mechanical functions of isolated hearts of various species including cat (1,2), rabbit (1-3), guinea pig (2,4-6) and frog (7). Unlike the information available from other species, the observations of the cardiac actions of histamine in rat atria are very few and not consistent. For example, in Langendorff

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preparations, it has been reported that histamine produces negative inotropic and chronotropic actions (6), negative inotropic followed by marked positive inotropic action (8,9) and in isolated spontaneously beating right atria, a positive chronotropic action (10).

At present it is generally believed that the cardiac stimulant actions of histamine are mediated by its direct action on specific histamine receptors (2,5,11). However, in rats, it has been suggested that the effects of histamine were mainly a result of catecholamine release (8,9). Later it was reported that histamine released catecholamines in the dog heart-lung preparation, but only at high concentrations (12).

In the present work, we attempted to resolve these conflicting reports by using paced left atria to study the inotropic action of histamine and spontaneously beating right atria for studying the chronotropic action. Pharmacological intervention, including the use of reserpine, propranolol and antihistaminic agents were also employed in this study to elucidate whether the cardiac actions of histamine observed in rat atria were mediated by specific histamine receptors or by release of endogenous catecholamines.

MATERIALS AND METHODS

General

Experiments were performed on isolated left and right atria from Sherman rats (250-300 g) of either sex. Rats were sacrificed by dislocation of the neck. The hearts were removed quickly and placed in a saturated 95% O_2 and CO_2 Kreb's solution at ambient temperature ($22-24^{\circ}C$). The atria were separated carefully from the ventricles and further divided into right and left atria. The tissue then was suspended vertically in an isolated organ bath (5 ml volume) containing a Kreb's solution of the following composition (mM): 118 NaCl, 4.7 KCl, 0.4 $MgSO_4$, 1.2 KH_2PO_4 , 2.8 $CaCl_2 \cdot 2H_2O$, 25 $NaHCO_3$, 11 glucose. The bathing solution

was maintained at 35° C and was bubbled with a 95% O₂, 5% CO₂ mixture. The pH of the solution was measured before and after the addition of drug solutions and ranged between 7.3 - 7.4.

Experimental Procedure

Isometric contraction was measured with a force displacement transducer (Grass FT-03) and recorded on a curvilinear recorder (Grass 79B). A preload of 0.75 g was applied to both right and left atria. Square wave pulses of 1 msec duration, double threshold voltage and at a frequency of 270 beats/min were applied across bipolar platinum electrodes to pace the left atria. The frequency of spontaneous contractions of the right atria was measured on an oscilloscope.

Isolated atria were allowed to equilibrate for 45 min in the organ bath during which time the bath solution was changed every 10 min. In a few experiments the right and left atria were maintained for about 30 min without changing the bathing solution. No significant changes were observed in either force or rate of contraction. Cumulative dose-response curves were obtained by exposing the tissues to stepwise increments in drug concentration. At the maximum response to each dose the next higher concentration was added. The maximal response to a given drug was reached when a 3-fold increase in concentration failed to elicit a further response.

The concentrations of drugs used to antagonize the cardiac action of histamine were selected based on preliminary trials of each drug at several concentrations. The highest concentration that did not affect either force or rate of contraction was selected. Reserpine (5 mg/kg) was administered intraperitoneally in a single dose 24 hrs before the study to reduce the catecholamine content of the isolated heart (13).

In some experiments the blocking agent (propranolol, diphenhydramine or metiamide) was added to the bath initially for 20 min followed by a change in the bath solution with the same drug concentration added a

second time for 15 min before a cumulative dose response-curve for histamine was obtained. The results were not statistically different from those treated for only 15 min. Since the volume of the bath solution was small (5 ml), care was taken to change the Kreb's solution at specified intervals. Therefore, the minimal effective pretreatment time of 15 min was preferred.

Either single or cumulative doses of histamine were employed in the experiments using the following protocols. If histamine was given in cumulative doses, only a single dose response curve was made for each tissue. When histamine was given in one single dose, the control response was made prior to studying the influence of an antagonist. If the antagonist could block the effect of histamine, then further experiments were performed without a control histamine response to ensure that the antagonism observed was not due to desensitization of the tissue.

Data Analysis

Statistical analysis was performed by using either a paired two-tailed t-test or unpaired two-tailed t-test and a probability of $p < 0.05$ was considered significant. The results were expressed as the mean \pm standard error of the mean. All the chronotropic actions of histamine were plotted as the actual measured heart rate.

Drugs

The following substances were used: histamine dihydrochloride and diphenhydramine (Sigma), reserpine (Ciba), propranolol (Ayerst) and metiamide (Smith, Kline and French). All drugs except reserpine were prepared the day of the experiment in triple distilled water. Concentrations of each drug were determined so that during sequential addition of the drug to the bath, the total volume of the bath solution changed less than 5%.

RESULTS

Chronotropic Action of Histamine

Histamine, in the dose range of 5×10^{-4} M to 6×10^{-3} M, was added to the Kreb's solution bathing the spontaneously beating right atria. Two effects of histamine were noted. Histamine 5×10^{-4} M to 10^{-3} M produced only a positive chronotropic action with each dose reaching a maximal chronotropic effect within 3-4 min. At higher concentrations of histamine ($> 2.5 \times 10^{-3}$) a biphasic response was noted. That is, a brief reduction in rate occurred within 1 min, followed by a gradual increase in rate which reached the maximal response within 4-5 min as shown in Fig. 1. Associated with this transient fall in heart rate was a decreased force of contraction. Likewise, as the heart rate increased during the second phase of the response the force of contraction increased also.

Chronotropic Action of Histamine: Reserpine Pretreatment

Pretreatment of animals with reserpine was used to reduce cardiac levels of catecholamines prior to testing with histamine. The basal heart rate in the reserpine-treated rats was significantly decreased from untreated control animals ($p < 0.05$). The positive chronotropic action of histamine was abolished by pretreating the animals with reserpine. However, the negative chronotropic action of histamine at doses above 2.5×10^{-3} M remained unaffected by reserpine as demonstrated in Fig. 1.

Chronotropic Action of Histamine: Propranolol

Addition of propranolol (10^{-6} M) to the tissue bath caused a decrease in heart rate which was not significantly different from the basal heart rate prior to the administration of propranolol. Results similar to those seen with reserpine were also observed with propranolol (Fig. 1). That is, only the positive chronotropic action was antagonised by propranolol, whereas the negative chronotropic action of histamine was not affected.

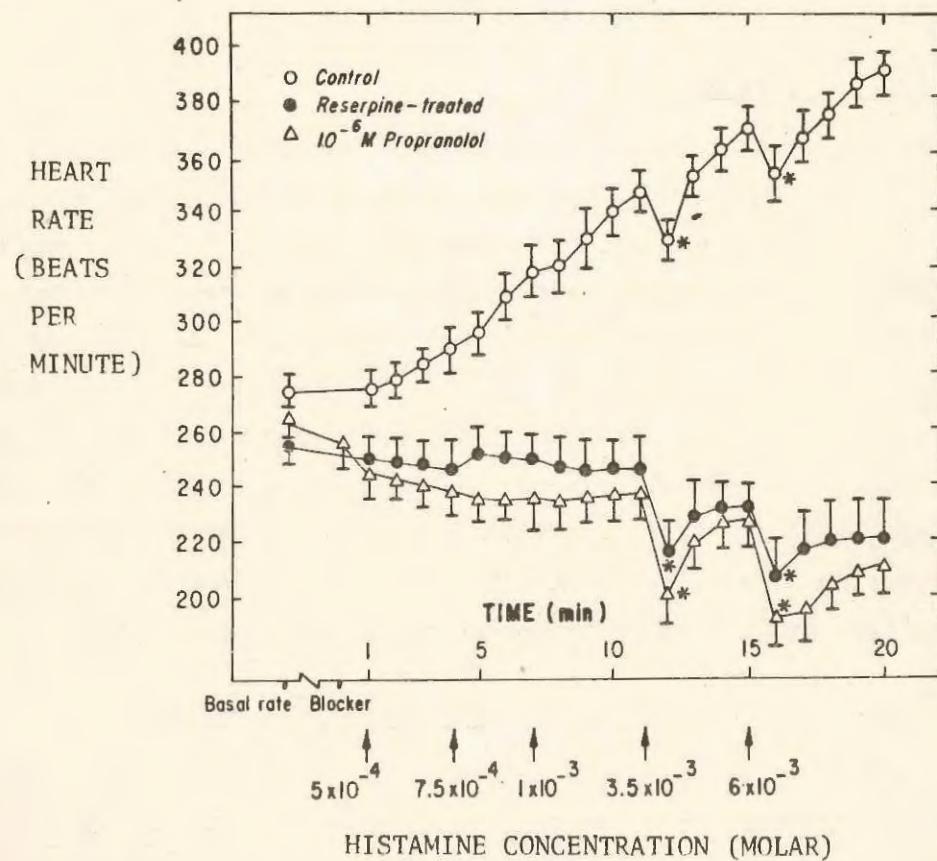


Figure 1 - Effects of reserpine and propranolol on the chronotropic actions of histamine. The chronotropic action of histamine on spontaneously beating right atria at 35° C is shown (O, n=9). Heart rate is displayed on the ordinate and time on the abscissa. Histamine concentrations were added at the time indicated by the arrows. While propranolol (10^{-6} M; Δ ; n=8) and reserpine (\bullet ; n=6) were effective in blocking the positive chronotropic action of histamine, they failed to block its negative chronotropic actions at high concentrations. The decrease in heart rate following high ($>2.5 \times 10^{-3}$ M) histamine concentrations was statistically significant ($*P < 0.001$) compared with the rate immediately prior to histamine administration. Significance was determined by a paired two-tailed t-test.

Positive Chronotropic Action of Histamine: Antihistamines

The positive chronotropic action of low doses of histamine was blocked by diphenhydramine (5×10^{-7} M). The characteristics of the biphasic response at high doses of histamine remained unchanged as shown in Fig. 2.

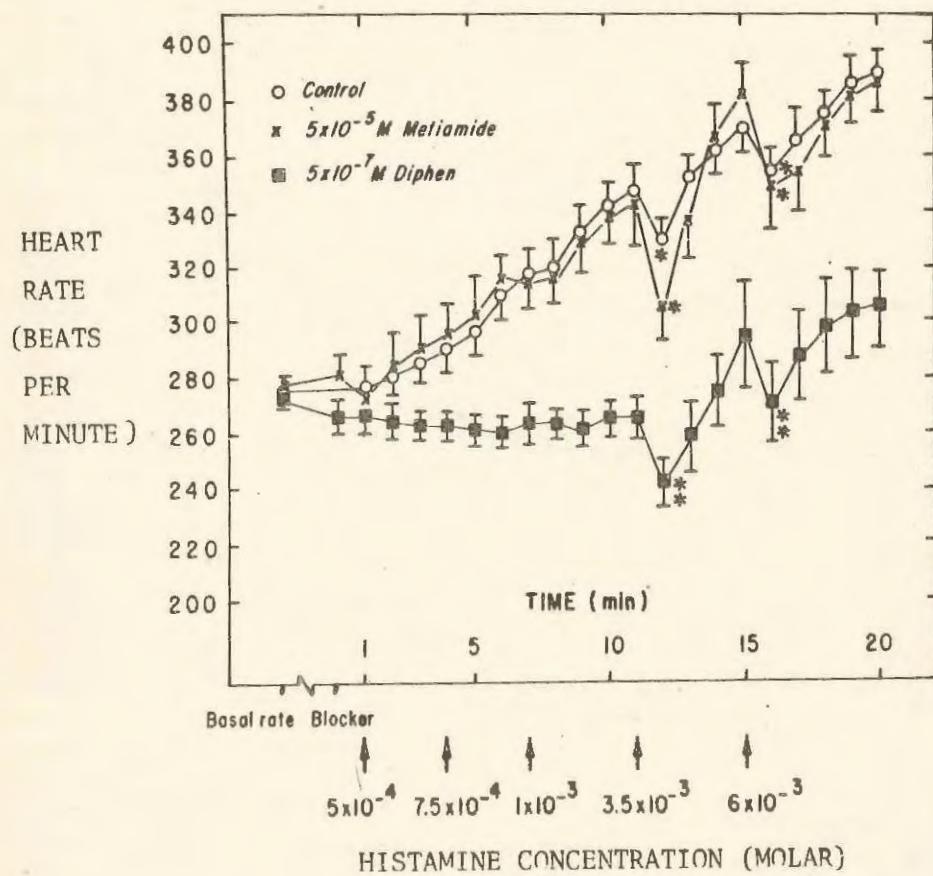


Figure 2 - Effects of metiamide and diphenhydramine on the chronotropic action of histamine. Histamine dose-response curve (O). Diphenhydramine (5×10^{-7} M; ■; n=5) is effective in blocking the positive chronotropic action of histamine while metiamide (5×10^{-5} M; ✕; n=6) is not effective. Both drugs fail to antagonize the negative chronotropic action of histamine. Statistical significance was determined as in Fig. 1 *P<0.001, **P<0.02

Higher concentrations of diphenhydramine ($>10^{-6}$ M) were not employed in this study because of a reduction in the basal heart rate.

Metiamide was employed at a concentration which, by itself, did not alter the basal heart rate. As shown in Fig. 2, metiamide (5×10^{-5} M) did not change the dose-response curve of histamine. Two further experiments using metiamide were performed at a concentration of 10^{-4} M. At this

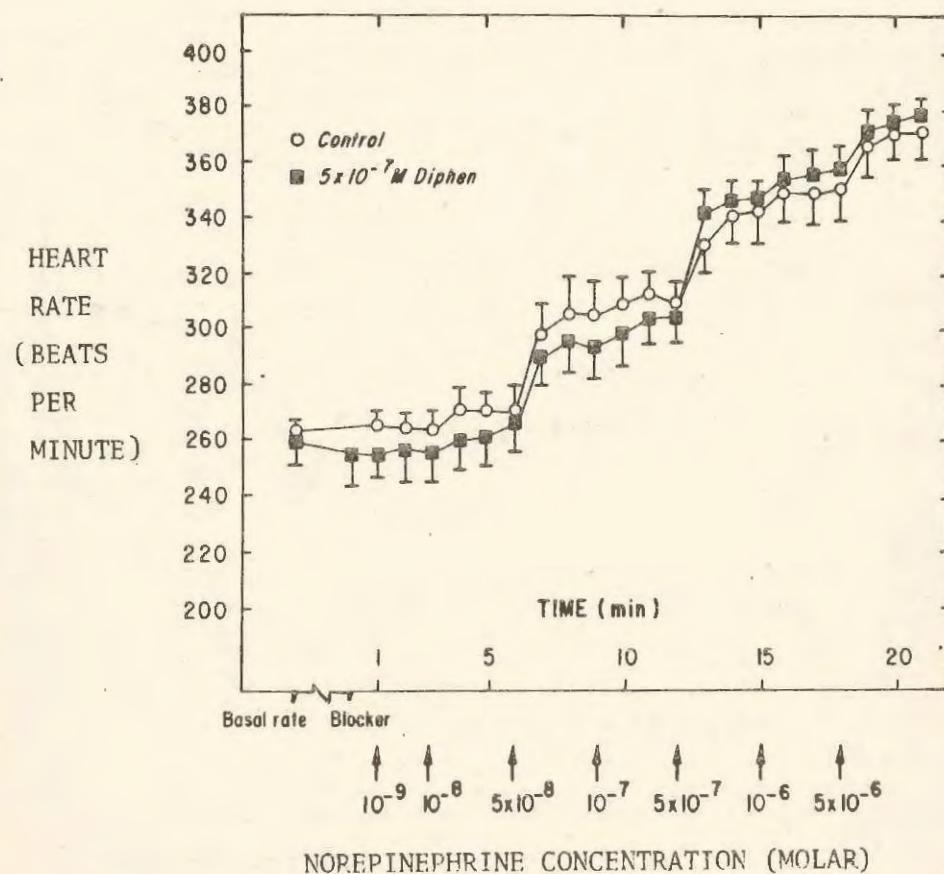


Figure 3 - Failure of diphenhydramine to antagonize the positive chronotropic action of norepinephrine. Open circle is the control dose response curve of norepinephrine at 35° C (n=6, O). Diphenhydramine (5×10^{-7} M; ■; n=6) does not block the positive chronotropic action of norepinephrine.

higher dose of metiamide there was neither a significant change in the basal heart rate, nor an antagonism of the response to histamine.

In six separate experiments, it was found that diphenhydramine (5×10^{-7} M), which was effective in blocking the positive chronotropic action of histamine, did not antagonize the positive chronotropic action of norepinephrine (Fig. 3).

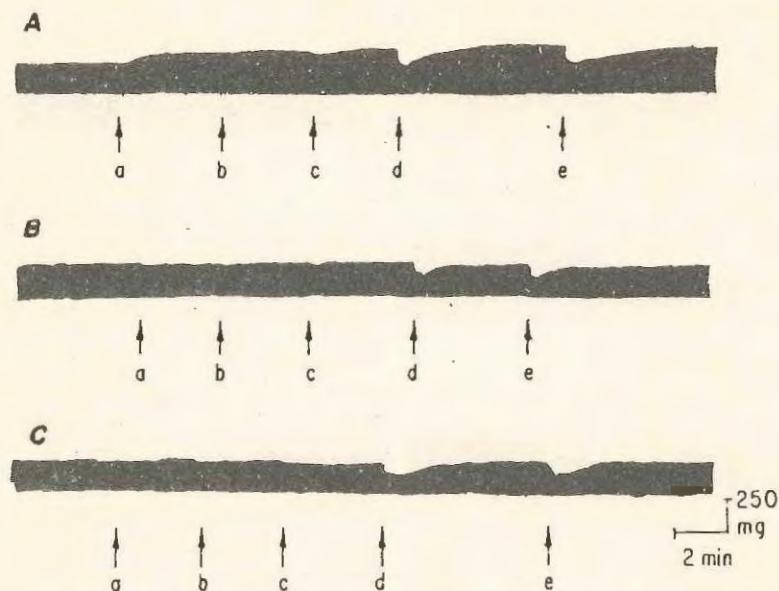


Figure 4 - Antagonism of the positive inotropic action of histamine by reserpine and propranolol. (A) Cumulative dose-response record during increasing doses of histamine. (B) Reserpine pretreatment resulted in loss of the positive inotropic action of histamine ($n=5$). (C) Pretreatment with propranolol (5×10^{-4} M) for 15 min also abolishes the positive inotropic action of histamine ($n=5$). Neither drug blocks the negative inotropic phase of histamine. Tracings A, B and C are from different preparations. Arrows indicate the time histamine was added to the tissue bath at the concentrations of a=5, b=7.5, c=10, d=35 and e= 60×10^{-4} M.

Inotropic Action of Histamine

In studies of the electrically paced left atria a similar dose range of histamine was employed. Histamine produced a positive inotropic action, with an initial slight decrease at low doses (Fig. 4A). At higher concentrations, a biphasic response characterized by a rapid decrease in force of contraction followed by positive inotropic effect was observed. The biphasic response to high doses of histamine was best observed when administered in a single dose as shown in Fig. 5, panel (a). In 24 single dose experiments, it was found that the rapid decrease in force of contraction reached about 30-50% of the basal force of contraction;

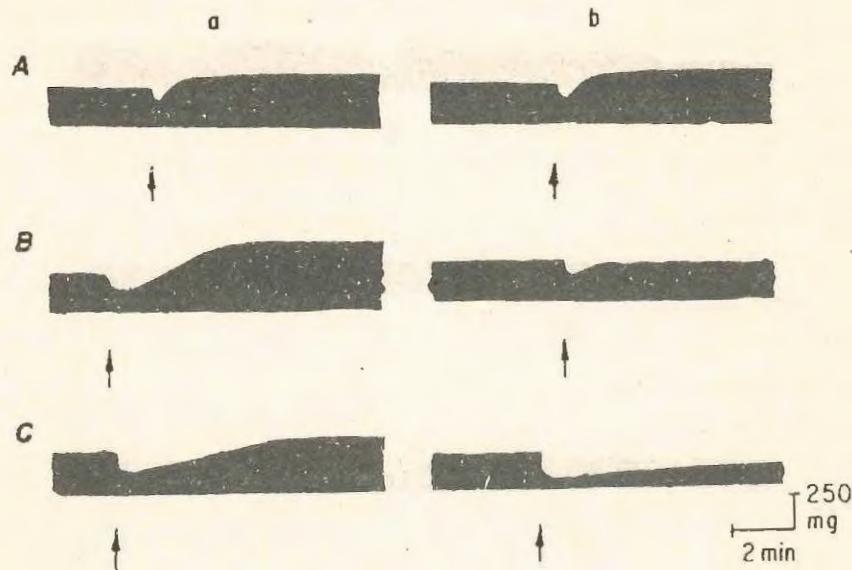


Figure 5 - The effects of reserpine and propranolol on the positive inotropic action of histamine administered in single dose. Panel (a) shows typical responses to histamine (2.5×10^{-3} M). Panel (b) shows responses to: (A) the same dose of histamine administered 20 min later, (B) histamine following pretreatment with reserpine, (C) histamine following pretreatment with propranolol (5×10^{-7} M). The responses to histamine in (A) and (C) are from the same preparation, while (B) are from different preparations. Arrows indicate the time at which histamine (2.5×10^{-3} M) was added to the tissue bath.

the subsequent positive inotropic action ranged from 35 to 100% above the basal force of contraction.

Positive Inotropic Action of Histamine: Reserpine and Propranolol

The positive inotropic action of histamine which was observed at all concentrations was abolished by pretreating the animals with reserpine or the addition of propranolol (5×10^{-7} M) to the tissue bath (Fig. 4B, 4C). However, these two drugs could not block the negative inotropic phase of the biphasic response of histamine. Furthermore, the negative inotropic phase occurred whether histamine was given in cumulative doses (Fig. 4B, 4C) or in a single high dose (Fig. 5B, 5C).

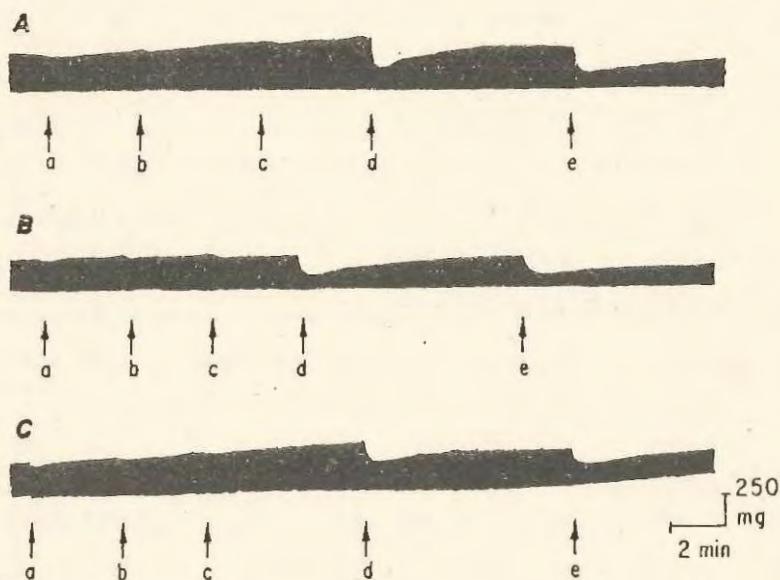


Figure 6 - Effects of antihistaminic agents on the inotropic action of histamine. (A) Cumulative dose response record during increasing doses of histamine. In 3 of 12 studies, a high dose of histamine (e) only produced a negative inotropic response. (B) Diphenhydramine (10^{-6} M) antagonizes the positive but not the negative inotropic actions of histamine. (C) Metiamide (5×10^{-5} M) does not block either the positive or the negative inotropic actions of histamine. Tracings A, B and C are from different preparations. Arrows indicate the time histamine was added to the tissue bath at the concentrations of a=5, b=7.5, c=10, d=35, and e= 60×10^{-4} M.

Positive Inotropic Action of Histamine: Diphenhydramine and Metiamide

The basal force of contraction and the positive inotropic response to histamine decreased if cumulative concentrations were given repeatedly. Therefore, in this study a control dose response curve to histamine was not performed. The blocking activity of an antagonist was determined by selecting the highest concentration that did not affect the basal force of contraction but could abolish the positive inotropic action of histamine. Using this criterion, it was found that out of 5 experiments diphenhydramine (5×10^{-7} M) blocked the positive inotropic action

of histamine in 2 experiments, 3 experiments were not effectively blocked. Diphenhydramine (10^{-6} M) was able to block the positive inotropic action of histamine in 3 out of 5 experiments (Fig. 6B). The other two experiments, the positive inotropic responses to low concentrations of histamine still could be noticed. Higher concentrations of diphenhydramine were not used because the positive inotropic action of histamine became irregular. The concentration of diphenhydramine used in this study was also unable to block the negative inotropic phase of the biphasic response to histamine.

Metiamide (5×10^{-5} M) did not antagonize the positive inotropic and the biphasic responses to histamine (n=6). When a high dose of histamine (60×10^{-4} M) was added to the tissue bath in the presence of metiamide (5×10^{-5} M), contracture developed as shown in Fig. 6C.

DISCUSSION

The results of the present investigation demonstrate that the cardiac actions of histamine in rat atria differ from other species in many aspects. First, in order to produce a cardiac response, histamine concentrations 1000 times higher are needed in the rat compared with the guinea pig (10). Second, the present data indicate that both the cardiac stimulant and depressant actions of histamine could be observed depending upon the dose and how the drug was administered (single dose versus cumulative dosing). Third, in the guinea pig there is evidence to indicate that the cardiac action of histamine is mediated by a direct action on specific histamine receptors (2,5,14). However, in rat and rabbit, it has been suggested that the cardiac action of histamine is mediated by release of endogenous catecholamines (8,9). The present investigation supports this observation in rat atria.

The data indicate that histamine produces both a cardiac stimulant and depressant action on rat atria. The results are similar to those reported earlier by Went and colleagues (8,9). That is, a negative

inotropic action followed by a marked positive inotropic action was always observed when histamine (2.5×10^{-3} M) was given in a single dose. Propranolol and reserpine could abolish the positive chronotropic and inotropic actions of histamine but not the negative chronotropic and inotropic actions of histamine at high doses. These results lead us to suggest that the effect of the cardiac stimulant actions of histamine on rat atria are mediated through released endogenous catecholamines.

Diphenhydramine (5×10^{-7} M) which is effective in antagonizing the positive chronotropic action of histamine failed to block the cardiac stimulant action of norepinephrine in rat atria. Hence, the antagonistic activity of diphenhydramine appears to be specific for histamine. Based on the differential sensitivity of histamine antagonists employed in this investigation, we suggest that histamine combines with an H_1 -receptor in the heart, probably in noradrenergic nerve endings, to initiate the release of endogenous catecholamines. This H_1 -histamine receptor is blocked by diphenhydramine but not metiamide.

In the present investigation, it was found that none of the antagonists used; propranolol, diphenhydramine or metiamide, or pretreating the animals with reserpine, could block the negative chronotropic and inotropic actions of histamine. The negative inotropic and chronotropic actions of histamine are interesting; further study will be needed to elucidate the mechanism associated with this effect of histamine.

ACKNOWLEDGEMENTS

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ANNUAL MEETING

The Pharmacological & Therapeutic Society of Thailand

May 2-4, 1983

Golden Beach Resort, Pattaya

Scientific program

A. การบรรยายพิเศษ (Invited Lecture)

1. เกล็ดวิทยาของไทยในอดีต ปัจจุบัน และอนาคต
2. Recent advances in the development of new antimalarial drugs

B. Symposium :

Prostaglandins, thromboxanes
and leukotrienes

C. Workshop :

Pharmacokinetics : concepts and
applications

D. Oral Presentation