SPECIES VARIATIONS IN CARDIAC EFFECTS OF HISTAMINE

by

ISMAIL LAHER

B.Sc., University of London, England, 1978

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Division of Pharmacology and Toxicology
of the Faculty of Pharmaceutical Sciences

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA, 1979

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmaceutical Sciences

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date 16 November 79.

ABSTRACT

The cardiac effects of histamine and its analogs were studied in tissues isolated from kittens, guinea-pigs and rats. Rate changes were recorded in right atria, while inotropic responses were recorded from electrically paced (1Hz) left atria, right ventricle strips and right papillary muscles.

In the kitten isolated right atrium, both histamine and 4-methylhistamine, a specific histamine H_2-receptor agonist, caused dose-dependent changes in rate. These responses were not affected by a histamine H_1-receptor antagonist (promethazine). Both cimetidine (a histamine H_2-receptor antagonist) and propranolol (a β-adrenoceptor antagonist) caused significant reductions in the chronotropic responses produced by histamine and 4-methylhistamine.

In the electrically paced preparations from kitten hearts, the inotropic responses of both histamine and 4-methylhistamine were observed at only high doses of agonist. The inotropic responses to these agonists were not affected by either promethazine or cimetidine. Propranolol caused marked inhibition of the inotropic response produced by histamine and 4-methylhistamine in the kitten left atrium, right ventricle strip and right papillary muscle.

Based on these results, it was concluded that the cardiac effects of histamine in the kitten were predominantly due to an indirect β-adrenoceptor stimulation. In addition, it was demonstrated that part of the chronotropic effect of histamine and 4-methylhistamine was due to histamine H_2-receptor stimulation.

The use of 2 - (2-pyridyl) ethylamine (PEA) in the study of cardiac histamine H_1-receptors was next investigated using guinea-pig right atria, left atria and right ventricle strips. The chronotropic response produced by PEA was not due to interaction with either histamine H_1- or H_2-receptors, as indicated by experiments with the specific histamine receptor antagonists. In atria depleted of endogenous catecholamines (using reserpine-pretreated guinea-pigs), the chronotropic response of PEA was abolished.

In guinea-pig left atria and right ventricle strips, inotropic responses of low doses of PEA were antagonised by promethazine. However, at higher doses of PEA, promethazine was unable to antagonise the increases in force of contraction in both the tissues used. The inotropic response due to high doses of PEA were reduced by propranolol or following reserpine pretreatment.

The absence of histamine H_1-receptors in the guinea-pig right atrium was confirmed. The results obtained also indicate that PEA has a dual action, by causing an initial direct histamine H_1-receptor stimulation at low doses, while at higher doses causing an indirect β-adrenoceptor stimulation.

Finally, the effect of histamine in isolated rat atria (right and left) was studied. Large doses of histamine produced changes in rate and force that were unaffected by promethazine or cimetidine. Following pretreatment of rats with reserpine (or by using propranolol in untreated tissues) the inotropic response due to histamine was abolished. At the same time, a negative chronotropic response (reversed by addition of atropine) revealed a cholinergic component in addition to an indirect adrenergic component in the cardiac effects of histamine in the rat.

In summary, the cardiac effects of histamine and its analogs show tremendous species variability. The results indicate that some responses were due to direct histamine H_1- or H_2-receptor stimulation. In addition, it was also demonstrated that the cardiac responses of histamine were not always due to such receptor interactions.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

ACKNOWLEDGMENT

I am indebted to Dr. J.H. McNeill for his valuable guidance and encouragement throughout this study. I would like to thank the committee members for their assistance, and I would also like to acknowledge the financial support of the Canadian Heart Foundation.

INTRODUCTION

The physiological importance of histamine in mammalian cardiovascular systems is unclear. It is known that histamine is stored in large amounts in cardiac tissue (Vergman and Rocha e Silva, 1966). High levels are to be found particularly in the regions of the right atrium of the rat heart, which comprise the sinoatrial and atrioventricular nodes (Harvey, 1978). Even though a role for cardiac histamine is not known, histamine could be of pathological importance, since it is known that mobilisation of histamine from storage sites can be induced by stimuli such as drugs (Douglas, 1975), immunological mechanisms (McIntire, 1973) and numerous surgical and diagnostic procedures (Lorenz, 1975). In addition, it is possible that the cardiac effects of histamine may be potentiated by some drugs, eg. digitalis (Levi and Capurro, 1975) and by disease states such as hyperthyroidism (McNeill and Schulze, 1972; Lee and Levi, 1977).

Histamine was synthesised over 70 years ago as an exercise in chemistry; the relatively simple organic molecule, β-imidazolyethylamine, was prepared in the laboratory by Windaus and Vogt (1907) before its discovery in nature. In the pioneering investigations published in the period 1910 to 1920, Dale and his coworkers Richards and Laidlaw revealed the diverse effects of histamine in various species (reviewed by Dale, 1953). The study of the actions of histamine at the receptor level has continued utilising mainly specific histamine receptor agonists and antagonists.

Bovet and Straub (1937) modified an existing compound to provide the first synthetic histamine antagonist. It was apparent to Ash and Schild (1966) that there existed at least two histamine receptor types. They

designated as H_1-receptors those sites involved in the responses to histamine that could be competitively blocked by mepyramine and related antihistaminic agents, and as non-H_1 receptors (later to be termed H_2 receptors) those which mediated responses to histamine that were refractory to mepyramine.

Trendelenburg (1960) had earlier reported that the classical antihistamines (mepyramine and tripelennamine) did not antagonise the cardiac effects of histamine. Other actions of histamine such as contraction of guinea-pig ileum and bronchi (Arunlakshana and Schild, 1954) could be blocked by these antihistaminic drugs — and were therefore classified as tissues with H_1-receptors according to the definition suggested by Ash and Schild (1966).

Like the cardiac histamine effects, histamine effects on gastric acid secretion and relaxation of the rat uterus were insensitive to the classical antihistamine drugs. (These sites were subsequently designated as containing H_2-receptors.) The characteristics of histamine H_2-receptors have been defined by Black et al. (1972). Careful re-evaluation of the structure-activity relations in a series of imidazole derivatives led, in 1972, to development of the first H_2-receptor antagonist, burimamide, by Black and his co-workers. Thus, classical antihistamines (mepyramine, tripelennamine, and promethazine) are now accurately defined as histamine H_1-receptor antagonists (Ash and Schild, 1966), whereas burimamide (Black et al., 1972), metiamide (Black et al., 1973) and cimetidine (Brimblecome et al., 1975) are histamine H_2-receptor antagonists. 2-methylhistamine (Black et al., 1972), 2-(2-amioethyl) pyridine (or 2-(2-pyridyl) ethylamine, PEA) and 2-(2-amioethyl) thiazole (Durant et al., 1975) are selective

histamine H_1-receptor agonists and 4-methylhistamine (Black et al., 1972) and dimaprit (Parsons et al., 1977) are selective histamine H_2-receptor agonists.

The availability of selective receptor agonists and antagonists has stimulated new interest in the nature of responses to histamine in many systems. The next section is an attempt to discuss the cardiovascular response to histamine, with particular reference to the receptors associated with the various responses to histamine.

Histamine and the Heart

The effect of histamine on the heart in vivo is complex, and although histamine can change cardiac function in vivo, distinction between the direct cardiac effects of histamine and those as a consequence of indirect mechanisms (eg. reflex responses to hypotension) is often very difficult. In a very extensive review on the cardiac actions of histamine, Altura and Halevy (1978) criticize such experiments, mainly because of inter- and intra-species variability. In addition, as pointed out by Altura and Halevy, the type of response to histamine under those conditions (ie. intact animal experiments) could also depend on experimental protocol.

In 1910, Dale and Laidlaw published the first report on the cardiac effects of histamine in isolated cat and rabbit hearts. The cardiac actions of the amine were then mainly ignored. In 1960, the studies of Mannaioni first suggested that histamine produced an increase in contractility by combining with specific histamine receptors in the myocardium.

Also in 1960, Trendelenburg reported on the effects of histamine and 5-hydroxytryptamine on atria isolated from cats, rabbits and guinea-pigs.

In this study, the effects of histamine and 5-hydroxytryptamine were compared to the responses produced by noradrenaline and nicotine. Based on the results with a variety of pharmacological agents known to affect neurotransmitter function, Trendelenburg concluded that the effects of histamine in the isolated kitten heart were due to an interaction with specific histamine receptors. Since neither reserpine pretreatment (3 mg/kg) nor the presence of a β-adrenoceptor blocking agent, dichloroisoprenaline altered the responses to histamine, Trendelenburg concluded that no indirect mechanisms were involved in the chronotropic responses due to histamine in the kitten heart. According to Trendelenburg, the reduction of the histamine response seen with dichloroisoprenaline was due to the positive chronotropic effect of the antagonist rather than due to an actual blockade of β-adrenoceptors. The author suggested that since dichloroisoprenaline itself produced a marked elevation of heart rate, the slight response due to histamine would be masked, and thus produced an apparent blockade.

When histamine antagonists (H_1) were used in that study, both pyrilamine and tripelennamine failed to inhibit the effects of histamine on contractility, except at high doses of antagonist. Using high doses of histamine (H_1) antagonists where significant depression of basal rates were produced, Trendelenburg reported a pA_2 value for the pyrilamine-histamine antagonism in the cat right atrium of 5.1 and 5.3 for guinea-pig right atria. Except for the study of pA_2 values, the results reported by Trendelenburg were based on single doses of agonists.

In addition to the report by Trendelenburg (1960), numerous other investigators (Ash and Schild, 1966; Flacke et al., 1967; Hughes and Coret,

1972; Levi and Kuye, 1974; McNeill and Mushek, 1972) have reported the ineffectiveness of the classical antihistamines in antagonising the cardiac effects of histamine.

However, with the introduction of specific histamine H_2-receptor antagonists, Reinhardt et al., (1974) as well as Steinberg and Holland (1975) clearly demonstrated that the inotropic effects of histamine in the guinea-pig left atrium were blocked by histamine H_1-receptor antagonists such as promethazine or tripelennamine, but not by H_2 blocking agents. On the other hand, the chronotropic effect of histamine in the guinea-pig right atrium was blocked by burimamide and metiamide, but not by histamine H_1-receptor antagonists.

These data contradicted the findings of McNeill and Verma (1974) in the perfused guinea-pig heart and also those of Moroni et al. (1973) and Ledder et al. (1974) in the guinea-pig right ventricle strip. All of these authors demonstrated, by using appropriate blocking agents, that the cardiac receptors mediating changes in contractile force were of the histamine H_2-receptor type.

In a later study by Verma and McNeill (1976), utilising a specific histamine H_1-receptor agonist, PEA, and a specific histamine H_2-receptor agonist, 4-methylhistamine, demonstrated that the type of histamine receptor in the guinea-pig heart depended on the part of the heart examined.

Whereas histamine H_2-receptors were found mainly in the guinea-pig right atrium, the left atrium contained H_1-receptors while the right ventricle strip contained a mixture of both H_1- and H_2-receptors (Verma and McNeill, 1976). In the spontaneously beating Langendorff guinea-pig heart preparation used by McNeill and Verma (1974), the blockade of the

inotropic response of histamine reported was therefore a blockade of the increased rate produced by histamine as well as an increased force of contraction in the right ventricle, both histamine H_2-receptor mediated effects (Verma and McNeill, 1977).

In a more recent study, NcNeill and Verma (1978), using isolated rabbit hearts, demonstrated that the left atrium, right ventricle strip and right papillary muscle contain histamine H_1-receptors. In the right atrium of the rabbit, however, both histamine H_1- and H_2-receptors were associated with chronotropic responses to histamine-like drugs.

These results and others (for reviews see Verma and McNeill, 1976 and Altura and Halevy, 1978), clearly indicate that the type of cardiac histamine receptor depends on both the species and the part of the heart examined.

A great deal of evidence has accumulated to support the hypothesis that the cardiac effects of histamine were mediated by cyclic AMP through a histamine-sensitive adenylate cyclase. In 1967, Pöch and Kukovetz first suggested that cardiac histamine receptors in guinea-pig heart were associated with adenylate cyclase. Dean, in 1968, also postulated that cyclic AMP mediated the cardiac effects of histamine by stimulating adenylate cyclase in a manner analogous to that reported by Robison et al. (1965) for the adrenergic amines. Klein and Levey (1971) published the first report showing an increase in cardiac cyclic AMP levels following histamine administration.

According to Klein and Levey (1971), the histamine-stimulated increases of cyclic AMP in the guinea-pig, cat and human heart were unaffected by the β-adrenoceptor antagonist, DL-propranolol. In contrast, Klein and Levey

also reported that diphenhydramine, a histamine H_1-receptor antagonist, could block the histamine-induced increases in cyclic AMP while not affecting increases produced by noradrenaline.

McNeill and Mushek (1972) performed a more extensive study of the effects of histamine on contractility, phosphorylase and adenylate cyclase in isolated, perfused guinea-pig hearts. These authors clearly showed that the blockade of histamine activation of adenylate cyclase by classical antihistamines (tripelennamine and diphenhydramine) was nonspecific. McNeill and Mushek (1972) therefore discussed the conclusion that not only were the mechanical responses due to histamine in the heart unaffected by histamine H_1-receptor antagonists, but that the biochemical responses (phosphorylase and adenylate cyclase activation) observed following histamine receptor stimulation were also not competitively antagonised by the histamine antagonists available at the time.

Verma and McNeill (1979) have since repeatedly demonstrated that stimulation of histamine H_2-receptors (eg. guinea-pig right atria, guinea-pig right ventricle strip and rabbit right atria) is associated with increases in cyclic AMP. On the other hand, stimulation of histamine H_1-receptors (guinea-pig and rabbit left atria, rabbit ventricle strip) did not result in increases of cyclic AMP. Therefore, increases in cyclic AMP are not absolutely necessary for the inotropic action of histamine.

Though the majority of literature on the cardiac effects of histamine is based on the guinea-pig (Altura and Halevy, 1978), the presence of histamine receptors has also been reported in numerous other species. Dale and Laidlow (1910) reported on the effects of histamine on cardiac contractility in the isolated heart of the cat and rabbit. Trendelenburg (1960)

further investigated the effects of histamine in the cat, guinea-pig and rabbit heart. Verma and McNeill (1977) and McNeill and Verma (1979) have studied the regional distribution of both histamine H_1- and H_2-receptors in the guinea-pig and rabbit myocardium. Chiba (1976) reported that the effects of histamine on inotropic and chronotropic activity in isolated canine atrial preparations were blocked by histamine H_1-receptor antagonists.

Using large doses of histamine, Korasěc and Erjavěc (1978) recently suggested that the effects of histamine in the rat heart were due to stimulation of both histamine H_2-receptors and β-adrenoceptors. These workers also claimed that the increases in cardiac cyclic AMP recorded were due to stimulation of histamine H_2-receptors. An earlier study (Satayavivad et al., 1977) reported that the chronotropic effect of histamine in both guinea-pig and rat atria was potentiated by the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine. However, at least in the rat atrial preparations, very high doses of histamine had to be used again. The ED_{50} values reported by Satayavivad et al., (1977) for the chronotropic effects of histamine in the guinea-pig was $2.3 \pm 0.2 \times 10^{-6}$M while that for the rat was $1.7 \pm 0.7 \times 10^{-3}$M.

At such high doses (eg. ED_{50} $1.7 \pm 0.7 \times 10^{-3}$M histamine) histamine would be expected to exert non-specific effects. Hood et al. (1975) have demonstrated that high doses of burimamide (34-1080 μM) produced a dose-dependent indirect sympathomimetic effect in kitten atria. Similarly, Broadley and Wilson (1978) have recently stated that high doses of PEA had both a direct effect (stimulation of histamine H_1-receptors) as well as an indirect effect (release of catecholamines).

In addition to the positive chronotropic and inotropic effects of histamine discussed already, it has recently been reported that histamine can also alter left intraventricular pressure, coronary flow, aortic flow, total cardiac output and external pressure-volume work in the isolated working heart preparation of the guinea-pig (Flynn et al., 1979).

In summary, the inotropic and chronotropic effect of histamine in a variety of species is well documented. Responses due to histamine H_2-receptor stimulation result in increases in cyclic AMP while those due to histamine H_1-receptor stimulation are not associated with cyclic AMP. In some species (eg. rat), however, mechanical and biochemical responses in the heart are produced with only high doses of histamine.

PURPOSE OF THE PRESENT INVESTIGATION

This study set out to investigate the chronotropic and inotropic response in the isolated kitten heart. It was hoped to characterise further the nature of these responses utilising specific histamine H_1- and H_2-receptor antagonists and agonists, and thereby report on the distribution of such receptors in this species. Previous work by Trendelenburg (1960) was reported for only the effects of single doses of histamine, and it was hoped to perform similar studies using complete dose-response curves.

We also wished to reinvestigate earlier reports on the effects of histamine on the isolated rat heart (Satayavivad *et al.*, 1977) since at the high doses used in that study (ED_{50} $1.7 \pm 0.7 \times 10^{-3}$M) it is quite likely that the effects of histamine would be non-specific. We hoped to repeat these studies with histamine, and, in addition, to characterise the nature of the receptors involved in the responses to histamine in the rat heart.

Finally, it was hoped to reinvestigate the effects of PEA in the isolated guinea-pig heart, since this histamine H_1-receptor agonist enjoys a widespread usage in the study of such systems (ie. H_1-receptors). The study was prompted by the preliminary report of Broadley and Wilson (1978) stating that this agonist released catecholamines in addition to its direct action on histamine H_1-receptors. Since the guinea-pig left atrium and right ventricle are known to contain histamine H_1-receptors (Verma and McNeill, 1977), this species was chosen to study whether the release of catecholamines was mediated by histamine H_1-receptors in the heart, as is the case in the adrenal medulla (Emmelin and Muren, 1949).

METHODS

Cardiac preparations were obtained from three species: cat (1-2kg), guinea-pig (300-400g) and rat (150-300g). Animals of either sex were used and they received food and water ad. libitum.

In studies with the isolated cat heart, right atria, left atria, right ventricle strips and right papillary muscles were dissected free and suspended in Chenoweth-Koelle solution (1946) at 37^{o}C under a 1 gram basal tension. When isolated guinea-pig and rat hearts were used, only the right and left atria were used from each animal. The buffer solution was aerated with a 95% oxygen, 5% carbon dioxide mixture. Following an initial equilibration period of 30 minutes, all quiescent tissues were electrically stimulated at 1Hz and twice the threshold voltage using a Grass model S6 stimulator.

Complete dose-response curves were constructed using the cumulative method as described by Van Rossum and Van der Brink (1963). When antagonists were used, these were added to the buffer an hour prior to the addition of agonist. In some studies, animals were pretreated with reserpine (2.5mg/kg i.p. or 3.0mg/kg i.p.) 24 hours prior to the experiment.

Each tissue was used for only one complete dose-response curve. Because of the gradual nature of all of the responses (rate and force responses), measurements were made 3 minutes after the addition of agonist.

Contractile force in the left atrium, right ventricle strip and right papillary muscle was measured isometrically by placing a Palmer clip at the apex of the tissue and connecting this to a Grass force displacement transducer. All recordings were made on a Grass model 79D polygraph.

Statistical Methods

Statistical analysis was done by the student's t-test for unpaired data. A probability of $p \leq 0.05$ was taken as the criterion of significance.

Materials: The following drugs were employed in the study.

Atropine Sulfate	(Sigma, St. Louis, U.S.A.)
Histamine Dihydrochloride	(Sigma, St. Louis, U.S.A.)
DL-Propranolol HCl	(Sigma, St. Louis, U.S.A.)
Reserpine (Serpasil®)	(CIBA-GEIGY Canada)

Promethazine, Cimetidine, 4 methyl-histamine and 2-pyridylethylamine were gifts from Smith Kline and French Laboratories, Welwyn Garden City, Herts., England.

RESULTS

Chronotropic Effect of Histamine in Kitten Right Atria

The dose-response curve for the chronotropic effect of histamine in the kitten right atrium is shown in Figure 1 and Table 1. The threshold of the response occurs at approximately 10^{-7}M histamine, and at a dose of 10^{-3}M, histamine was able to produce an increase in rate of about 87 beats per minute (Table 1). Promethazine at low doses (10^{-7}M) was unable to significantly reduce the changes in heart rate produced by histamine at any dose (Table 1). When higher doses of promethazine were applied, a depression of basal heart rate was noted. In addition, the antagonism produced by higher doses of promethazine was not surmountable by increasing the dose of histamine. This observation is in agreement with the data of McNeill and Verma (1974) who demonstrated that at higher concentrations (greater than 4μM), promethazine was able to interact with the histamine receptor in either a non-competitive or competitive non-equilibrium manner.

Cimetidine, at doses of 1 x 10^{-6}M and 1 x 10^{-5}M, caused a displacement of the histamine curve to the right hand side in a dose-dependent manner. Though the same maxima (as the control maximal histamine effect) were not reached, preliminary studies with higher doses of agonist showed that the antagonism was surmountable. With each increasing dose of cimetidine, the threshold for the histamine response was also increased (Figure 1).

When propranolol (10^{-7}M) was added to the buffer solution, a slight elevation of the basal heart rate was observed. This dose of propranolol was sufficient to produce a significant antagonism of the chronotropic

responses to doses of histamine greater than 1 x 10^{-5}M (Table 1).

Inotropic Effect of Histamine in Isolated Kitten Heart

The inotropic responses of histamine in the isolated kitten heart were only demonstrable at high doses (at or greater than 10^{-4}M). For this reason, it was decided to express the effect of histamine on the isolated kitten left atrium (Figure 3), right ventricle strip (Figure 4) and right papillary muscle (Figure 5) by means of histograms. Results for the complete dose-response curves are summarised in Tables 2, 3 and 4.

At a dose of 10^{-3}M histamine, histamine caused a significant increase in contractile force in all three tissues studied — left atrium, right ventricle strip and right papillary muscle (Tables 2, 3 and 4). In the presence of either cimetidine (10^{-5}M or 10^{-6}M) or promethazine (10^{-7}M), histamine (10^{-3}M) was able to cause an increase in force of contraction that was not significantly different from control conditions. However, when propranolol (10^{-7}M) was added to the perfusing buffer, the inotropic effect of histamine in the kitten left atrium (Figure 3), right ventricle strip (Figure 4) and right papillary muscle (Figure 5) was reduced by 50% or more.

Chronotropic Effect of 4-methyl Histamine in Kitten Right Atrium

4-methyl histamine, a specific histamine H_2-agonist, produced a positive chronotropic effect in spontaneously beating kitten right atria (Figure 6, Table 5). Again, as with histamine, the onset of action was delayed. However, when compared to the effect of histamine in the kitten

right atrium, 4-methyl histamine had a higher threshold of response (10^{-5}M 4-methyl histamine compared to 10^{-6}M histamine). But it should be noted that the mean of the maximal changes produced by either agonist is approximately equal (87 beats per minute change with histamine compared to 78 beats per minute change with 4-methyl histamine).

Both doses of cimetidine used (1×10^{-6}M and 1×10^{-5}M) produced a displacement of the control dose-response curve to the right hand side. The inhibition of the 4-methyl histamine effect was dependent on the dose of antagonist used, so that the inhibition of the maximal responses occurring at 3×10^{-4}M and 1×10^{-3}M 4-methyl histamine were only significant with the higher dose of cimetidine (10^{-5}M) (Table 5).

Again, as with the histamine chronotropic responses, when propranolol was added to the perfusate, a significant inhibition of the chronotropic response to 4-methyl histamine occurred (Figure 6). Whereas the mean maximal change produced by 10^{-3}M 4-methyl histamine under control conditions is 78 beats per minute, this value was reduced to 37 beats per minute when propranolol was included in the perfusate.

These results are more clearly demonstrated when the response to a single dose of 4-methyl histamine (10^{-3}M) under various conditions is represented by histograms, as in Figure 7. As is demonstrated in Figure 7, the response to 10^{-3}M 4-methyl histamine was significantly reduced by both cimetidine (10^{-5}M) and propranolol (10^{-6}M).

Inotropic Effect of 4-methyl Histamine in Isolated Kitten Heart

4-methyl histamine (10^{-3}M) produced an inotropic effect in the kitten left atrium (Figure 8), right ventricle strip (Figure 9) and right papillary muscle (Figure 10). Again, as with the histamine inotropic effect, these responses were only obtained at high doses of agonist.

Cimetidine (10^{-6}M) was without significant effect on the inotropic response to 4-methyl histamine in the kitten left atrium (Table 6), right ventricle strip (Table 7) and right papillary muscle (Table 8). However, in the presence of 10^{-7}M propranolol, the inotropic effect of 10^{-3}M 4-methyl histamine was reduced by more than 50% of the control response in all three tissues (Tables 6, 7 and 8).

It was reasoned that since promethazine had no significant effect on either the chronotropic (Table 1) or inotropic (Tables 2, 3 and 4) effects of histamine in the kitten heart, it was unlikely to alter these responses when 4-methyl histamine was used as the agonist.

Chronotropic Effect of PEA in Guinea-Pig Isolated Right Atria

PEA produced a dose-dependent increase in rate in atria obtained from untreated guinea-pigs (Figure 11). The threshold of this response occurred at 10^{-6}M PEA, and the maximal change recorded under control conditions was 126 beats per minute with 10^{-3}M PEA (Table 9).

When either promethazine (3×10^{-6}M) or cimetidine (3×10^{-6}M) were added to the perfusate, the dose-response curve for PEA was not altered by either treatment (Figure 11). In other words, neither promethazine (3×10^{-6}M) or cimetidine (3×10^{-6}M) significantly affected the basal

heart rate (176 beats per minute) or the maximal change produced by 10^{-3}M PEA (126 beats per minute) (Table 9).

The chronotropic effects of PEA were also studied in atria obtained from guinea-pigs pretreated with reserpine (2.5 mg/kg, 24 hours prior to sacrifice). The results of these studies are illustrated in Figure 12. Reserpine pretreatment significantly reduced the maximal rate change obtained with PEA from 126 beats per minute to 44 beats per minute. The increases in heart rate with 10^{-4}M and 10^{-3}M PEA in atria from reserpine pretreated guinea-pigs were significant, and consequently propranolol (10^{-6}M) was also included in the buffer in another series of experiments (Figure 12).

When propranolol was added to the perfusate, the rate responses to PEA in atria obtained from reserpine-pretreated guinea-pigs were significantly depressed (Figure 12). In other words, PEA was unable to cause a significant increase in heart rate under these conditions.

Inotropic Responses to PEA in Isolated Guinea-Pig Left Atria

PEA had a dose-dependent positive inotropic effect in left atria from untreated guinea-pigs (Figure 13), increasing the force of contraction from an average basal value of 0.6g to a mean developed tension of 2.8g, an increase of 2.2g (Table 10).

In the presence of a non-depressant dose of promethazine (3×10^{-6}M) (Table 10), PEA was still able to produce an increased force of contraction at doses equal to or greater than 10^{-6}M. However, the inhibition of the PEA response in the guinea-pig left atrium was significant only at two doses, 10^{-6}M and 10^{-5}M PEA (Table 10). The responses to PEA at higher

doses (10^{-4}M and 10^{-3}M) were not significantly altered by this antagonist (promethazine).

Cimetidine (3×10^{-6}M) had little effect on the dose-response curve for the inotropic effect of PEA in the guinea-pig left atrium (Figure 13) and affected neither the slope nor the maximal response. As is clear from Figure 13, propranolol at a dose of 10^{-6}M in the perfusing medium, had a significant effect on the maximal effect of PEA in the guinea-pig left atrium, reducing the inotropic effect of 10^{-3}M PEA from a developed tension of a mean 2.2g to that of 1.4g. But it will be noted that at doses of PEA less than 10^{-5}M propranolol had little effect on the slope of the dose-response curve (Figure 13).

Experiments similar to those just described above were next repeated in left atria isolated from reserpine-pretreated guinea-pigs (2.5 mg/kg, 24 hours prior to sacrifice). The results of these experiments are summarised in Figure 14 and Table 11, and for comparative purposes, a control (ie. untreated) dose-response curve is also included. In left atria from reserpine-pretreated guinea-pigs, PEA was still able to produce a dose-dependent positive inotropic effect, but compared to the inotropic effect of PEA in left atria from untreated guinea-pigs, the maximal response obtained with 10^{-3}M PEA was significantly depressed (Table 11). In addition, the responses to lower doses of PEA were also depressed, though this was not significant at $p \leq 0.05$. A rightward shift of the dose-response curve to PEA in left atria obtained from reserpine-pretreated guinea-pigs (as compared to atria from untreated animals) appears to be the trend at doses greater than 10^{-5}M PEA.

As with the response to PEA in left atria from untreated guinea-pigs

(Table 10), cimetidine (3×10^{-6}M) was unable to alter the positive inotropic effect of PEA in left atria from reserpine-pretreated guinea-pigs (Table 11, Figure 14). When promethazine (3×10^{-6}M) was added to the perfusate, a further rightward shift of the dose-response curve to PEA was observed (Figure 14). Under these conditions, promethazine caused a statistically significant inhibition of the PEA-induced increases in force of contraction at doses greater than 10^{-5}M (based on a comparison with the responses obtained in atria from untreated animals (Table 11)).

Inotropic Response of Guinea-Pig Right Ventricle Strip to PEA

At doses greater than 10^{-6}M PEA, the agonist produced a positive inotropic response in right ventricle strips from untreated guinea-pigs (Figure 15). Inclusion of either promethazine (3×10^{-6}M) or propranolol (10^{-6}M) significantly reduced the inotropic effects of both 10^{-4}M and 10^{-3}M PEA in ventricle strips obtained from untreated guinea-pigs (Table 12). When the response of ventricle strips from reserpine-pretreated guinea-pigs to PEA was studied, it was observed that under these conditions, the increases in tension produced by 10^{-4}M and 10^{-3}M PEA were significantly reduced (Figure 15), whereas propranolol (10^{-6}M) and promethazine (3×10^{-6}M) reduced the PEA-induced increases in tension by 42% and 29% respectively (average values); following reserpine-pretreatment the reduction in the maximal response to 10^{-3}M PEA was a mean of 52% (Table 12).

Chronotropic Effect of 4-methylhistamine in Guinea-Pig Right Atria

The response of the guinea-pig right atrium to 4-methylhistamine was

also studied, and the results are summarised in Table 13 and Figure 16. The maximal effect with this agonist occurred at 10^{-4}M 4-methylhistamine, when the spontaneous rate of the right atrium was increased from a mean of 185 beats per minute to a mean of 319 beats per minute (Table 13). Addition of 10^{-7}M propranolol affected neither the slope nor the maximal response of the dose-response curve (Figure 16). In the presence of 10^{-6}M cimetidine, the rate changes seen with 4-methylhistamine were reduced, and these became significant at 10^{-6}M and 10^{-5}M 4-methylhistamine (Table 13). However, this inhibition due to cimetidine was overcome at higher doses of 4-methylhistamine (10^{-4}M and 10^{-3}M), with the maximal chronotropic effect in the presence of 10^{-6}M cimetidine now occurring at 10^{-3}M 4-methylhistamine (Figure 16).

Effect of Histamine on Spontaneously Beating Isolated Rat Right Atria

In Figure 17 and Table 14 are summarised the effects of histamine on the spontaneous rate of contraction of isolated rat right atria under various conditions. It is immediately apparent that any chronotropic response due to histamine was noted only at doses greater than 10^{-4}M (Figure 17), so that at 10^{-2}M histamine, a mean increase in rate of 82 beats per minute was recorded.

This response seen with histamine was unaffected by 10^{-7}M cimetidine, when a mean maximal change of 96 beats per minute was recorded with 10^{-2}M histamine (Table 14). However, in the presence of 10^{-7}M promethazine, a statistically significant potentiation of the response due to 10^{-2}M histamine was observed (Table 14), with no other changes evident.

Following either the addition of propranolol (10^{-6}M) or pretreatment

of rats with reserpine (3.0 mg/kg, 24 hours prior to sacrifice), histamine (at doses of 10^{-3}M and 10^{-2}M) produced a statistically significant and dose-dependent negative chronotropic response. In the presence of propranolol, the mean change with 10^{-2}M histamine was (-)39 beats per minute, and the corresponding value in atria from reserpine-pretreatment rats was (-)55 beats per minute.

However, the negative chronotropic response seen with high concentrations of histamine when propranolol was added to the perfusate appeared to be reversed when atropine was also included in the buffer (Figure 17). Under these conditions, (ie. 10^{-7}M atropine and 10^{-7}M propranolol) only the highest dose of histamine used (10^{-2}M) caused a significant increase in heart rate over basal value.

Effect of Histamine on Force of Contraction of Electrically Stimulated (1Hz) Rat Left Atria

Increases in force of contraction was measured following only high doses of histamine. Consequently, the results express only the per cent increases in force following 10^{-3}M histamine (Table 15, Figure 18).

A mean increase of 80% over control tension was observed following the addition of 10^{-3}M histamine (Table 15). The increase in contractile force was not significantly altered by the addition of either 10^{-7}M cimetidine or 10^{-7}M promethazine to the perfusate.

When histamine (10^{-3}M) was added to rat left atria in the presence of 10^{-6}M propranolol, a significant reduction in the inotropic effect of the agonist was observed, with histamine producing a mean increase in force of only 15%. Similarly, histamine (10^{-3}M) produced a mean increase in

force of only 10% when its inotropic response was studied in left atria obtained from reserpine-pretreated rats (Table 15).

TABLE 1: The effect of histamine on isolated spontaneously beating kitten right atria. Values are absolute heart rates (beats/min) under control conditions (basal heart rate, basal H.R.) and in the presence of various antagonists.

Results expressed as mean rate (beats/min) $\pm$S.E.M. for 7-10 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

		conc. of histamine (M)					
	basal H.R.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (10)	125±4	125±4	125±4	131±4	169±11[a]	202±10[a]	212±7[a]
$+10^{-7}$ Propran. (7)	133±4	133±4	133±4	133±4	136±6[b]	159±6[a,b]	157±14[a,b]
$+10^{-5}$ Cimet. (10)	124±5	124±5	124±5	124±5	124±5[b]	133±5[a,b]	175±8[a,b]
$+10^{-6}$ Cimet. (8)	123±5	123±5	123±5	123±5	130±7[b]	170±13[a]	186±14[a]
$+10^{-7}$ Promet. (8)	125±11	125±11	125±11	125±11	149±11	172±10[a]	195±6[a]

TABLE 2: The effect of histamine on electrically paced (1Hz) isolated kitten left atria. Results are expressed in absolute tension (g) both in the absence of histamine (basal developed force, B.D.F.) and following administration of histamine under various conditions.

Results expressed as mean tension (g) $\pm$ S.E.M. for 7-10 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

		conc. of histamine (M)					
	B.D.F.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (10)	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.7±0.2	1.8±0.2	2.2±0.2[a]
$+10^{-7}$ Propran. (7)	1.5±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.7±0.2	1.7±0.2	1.8±0.2[a,b]
$+10^{-5}$ Cimet. (10)	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.6±0.3	2.0±0.3[a]
$+10^{-6}$ Cimet. (8)	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.6±0.2[a,b]
$+10^{-7}$ Promet. (8)	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.3±0.3	1.3±0.2	1.8±0.2[a]

TABLE 3: The effect of histamine on electrically paced (1Hz) kitten right ventricle strips. Results are expressed in absolute tension (g) both in the absence of histamine (basal developed force, B.D.F.) and following administration of histamine under various conditions.

Results are expressed as mean tension (g) $\pm$ S.E.M. for 7-10 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	B.D.F.	conc. of histamine (M) 10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (10)	1.6±0.3	1.6±0.3	1.6±0.3	1.6±0.3	1.8±0.3	1.8±0.3[a]	2.2±0.3
$+10^{-7}$ Prop. (7)	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.7±0.2[b]
$+10^{-6}$ Cimet. (10)	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.7±0.3	1.7±0.3[a]	2.2±0.4
$+10^{-5}$ Cimet. (8)	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2[a]	2.1±0.2

TABLE 4: The effect of histamine on electrically paced (1Hz) kitten right papillary muscles. Results are expressed in absolute tension (g) both in the absence of histamine (basal developed force, B.D.F.) and following administration of histamine under various conditions.

Results are expressed as mean tension (g) $\pm$ S.E.M. for 7-10 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	conc. of histamine (M)						
	B.D.F.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (10)	1.0±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1[a]	1.5±0.2
$+10^{-7}$ Prop.(7)	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1	1.2±0.1	1.4±0.2[b]
$+10^{-5}$ Cimet.(10)	1.4±0.3	1.4±0.3	1.4±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.8±0.3
$+10^{-6}$ Cimet.(8)	1.5±0.2	1.7±0.2	1.7±0.2	1.7±0.2	1.7±0.2	1.7±0.2[a]	2.1±0.3
$+10^{-7}$ Promet.(8)	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2[a]	1.5±0.4

TABLE 5: The effect of 4-methylhistamine (4MH) on rate of spontaneously beating isolated kitten right atria. Results express the rates (beats/min) under control conditions (basal heart rate) and following administration of 4MH under various conditions.

Results represent the mean rate (beats/min) $\pm$ S.E.M. for 8-12 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	4-methylhistamine (M)					
	basal H.R.	10^{-5}	$3x10^{-5}$	10^{-4}	$3x10^{-4}$	10^{-3}
Control (10)	112±6	114±3	124±6	157±4[a]	181±9[a]	192±11[a]
$+10^{-6}$ Cimet.(10)	111±4	115±3	121±3	141±5[a]	161±4[a]	178±6[a]
$+10^{-5}$ Cimet.(8)	122±4	122±4	124±5	139±8	150±6[a,b]	158±7[a,b]
$+10^{-6}$ Propran.(12)	115±5	115±6	118±6	126±4[a]	143±6[a,b]	152±9[a,b]

TABLE 6: The effect of 4-methylhistamine (4MH) on electrically paced (1Hz) kitten left atria. Results express the tension (g) in the absence of 4MH (basal developed force, B.D.F.) and in the presence of 10^{-3}M 4MH. Also shown is the percent increase over B.D.F. following administration of 10^{-3}M 4MH.

Results represent the mean tension (g) $\pm$ S.E.M. of 10-12 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	B.D.F.	$+10^{-3}$M 4MH	% Δforce
Control (10)	1.8 ± 0.3	3.3 ± 0.3^{a}	86 ± 12
$+10^{-7}$ Prop. (12)	1.7 ± 0.3	2.1 ± 0.4^{b}	23 ± 6^{b}
$+10^{-6}$ Cimet. (10)	2.0 ± 5	3.6 ± 0.6^{a}	80 ± 11

TABLE 7: The effect of 4-methylhistamine (4MH) on electrically paced (1Hz) kitten right ventricle strips. Results express the tension (g) in the absence of 4MH (basal developed force, B.D.F.) and in the presence of 10^{-3}M 4MH. Also shown is the percent increase over B.D.F. following administration of 10^{-3}M 4MH.

Results represent the mean tension (g) $\pm$ S.E.M. of 10-12 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	B.D.F.	10^{-3}M 4MH	% Δ force
Control (10)	1.6±0.3	3.1±0.6[a]	94±11
$+10^{-7}$M Propr. (12)	1.4±0.4	1.7±0.4	21±6[b]
$+10^{-6}$M Cimet. (10)	1.8±0.3	3.2±0.4[a]	79±12

TABLE 8: The effect of 4-methylhistamine (4MH) on electrically paced (1Hz) kitten right papillary muscles. Results express the tension (g) in the absence of 4MH (basal developed force, B.D.F.) and in the presence of 10^{-3}M 4MH. Also shown is the percent increase over B.D.F. following administration of 10^{-3}M 4MH.

Results represent the mean tension (g) $\pm$ S.E.M. of 10-12 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

	B.D.F.	10^{-3}M 4MH	% Δforce
Control (10)	1.1±0.2	1.6±0.6	46±8
$+10^{-7}$M Propr. (12)	1.0±0.3	1.1±0.5	12±2[a]
$+10^{-6}$M Cimet. (10)	12.±0.3	1.7±0.5	42±9

TABLE 9: The effect of PEA on rate of spontaneously beating guinea-pig right atria. Results express the rates (beats/min) in the absence (basal heart rate) and presence of PEA under various conditions. Values express the mean rates (beats/min) $\pm$ S.E.M. for 12-28 preparations.

Atria were obtained from either control or reserpine-pretreated (2.5 mg/kg, 24 hours) guinea-pigs.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

		PEA (M)					
	basal H.R.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (28)	176±6	176±6	176±6	180±11	220±12[a]	276±10[a]	302±13[a]
+3x10^{-6}M Promet. (18)	170±11	170±1	170±1	170±11	198±9	257±10[a]	295±11[a]
+3x10^{-6}M Cimet. (18)	182±4	182±4	182±4	182±4	219±8[a]	270±4[a]	292±11[a]
+10^{-6} Propran. (20)	166±6	167±9	167±9	168±11	179±5[a]	219±8[a,b]	252±12[a,b]
Reserpine-pretreated (20)	179±8	179±8	181±5	181±5	193±6	207±3[a,b]	223±6[a,b]
+Propran. (10^{-6}M) (12)	168±9	168±9	168±9	168±6	172±6[b]	176±7[b]	181±6[b]

TABLE 10: The effect of PEA on contractile force of electrically paced (1Hz) guinea-pig left atria. Results express the absolute tension (g) for atria in the absence of PEA (basal developed force, B.D.F.) or following administration of PEA under various conditions.

Results express the mean tension (g) $\pm$ S.E.M. for 18-28 tissues.

a = sign. diff. ($p \leq 0.05$) compared to basal value.

b = sign. diff. ($p \leq 0.05$) compared to control curve.

		PEA (M)					
	B.D.F.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (28)	0.6±0.1	0.6±0.1	0.7±0.1	1.1±0.2[a]	1.8±0.1[a]	2.5±0.3[a]	2.8±0.2[a]
+3x10^{-6}M Cimet. (18)	0.5±0.1	0.5±0.1	0.6±0.1	1.1±0.2[a]	1.7±0.2[a]	2.6±0.3[a]	3.0±0.4[a]
+3x10^{-6}M Promet. (24)	0.4±0.2	0.4±0.2	0.4±0.2	0.6±0.2[b]	1.4±0.1[a,b]	2.2±0.4[a]	2.7±0.4[a]
+10^{-6}M Propran. (20)	0.5±0.1	0.5±0.1	0.6±0.1	0.8±0.2	1.4±0.1[a,b]	1.8±0.1[a.b]	1.9±0.2[a.b]

TABLE 11: The effect of PEA on contractile force of electrically paced (1Hz) guinea-pig left atria. Results express the absolute tension (g) for atria in the absence of PEA (basal developed force, B.D.F.) or following administration of PEA under various conditions.

Results express the mean tension (g) $\pm$ S.E.M. for 12-28 tissues obtained from either control or reserpine-pretreated animals (2.5 mg/kg, 24 hours).
a = sign. diff. ($p \leq 0.05$) compared to basal value.
b = sign. diff. ($p \leq 0.05$) compared to control curve.

		PEA (M)					
	B.D.F.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (28)	0.6±.1	0.6±0.1	0.7±0.1	1.1±0.2[a]	1.8±0.1[a]	2.5±0.3[a]	2.8±0.2[a]
Reserpine (20)	0.7±.1	0.7±0.1	0.8±0.1	1.2±0.2[a]	1.6±0.2[a]	1.9±0.2[a]	2.1±0.3[a, b]
Reserpine +3x10^{-6}M Cimet. (12)	0.8±.1	0.8±0.1	0.8±0.1	1.0±0.2	1.5±0.1[a]	2.0±0.1[a]	2.2±0.2[a]
Reserpine +3x10^{-6}M Prometh. (20)	0.5±0.2	0.5±0.2	0.5±0.2	0.9±0.2	1.3±0.2[a, b]	1.7±0.2[a, b]	1.9±0.2[a, b]

TABLE 12: The effect of PEA on contractile force of electrically stimulated (1Hz) guinea-pig right ventricle strips. Results express the per cent increase in force (over basal developed force) produced by PEA under various conditions, and represent the mean ± S.E.M. of 16-20 tissues, obtained from control or reserpine-pretreated animals (2.5 mg/kg, 24 hours).

a = sign. diff. ($p \leq 0.05$) from control values.

	PEA (M)					
	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (20)	0	0	11 ± 3	25 ± 4	90 ± 5	122 ± 20
$+10^{-6}$M Propran. (16)	0	0	5 ± 2	18 ± 5	63 ± 2^{a}	80 ± 5
$+3 \times 10^{-6}$M Promet. (16)	0	0	4 ± 2	10 ± 5	66 ± 4^{a}	93 ± 4
Reserpine-pre-treated (20)	0	0	12 ± 8	23 ± 4	58 ± 6^{a}	70 ± 6^{a}

TABLE 13: The effect of 4-methylhistamine on rate of spontaneously beating guinea-pig right atria under various conditions. Results express the mean rate (beats/min) $\pm$ S.E.M. of 11-14 tissues.

a = sign. diff. ($p \leqslant 0.05$) compared to basal value.

b = sign. diff. ($p \leqslant 0.05$) compared to control curve.

		4-methylhistamine (M)					
	basal H.R.	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Control (14)	177 ± 9	185 ± 10	219 ± 14^{a}	255 ± 12^{a}	309 ± 13^{a}	319 ± 5^{a}	318 ± 6^{a}
$+10^{-7}$M Propran. (11)	170 ± 6	179 ± 6	204 ± 6^{a}	248 ± 4^{a}	297 ± 6^{a}	315 ± 5^{a}	321 ± 8^{a}
$+10^{-6}$M Cimet. (12)	183 ± 9	185 ± 13	189 ± 6	$205 \pm 7^{a,b}$	$235 \pm 8^{a,b}$	289 ± 6^{a}	316 ± 8^{a}

TABLE 14: The effect of histamine on rate of spontaneously beating isolated rat right atria under various conditions. Rsults express the mean rate (beats/min) $\pm$ S.E.M. of 18-24 tissues obtained from control or reserpine pretreated animals (3.0 mg/kg, 24 hours).

a = sign. diff. ($p \leqslant 0.05$) compared to basal value.

b = sign. diff. ($p \leqslant 0.05$) compared to control curve.

	basal H.R.	10^{-4}	10^{-3}	10^{-2}
Control (20)	260 ± 6	260 ± 6	286 ± 8^{a}	342 ± 11^{a}
$+10^{-7}$M Prometh.(20)	258 ± 5	258 ± 8	289 ± 9^{a}	$384 \pm 9^{a,b}$
$+10^{-7}$M Cimet.(20)	262 ± 4	262 ± 4	280 ± 3^{a}	358 ± 8^{a}
$+10^{-6}$M Propran.(24)	240 ± 6	240 ± 6	235 ± 6^{b}	$202 \pm 8^{a,b}$
Reserpine pretreat.(18)	245 ± 3	240 ± 6	225 ± 8^{b}	$190 \pm 11^{a,b}$
10^{-7}M Propran.+ 10^{-7}M Atropine (18)	238 ± 6	238 ± 6^{b}	248 ± 10^{b}	$282 \pm 15^{a,b}$

TABLE 15: The effect of histamine (10^{-3}M) on contractile force of electrically paced (1Hz) isolated rat left atria under various conditions. Results express the per cent increase in force of over basal values $\pm$ S.E.M. of 18-24 tissues obtained from either control or reserpine pretreated (3.0 mg/kg, 24 hours) animals.

a = sign. diff. ($p \leqslant 0.05$) from control values.

Treatment	10^{-3} Histamine
Control (20)	80 ± 12
$+10^{-7}$M Cimetidine (20)	72 ± 18
$+10^{-7}$M Promethazine (20)	80 ± 20
$+10^{-6}$M Promethazine (24)	15 ± 7^{a}
Reserpine pretreated (18)	10 ± 12^{a}

FIGURE 1: The effect of histamine on the rate of spontaneously beating kitten right atria. Antagonists were added to the buffer one hour prior to addition of agonist.

y axis: absolute rate (beats/min).

x axis: log concentration histamine (M).

Results represent the mean $\pm$ S.E.M. of 7-10 tissues.

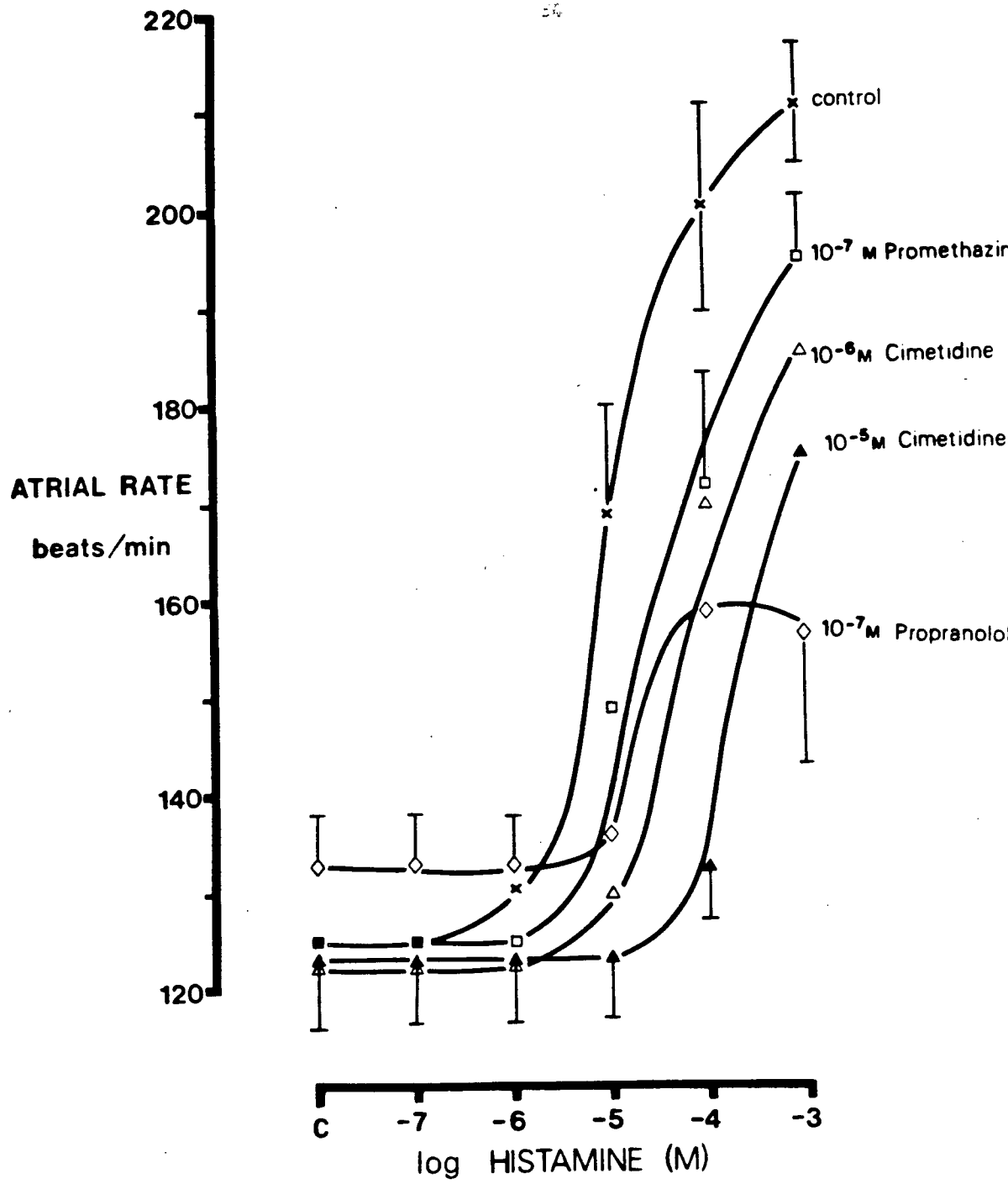

220
200
180
160
140
120
ATRIAL RATE
beats/min
control
10-7 M Promethazine
10-6M Cimetidine
10-5M Cimetidine
10-7M Propranolol
C
-7
-6
-5
-4
-3
log HISTAMINE (M)

FIGURE 2: Maximal changes in rate produced by 10^{-3}M histamine in isolated kitten right atria. Bars represent the means $\pm$ S.E.M. of basal rate (control), and maximal responses to 10^{-3}M histamine and the absence and presence of various antagonists (obtained from results in Fig. 1).

Asterisks indicate a significant decrease in response compared to the effect of 10^{-3}M in the absence of antagonist ($p \leqslant 0.05$).

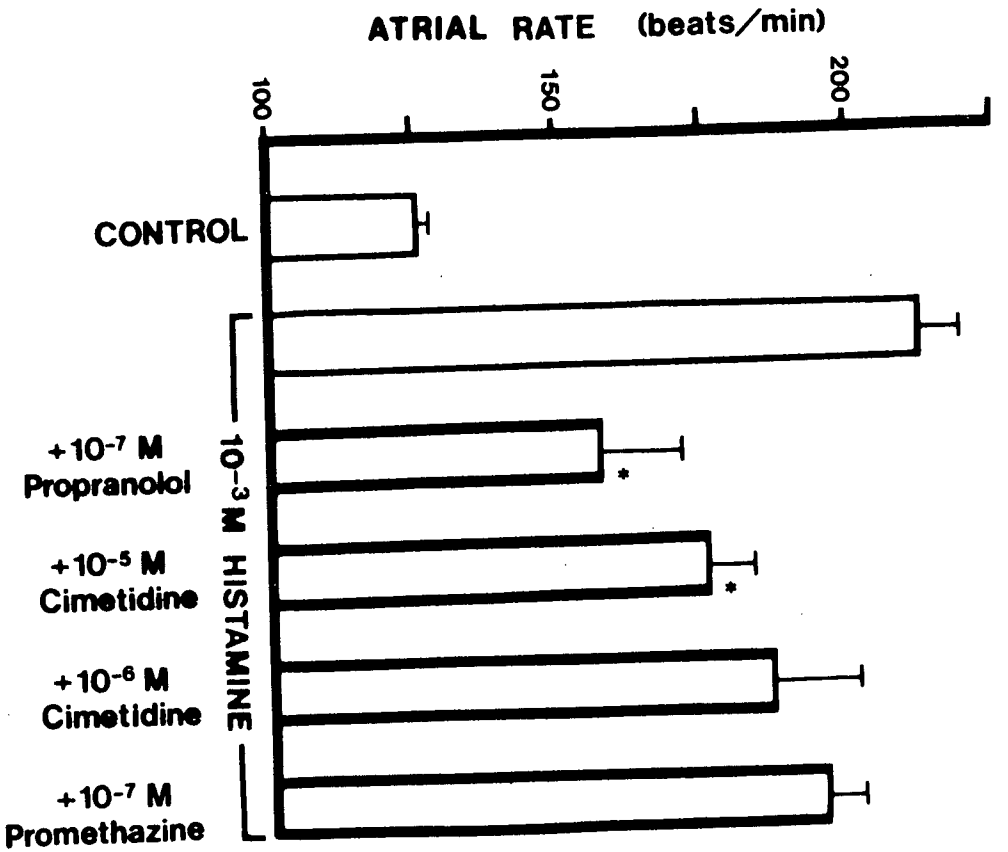

ATRIAL RATE (beats/min)
100
150
200
CONTROL
10⁻³ M HISTAMINE
+10⁻⁷ M Propranolol
+10⁻⁵ M Cimetidine
+10⁻⁶ M Cimetidine
+10⁻⁷ M Promethazine
*
*

FIGURE 3: Maximal changes in force of contraction (expressed as per cent increase over basal developed force) produced by 10^{-3}M histamine in the presence of various antagonists in isolated kitten left atria.

Asterisks represent a significant decrease in response compared to the effect of 10^{-3}M histamine in the absence of antagonist ($p \leqslant 0.05$). Results express the mean $\pm$ S.E.M. of 7-10 tissues.

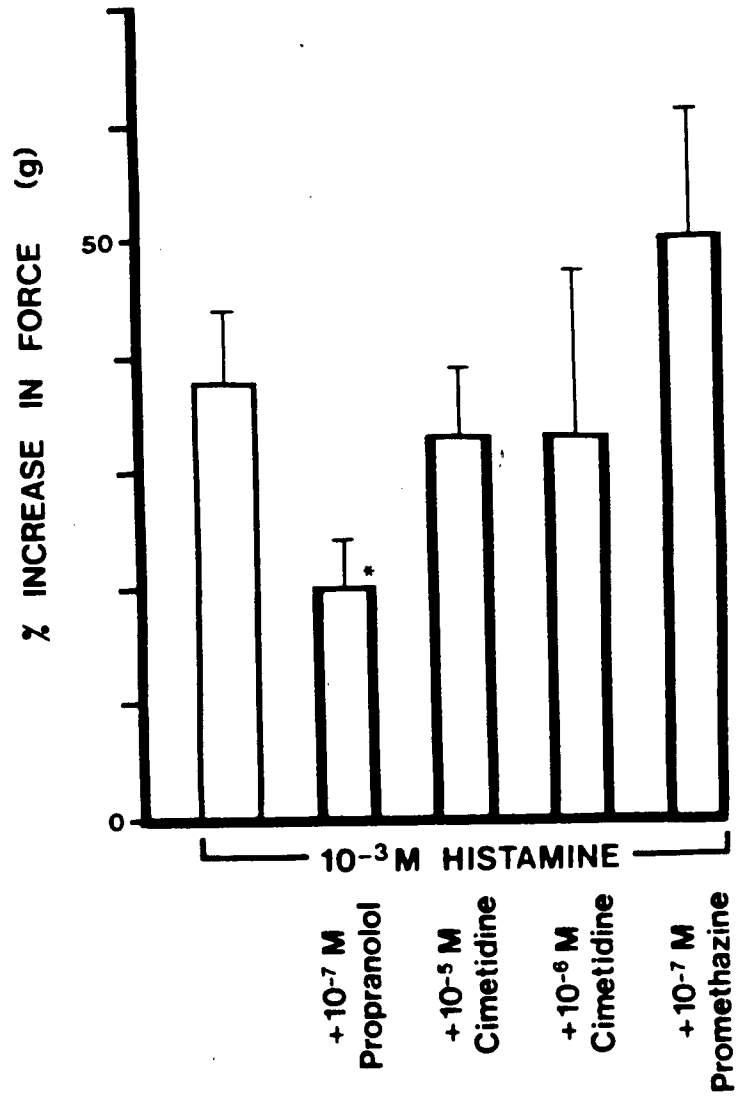

% INCREASE IN FORCE (g)
50
0
*
10⁻³ M HISTAMINE
+10⁻⁷ M Propranolol
+10⁻⁵ M Cimetidine
+10⁻⁶ M Cimetidine
+10⁻⁷ M Promethazine

FIGURE 4: Maximal increase in force of contraction of kitten right ventricle strips (expressed as per cent increase over basal developed force) produced by 10^{-3}M histamine in the presence of various antagonists.

Asterisk indicates a significantly decreased response compared to the effect of 10^{-3}M histamine in the absence of antagonist ($p \leq 0.05$). Results express the mean $\pm$ S.E.M. of 7-10 tissues.

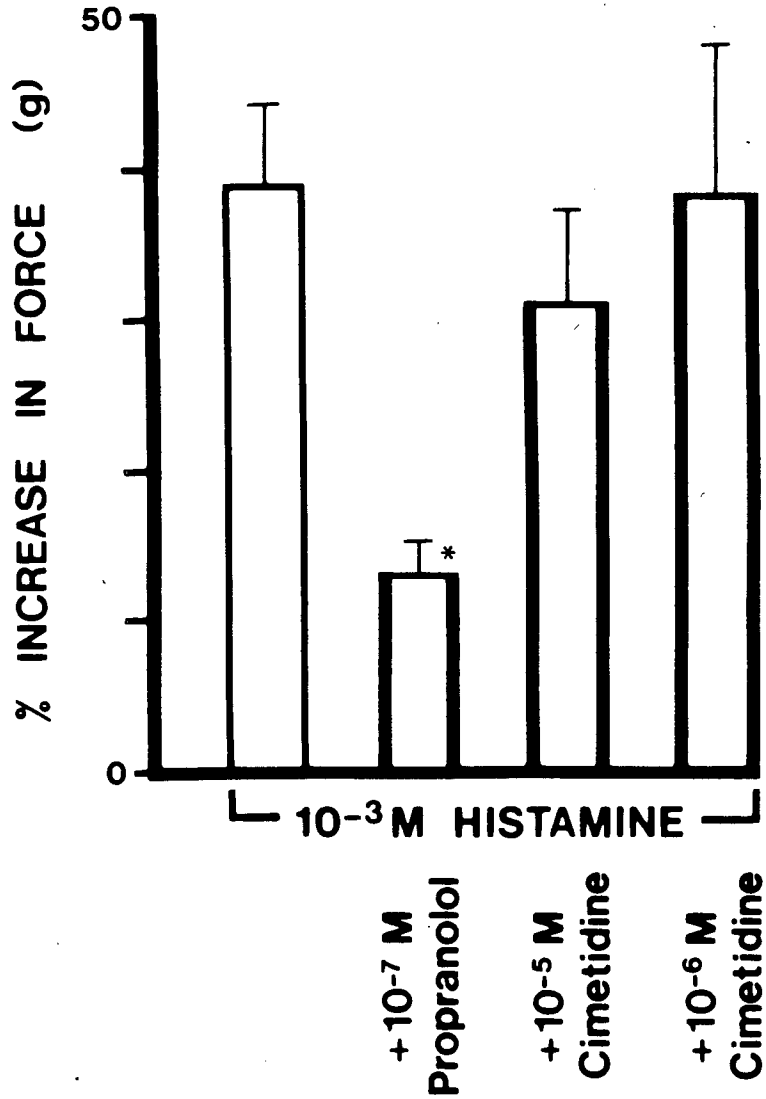

50
0
% INCREASE IN FORCE (g)
*
10-3 M HISTAMINE
+10-7 M Propranolol
+10-5 M Cimetidine
+10-6 M Cimetidine

FIGURE 5: Maximal increase in force of contraction of kitten right papillary muscles (expressed as per cent increase over basal developed force) produced by 10^{-3} M histamine in the presence of various antagonists.

Asterisk indicates a significantly decreased response compared to the effect of 10^{-3} M histamine in the absence of antagonist ($p \leq 0.05$). Results express the mean $\pm$ S.E.M. of 7-10 tissues.

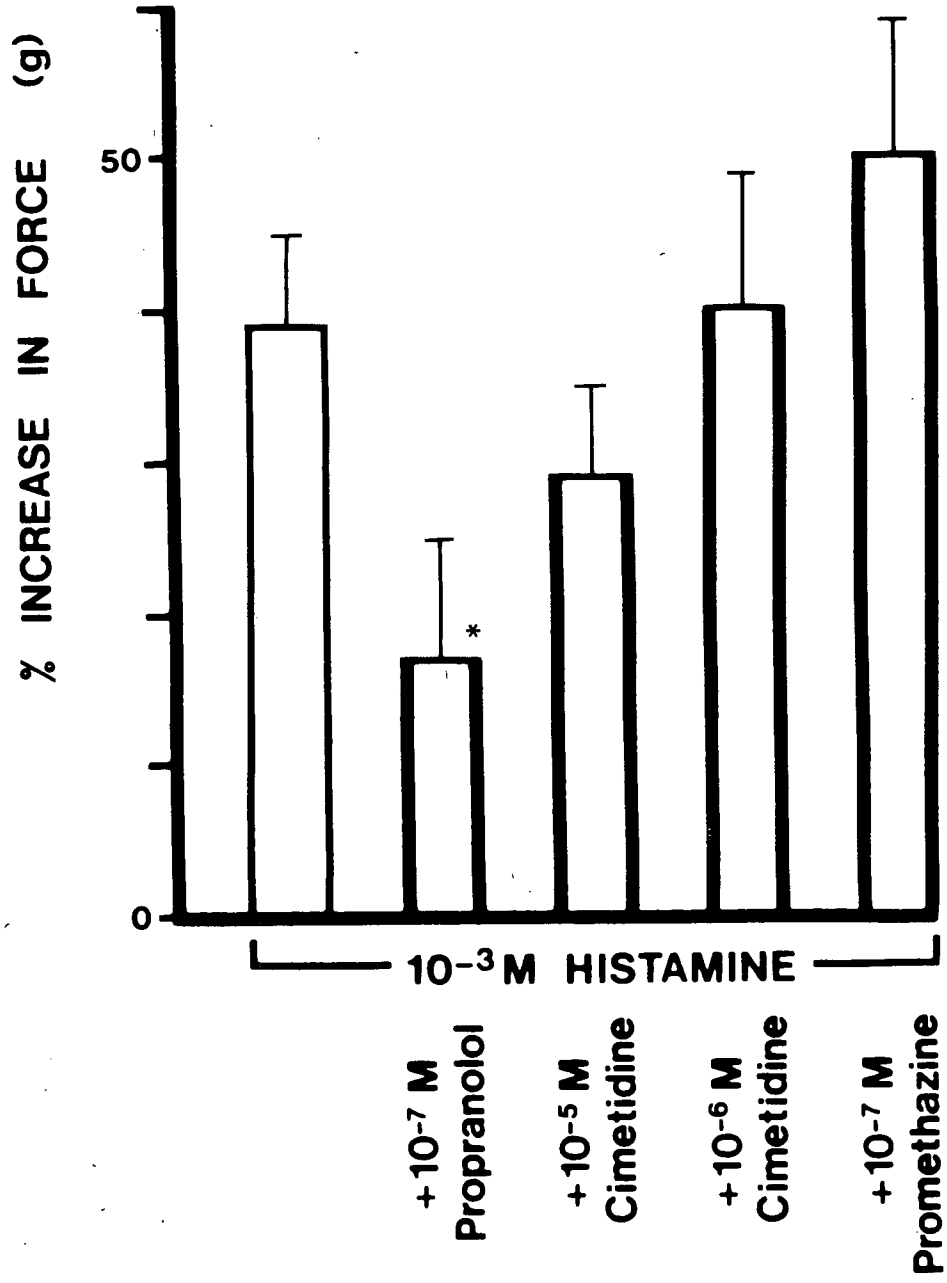

% INCREASE IN FORCE (g)
50
0
*
10⁻³ M HISTAMINE
+10⁻⁷ M Propranolol
+10⁻⁵ M Cimetidine
+10⁻⁶ M Cimetidine
+10⁻⁷ M Promethazine

FIGURE 6: The effect of 4-methylhistamine on the rate of spontaneously beating kitten right atria. Antagonists were added to perfusate one hour prior to addition of agonist.

y axis: absolute rate (beats/min)

x axis: log concentration 4-methylhistamine (M).

Results express the mean $\pm$ S.E.M. of 8-12 tissues.

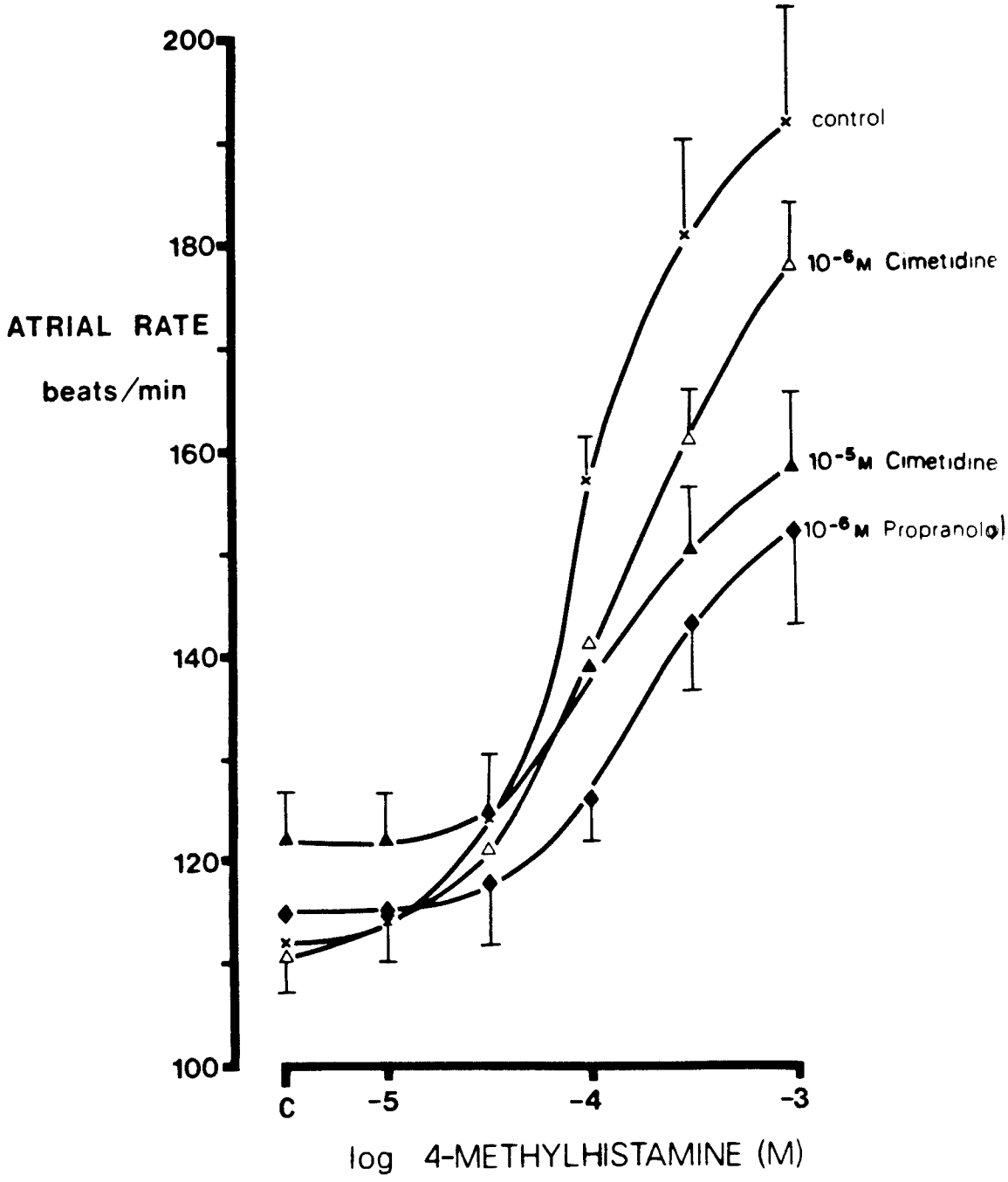

200
180
160
140
120
100
ATRIAL RATE
beats/min
control
10-6M Cimetidine
10-5M Cimetidine
10-6M Propranolol
C
-5
-4
-3
log 4-METHYLHISTAMINE (M)

FIGURE 7: Maximal changes in rate of spontaneously beating kitten right atria produced by 10^{-3}M 4-methylhistamine (4MH). Antagonists were added to perfusate one hour prior to addition of agonist, and bars represent the mean $\pm$ S.E.M. of basal rate (control) and the effect of 10^{-3}M 4MH in the absence or presence of antagonists.

Asterisks represent a significant decrease in response compared to the effect of 10^{-3}M 4MH in the absence of antagonist ($p \leq 0.05$).

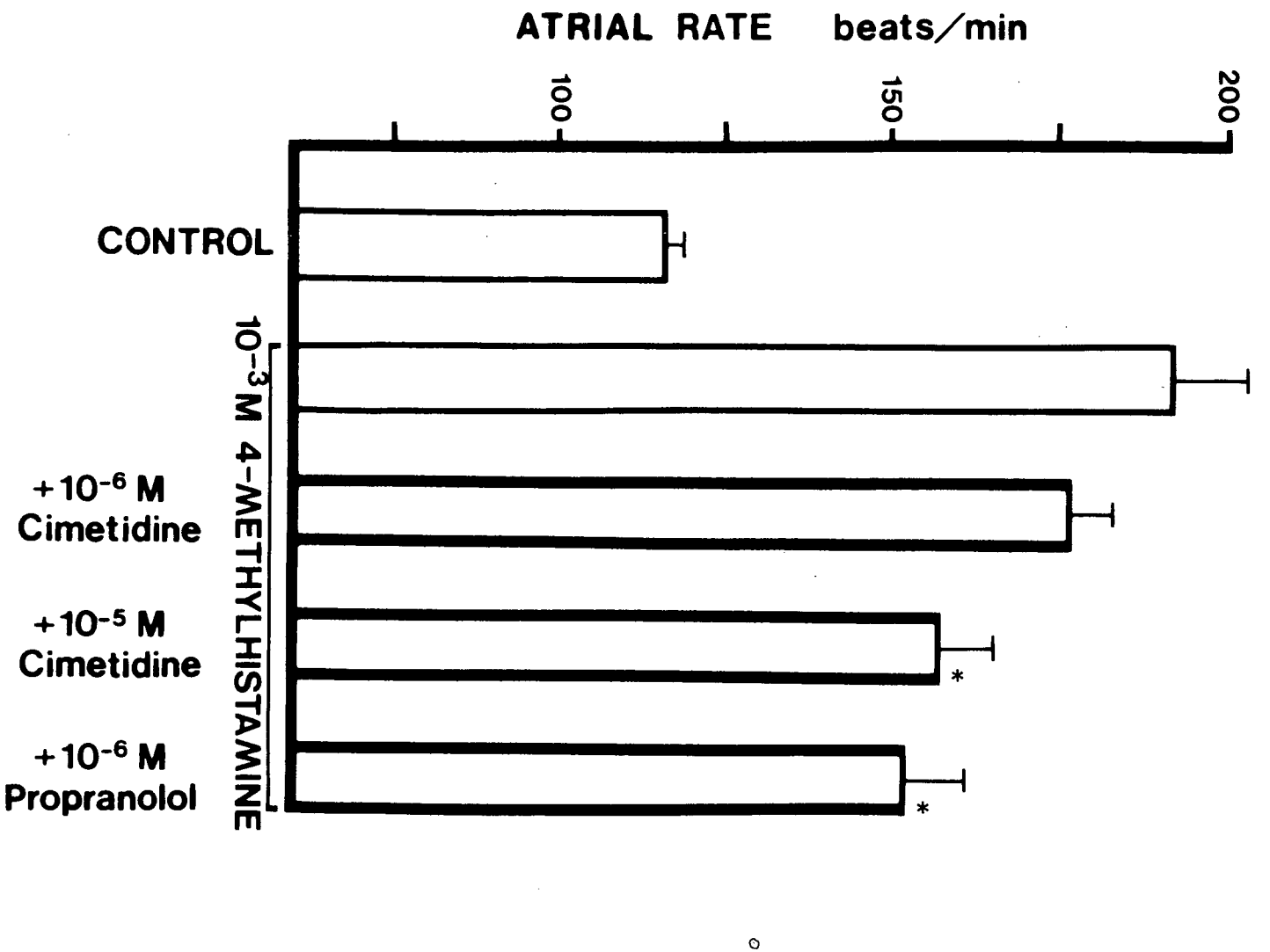

ATRIAL RATE beats/min
100
150
200
CONTROL
10⁻³ M 4-METHYLHISTAMINE
+10⁻⁶ M Cimetidine
+10⁻⁵ M Cimetidine
+10⁻⁶ M Propranolol
*
*

FIGURE 8: Changes in force of contraction in isolated kitten left atria produced by 4-methylhistamine (4MH) (expressed as per cent increase in force over basal developed force) in the absence or presence of antagonists.

Asterisk represents a significant decrease of response compared to the effect of 10^{-3}M 4MH in the absence of antagonist ($p \leq 0.05$).

Bars represent the mean $\pm$ S.E.M. of 10-12 determinations.

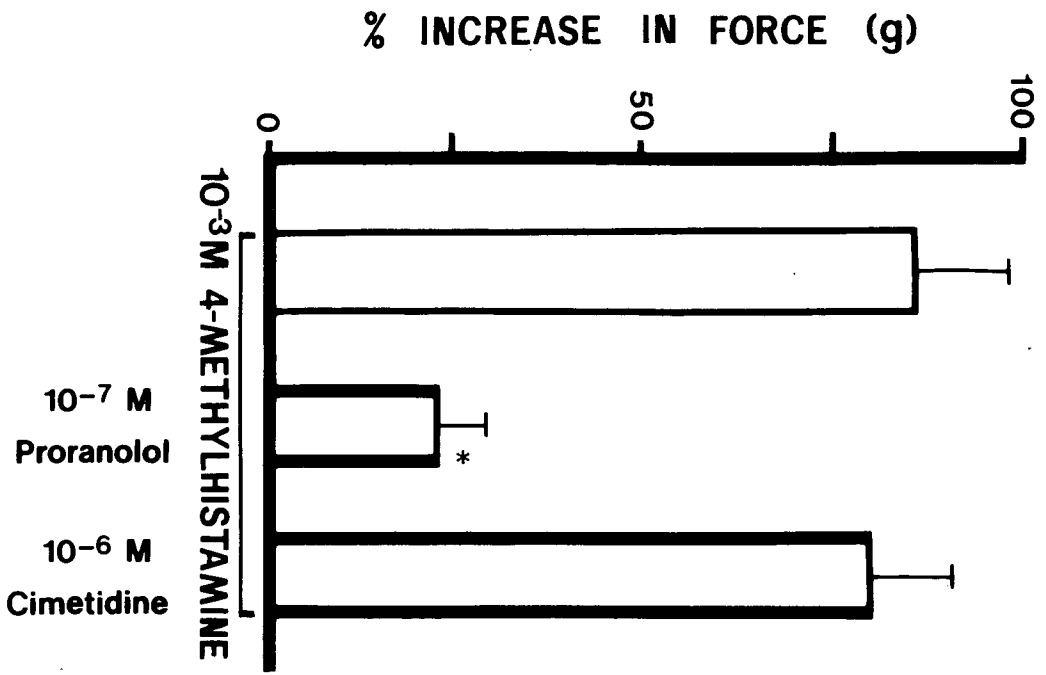

% INCREASE IN FORCE (g)
0
50
100
10-3M 4-METHYLHISTAMINE
10-7 M
Proranolol
10-6 M
Cimetidine
*

FIGURE 9: Changes in force of contraction in isolated kitten right ventricle strips produced by 4-methylhistamine (4MH) (expressed as per cent increase in force over basal developed force) in the absence or presence of antagonists.

Asterisk represents a significant decrease of response compared to the effect of 10^{-3}M 4MH in the absence of antagonist ($p \leq 0.05$).

Bars represent the mean $\pm$ S.E.M. of 10-12 determinations.

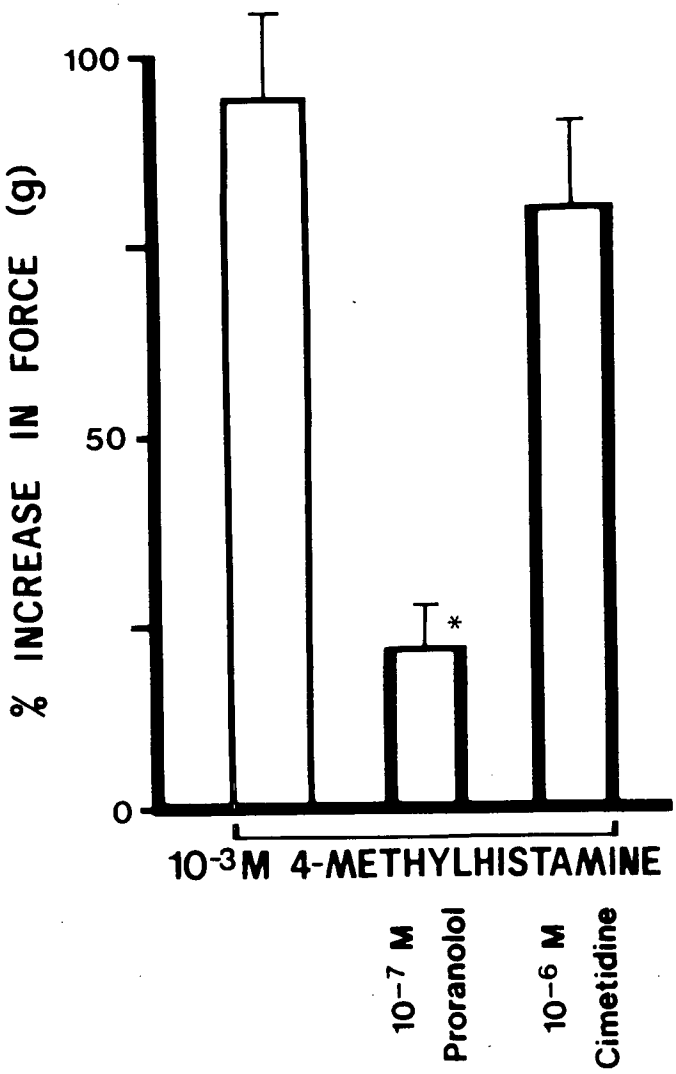

100
50
0
% INCREASE IN FORCE (g)
*
10-3M 4-METHYLHISTAMINE
10-7 M Proranolol
10-6 M Cimetidine

FIGURE 10: Changes in force of contraction in isolated kitten right papillary muscles produced by 4-methylhistamine (4MH) (expressed as per cent increase in force over basal developed force) in the absence or presence of antagonists.

Asterisk represents a significant decrease of response compared to the effect of 10^{-3}M 4MH in the absence of antagonist ($p \leq 0.05$).

Bars represent the mean $\pm$ S.E.M. of 10-12 determinations.

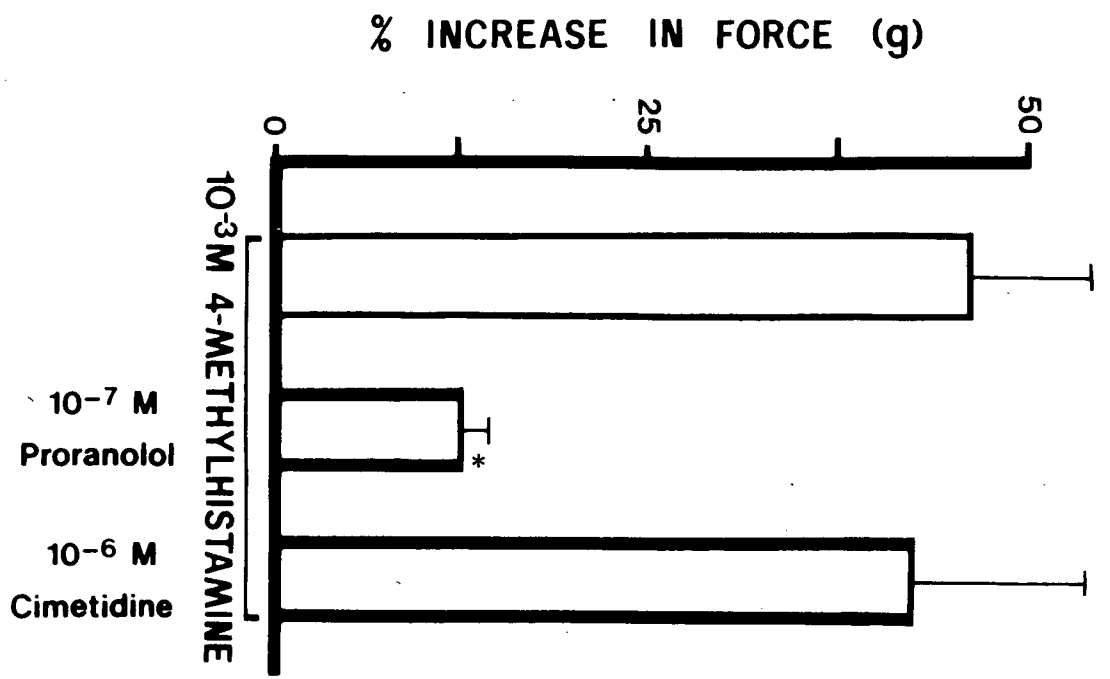

% INCREASE IN FORCE (g)
0
25
50
10-3M 4-METHYLHISTAMINE
10-7 M
Proranolol
10-6 M
Cimetidine
*

FIGURE 11: Changes in rate of isolated guinea-pig right atria produced by PEA in the absence or presence of antagonists.

y axis: absolute rates (beats/min)

x axis: log concentration PEA (M)

Results express the mean $\pm$ S.E.M. of 18-28 experiments.

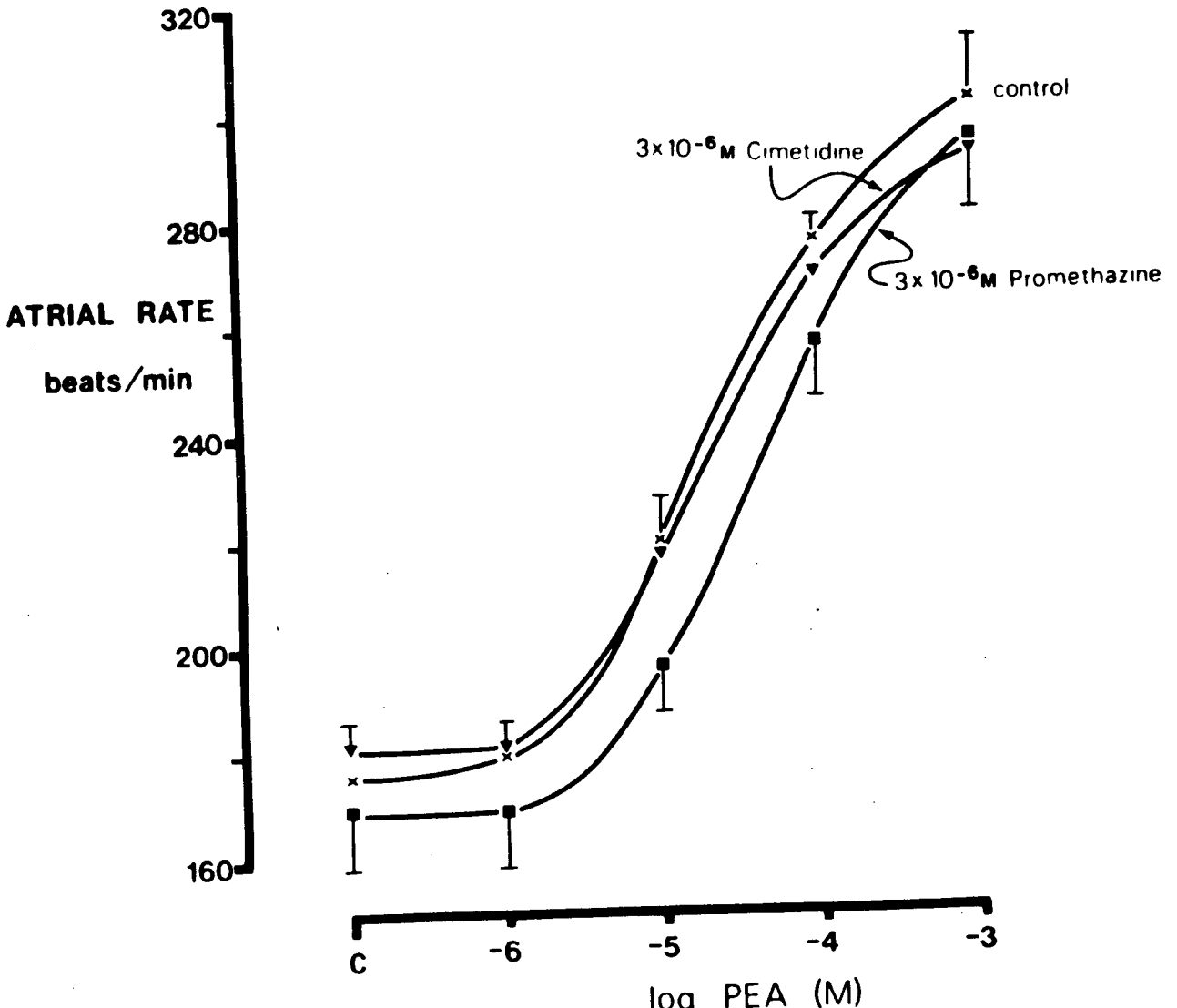

320
280
240
200
160
ATRIAL RATE
beats/min
control
3x10-6M Cimetidine
3x10-6M Promethazine
C
-6
-5
-4
-3
log PEA (M)

FIGURE 12: Effect of PEA on rate of spontaneously beating right atria from untreated (control) and reserpine-pretreated (2.5 mg/kg, 24 hours) guinea-pigs.

y axis: absolute rate (beats/min)

x axis: log concentration PEA (M)

Rsults express the mean ± S.E.M. of 12-28 experiments.

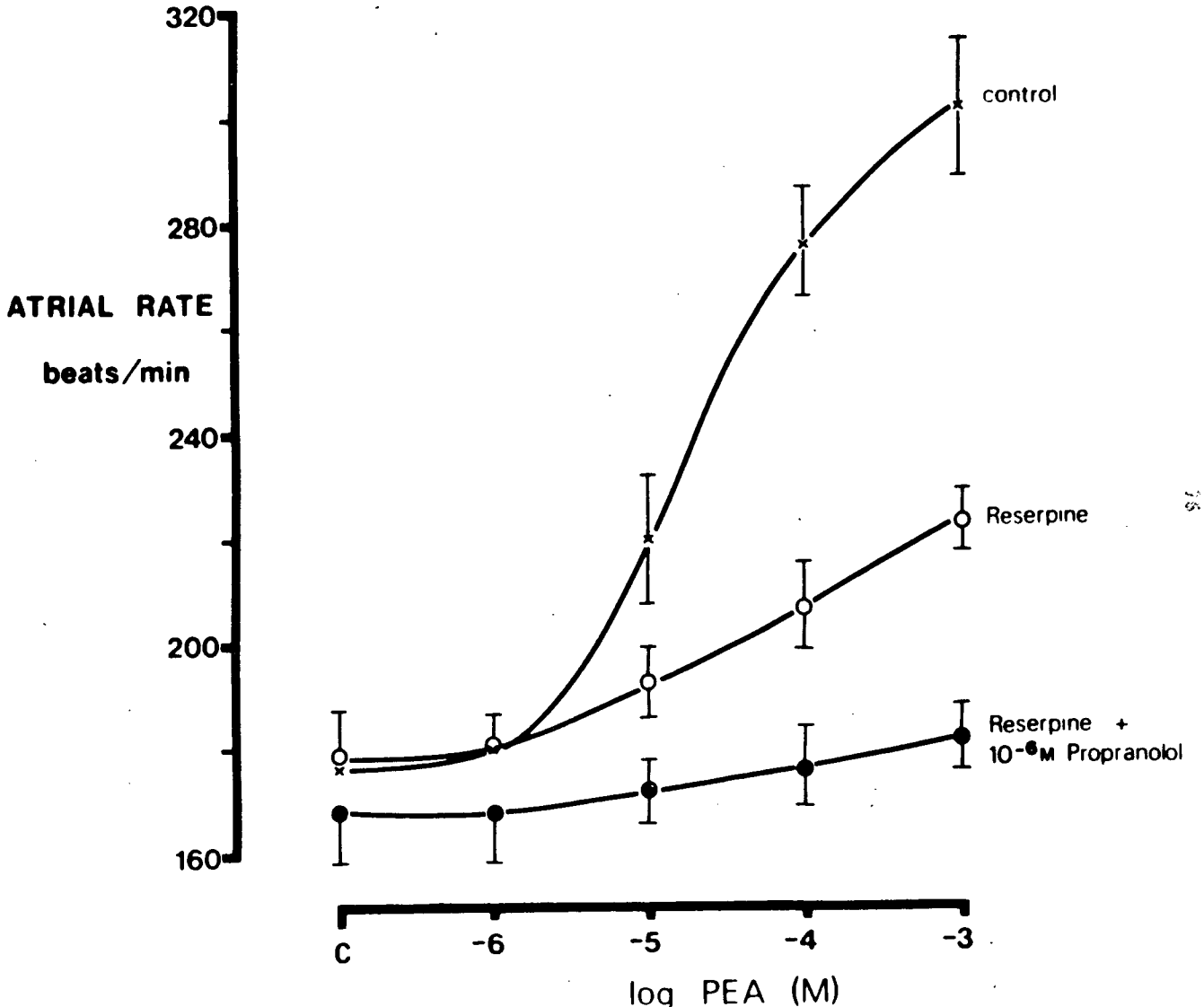

320
280
240
200
160
ATRIAL RATE
beats/min
control
Reserpine
Reserpine +
10-6M Propranolol
C
-6
-5
-4
-3
log PEA (M)

FIGURE 13: Increases in tension (g) of electrically paced (1Hz) guinea-pig left atria produced by PEA in the absence (control) or presence of various antagonists.

y axis: absolute developed tension (g)

x axis: log concentration PEA (M)

Results express the mean ± S.E.M. of 18-28 exerpiments.

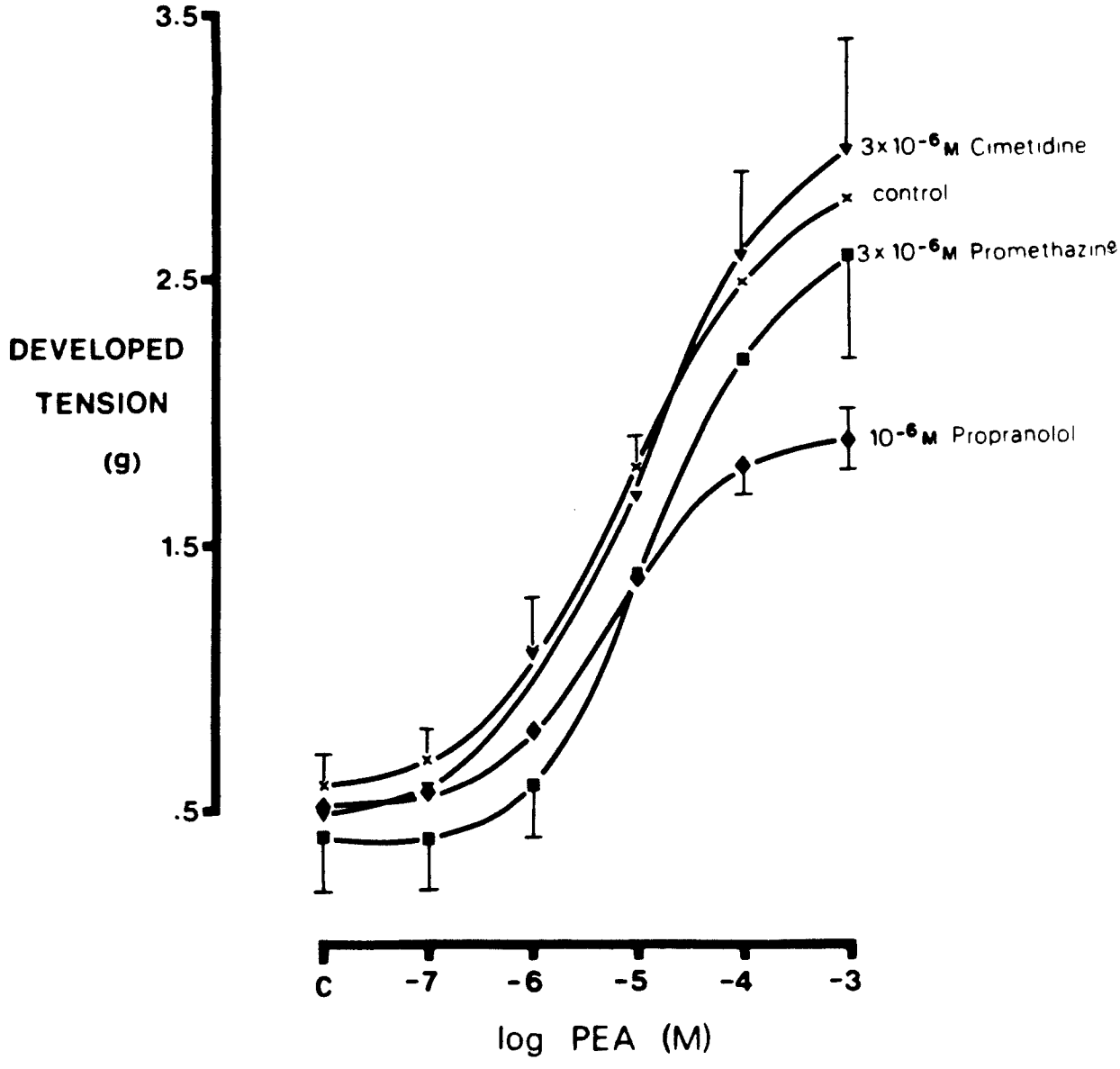

3.5
2.5
1.5
.5
DEVELOPED
TENSION
(g)
3x10-6M Cimetidine
control
3x10-6M Promethazine
10-6M Propranolol
C
-7
-6
-5
-4
-3
log PEA (M)

FIGURE 14: Increases in tension (g) produced by PEA in electrically paced (1Hz) left atria obtained from untreated (control) and reserpine-pretreated (2.5 mg/kg, 24 hours) guinea-pigs.

y axis: absolute developed tension (g)

x axis: log concentration PEA (M)

Results express the mean ± S.E.M. of 12-28 experiments.

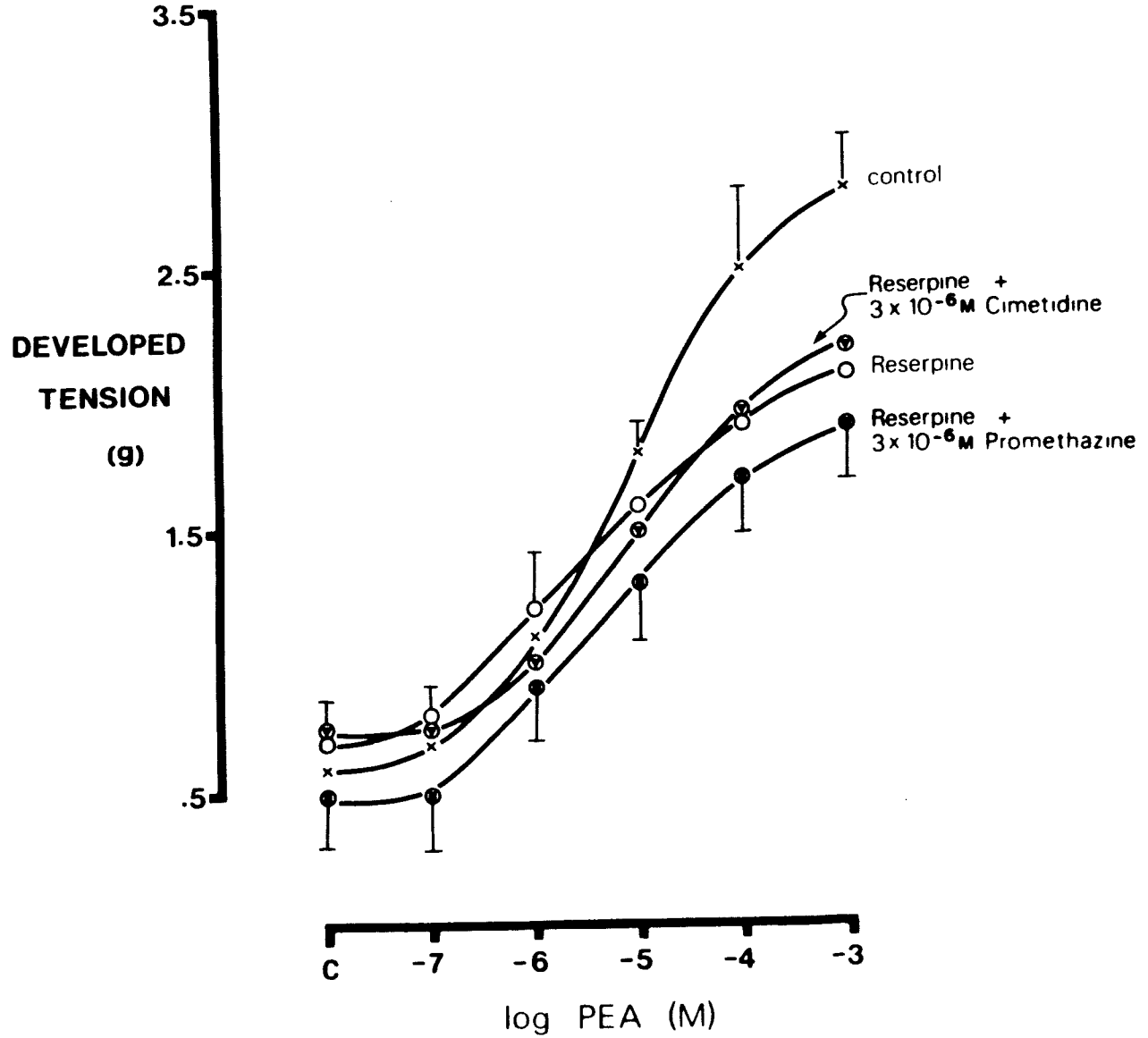

3.5
2.5
1.5
.5
DEVELOPED
TENSION
(g)
control
Reserpine +
3 x 10⁻⁶M Cimetidine
Reserpine
Reserpine +
3 x 10⁻⁶M Promethazine
C
-7
-6
-5
-4
-3
log PEA (M)

FIGURE 15: Increases in tension of guinea=pig isolated right ventricle strips produced by PEA in the absence or presence of antagonists. Responses were obtained in electrically paced (1Hz) ventricle strips from untreated and reserpine pretreated guinea-pigs (2.5 mg/kg, 24 hours).

y axis: per cent increase in force (over basal developed tension)

x axis: log concentration PEA (M)

Results express the mean $\pm$ S.E.M. of 16-20 experiments.

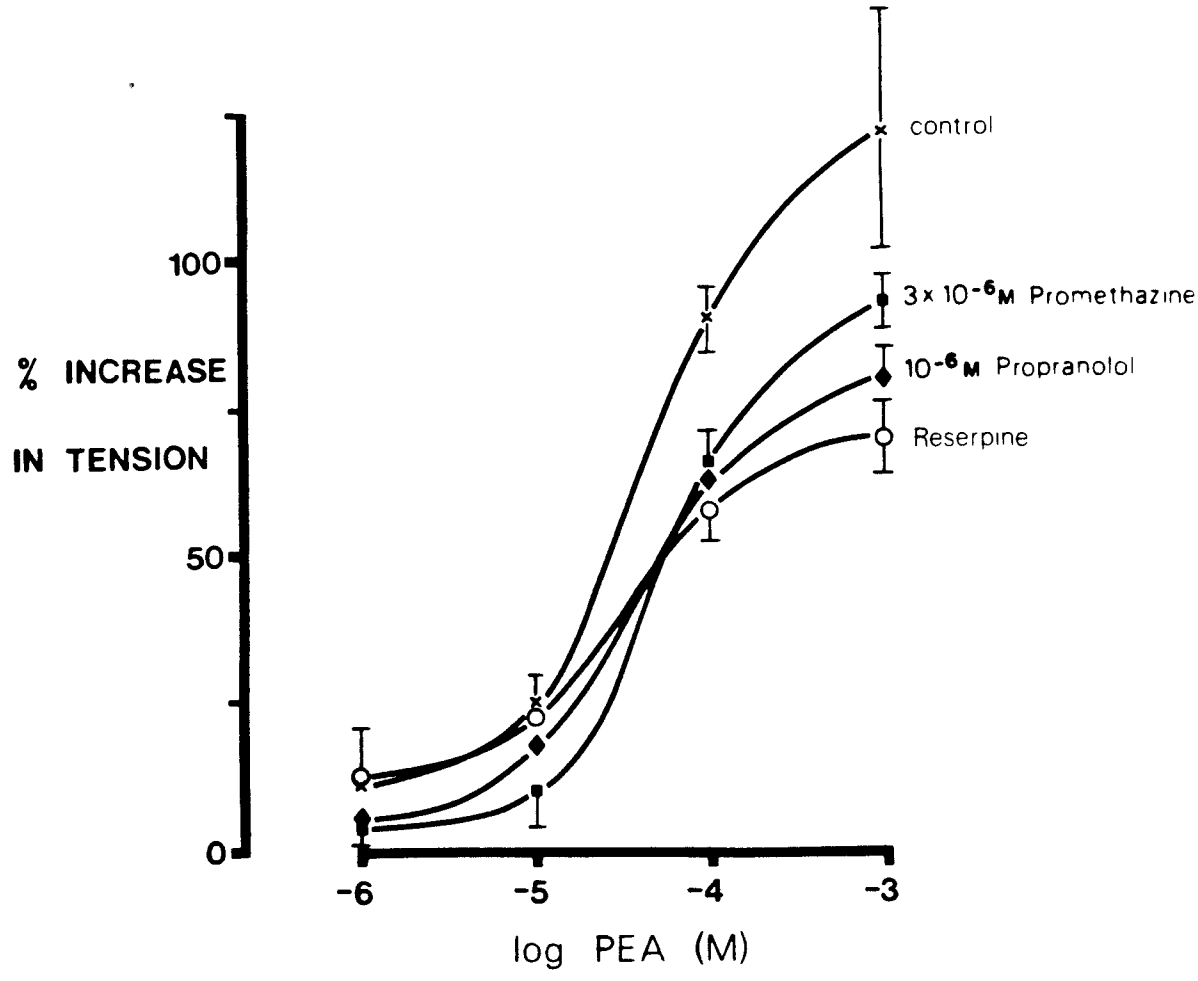

control
3 x 10-6M Promethazine
10-6M Propranolol
Reserpine
100
50
0
% INCREASE
IN TENSION
-6
-5
-4
-3
log PEA (M)

FIGURE 16: Increases in rate of isolated guinea-pig right atria produced by 4-methylhistamine in the absence (control) or presence of antagonists.

y axis: absolute rate (beats/min)

x axis: log concentration 4-methylhistamine (M)

Results express the mean $\pm$ S.E.M. of 12-14 experiments.

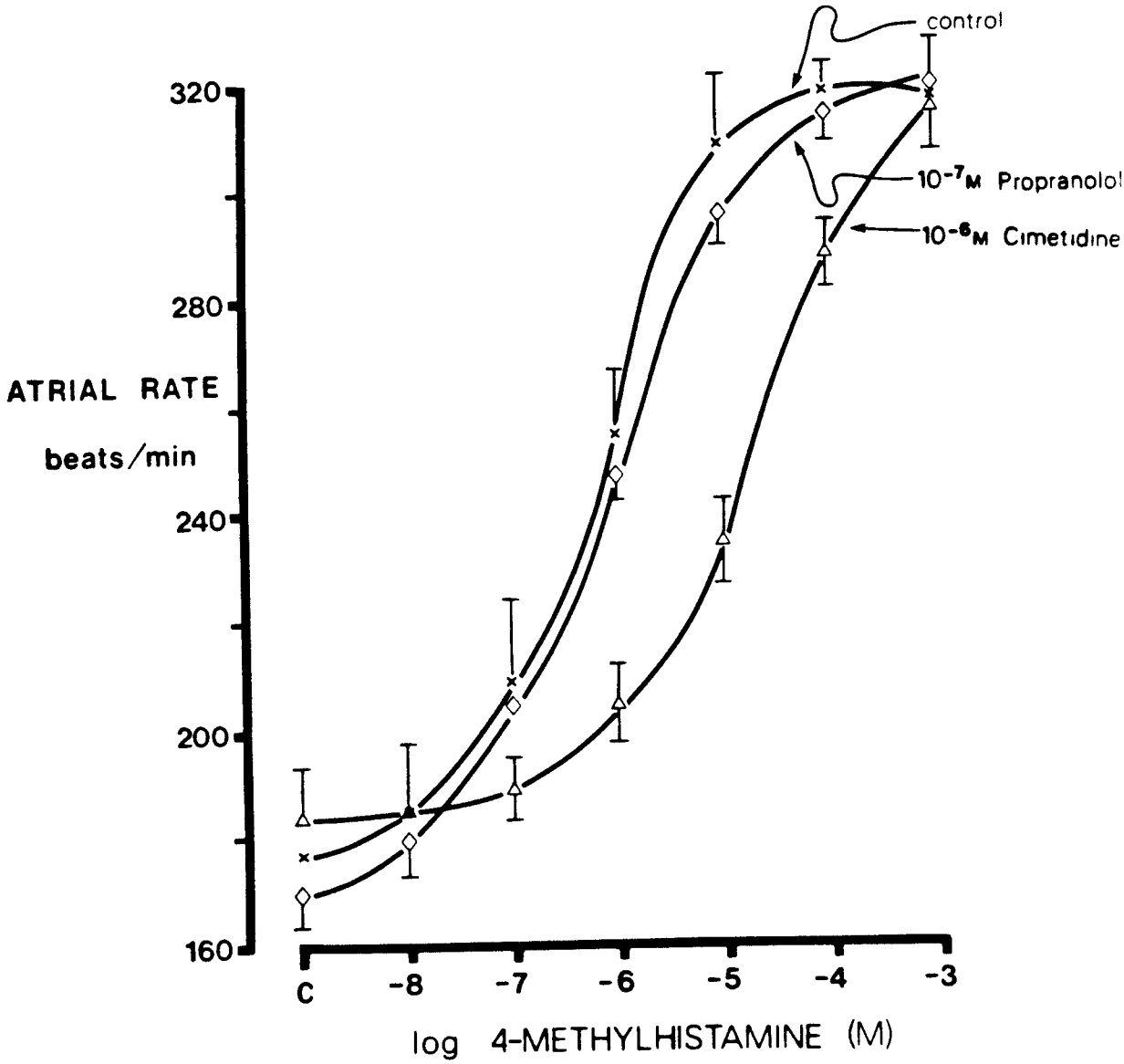

control
10-7M Propranolol
10-6M Cimetidine
320
280
240
200
160
ATRIAL RATE
beats/min
C
-8
-7
-6
-5
-4
-3
log 4-METHYLHISTAMINE (M)

FIGURE 17: Changes in rate of isolated rat atria produced by histamine in the absence (control) or presence of antagonists. Where atria were obtained from reserpine-pretreated animals, rats were pretreated 24 hours prior to experiment at a dose of 3.0 mg/kg.

y axis: absolute rate (beats/min)

x axis: log concentration histamine (M)

Results express the mean $\pm$ S.E.M. of 18-20 experiments.

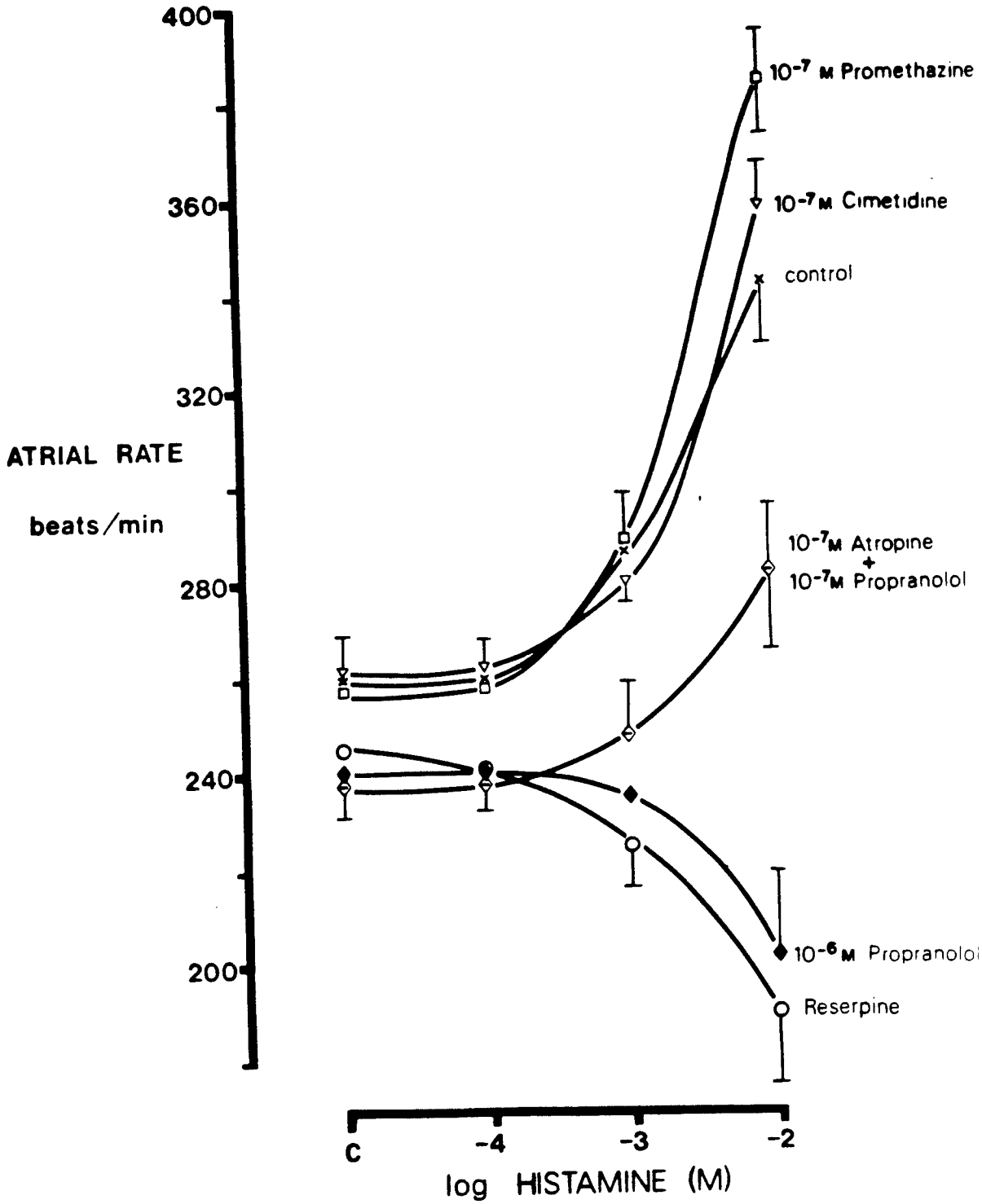
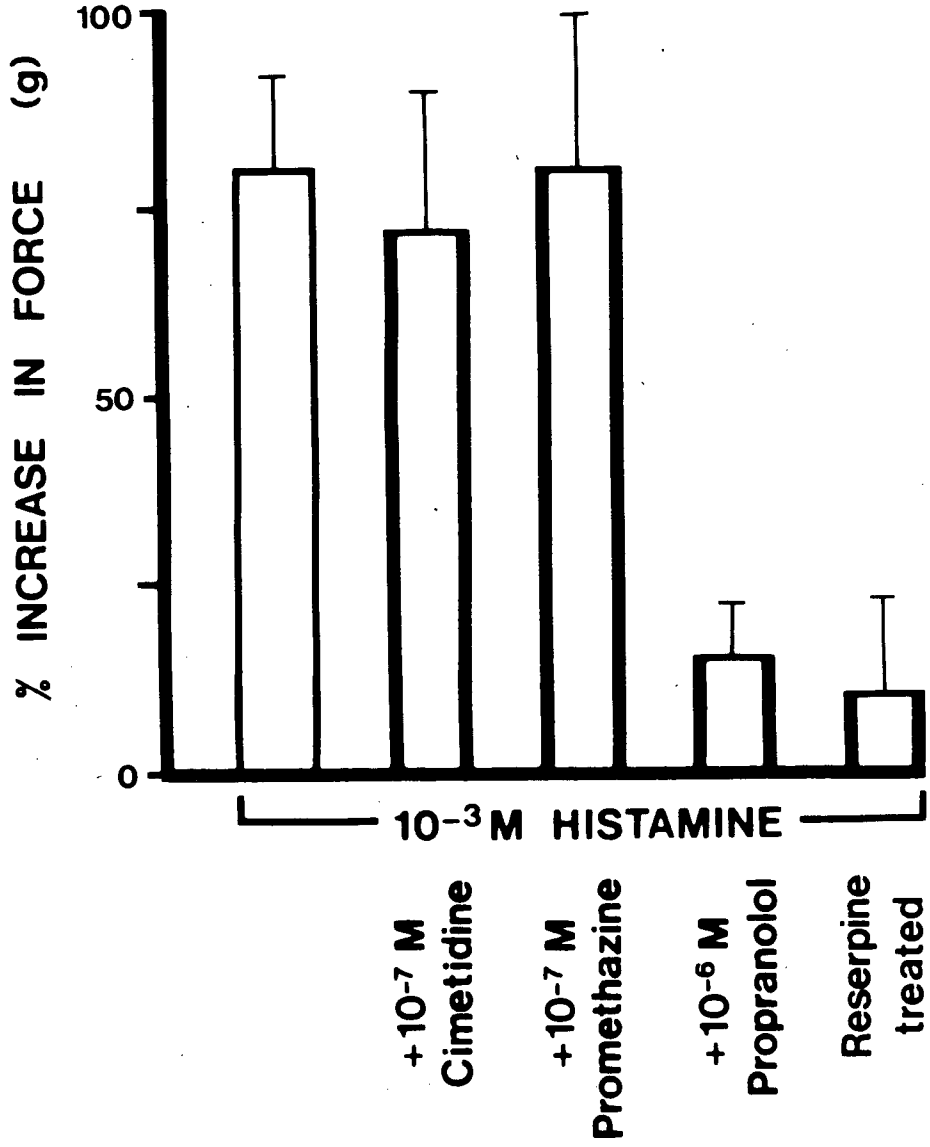

400
360
320
280
240
200
ATRIAL RATE
beats/min
10⁻⁷ M Promethazine
10⁻⁷M Cimetidine
control
10⁻⁷M Atropine
+
10⁻⁷M Propranolol
10⁻⁶M Propranolol
Reserpine
C
-4
-3
-2
log HISTAMINE (M)

FIGURE 18: Effect of 10^{-3} M histamine on force of contraction (expressed as per cent increase over basal developed tension) of isolated left atria obtained from untreated (control) and reserpine-pretreated rats (3.0 mg/kg, 24 hours).

Results express the mean $\pm$ S.E.M. of 18-24 experiments.


% INCREASE IN FORCE (g)
100
50
0
10⁻³ M HISTAMINE
+10⁻⁷ M Cimetidine
+10⁻⁷ M Promethazine
+10⁻⁶ M Propranolol
Reserpine treated

DISCUSSION

The positive inotropic and chronotropic effect of histamine has been demonstrated to be the result of an initial drug-receptor interaction (Ash and Schild, 1966; Black et al., 1972; Verma and McNeill, 1976 and Levi et al., 1976). The results of the present study indicate that in some species, the cardiac responses due to histamine are due to an interaction of the agonist with specific cardiac histamine receptors. In addition, the results obtained also demonstrate that cardiac responses due to histamine and its analogs are not always due to such drug-receptor interactions, but are due to histamine-induced release of endogenous noradrenaline.

Trendelenburg (1960) demonstrated that in the isolated kitten heart, histamine produced a positive chronotropic response. Since only changes in rate were reported by Trendelenburg, it was an aim of the present study to more clearly define any changes in both force and rate of contraction produced by histamine in the isolated kitten heart. To do this, a variety of tissues were used -- right atria to measure rate changes and left atria, right ventricle strips and right papillary muscles to measure changes in contractile force.

It is clear from this study that histamine produced pronounced changes in both rate and force of contraction in all preparations used from the kitten heart. The slow nature of the responses observed is in agreement with the finding by Trendelenburg that the full effect to any dose of histamine usually takes about 5 minutes to develop.

Closer examination of the Trendelenburg study reveals that when the

effect of histamine was studied in isolated kitten atria, only a single dose of histamine (6.7×10^{-6}M) was used throughout. The inappropriatness of such an experimental protocol is demonstrated when the results of this study are considered.

When histamine was added to isolated kitten right atria, a positive chronotropic response was recorded (Figure 1). Both the histamine H_2-receptor antagonist cimetidine and the β-adrenoceptor antagonist, propranolol caused an inhibition of the histamine effect. However, this inhibition was only significant at higher doses of histamine, and consequently may explain why Trendelenburg (1960) was unable to record any inhibition with a low test-dose of histamine in isolated kitten right atria.

Propranolol caused the greatest inhibition of the maximal chronotropic response to histamine (Figure 1). This indicates a strong involvement of β-adrenoceptors in the response to histamine, in addition to the activation of histamine H_2-receptors as suggested by the antagonism with cimetidine. However, Trendelenburg (1960) claimed that the inhibition of the rate response to histamine following administration of a β-adrenoceptor antagonist, dichloroisoprenaline, was due to an elevated basal heart rate because of the intrinsic activity of dichloroisoprenaline, thus reducing any chronotropic changes produced by histamine. The results obtained in the present study, using propranolol, a β-adrenoceptor antagonist with no intrinsic activity, indicate that the chronotropic action of histamine can be blocked by β-adrenoceptor antagonists. This suggests that the blockade of the histamine response produced by both dichloroisoprenaline and propranolol was due to β-adrenoceptor blockade

and that histamine produces part of its chronotropic response by releasing endogenous catecholamines.

The presence of histamine H_2-receptors in the kitten right atrium is indicated by a competitive blockade of the histamine dose-response curve by two doses of cimetidine (Figure 1). The pA_2 for the antagonism of histamine at histamine H_2-receptors (eg. guinea-pig right atria) using burimamide as the antagonist is 5.11 (Black et al., 1972). Trendelenburg (1960) reported a mean pA_2 value of 5.1 in cat right atria using pyrilamine as the antagonist. The pA_2 value in organs in which pyrilamine acts as a specific antagonist of the H_1 effecting histamine, such as the guinea-pig ileum, is approximately 9.3 (Arunlakshana and Schild, 1959). Based on these reported values, and on the results of this study indicating the presence of histamine H_2-receptors in the kitten right atrium, it would appear quite likely that the blockade observed by Trendelenburg (1960) using high doses of pyrilamine was a blockade of histamine H_2-receptors by a histamine H_1-receptor antagonist.

The reported effects of histamine H_1-receptor antagonists on cardiac contractility and adenylate cyclase activity is unclear. Several workers (Trendelenburg, 1960; Mannaioni, 1960; McNeill and Muschek, 1972) reported that high concentrations of histamine H_1-receptor antagonists resulted in a blockade of histamine H_1-receptor mediated contractility of the heart in a non-competitive or irreversible manner. When the effects of histamine on adenylate cyclase activity was measured, it was also found that high concentrations of diphenhydramine (Klein and Levey, 1971; McNeill and Muschek, 1972) and mepyramine (Weinryb and Michel, 1975) blocked this effect. The blockade of the effect of histamine on cardiac adenylate

cyclase by tripelennamine and diphenhydramine was through a non-competitive mechanism according to the study of McNeill and Muschek (1972). On the other hand, Johnson and Mizoguchi (1977) later reported that the inhibition of the effect of histamine on cardiac ventricular adenylate cyclase by tripelennamine was competitive.

Kanof and Greengard (1979) have recently studied the pharmacological properties of a histamine-sensitive adenylate cyclase preparation from guinea-pig cardiac ventricular muscle. This preparation compared favourably with the properties of other histamine H_2-receptors as defined by physiological experiments in peripheral tissues (Black et al., 1972; Verma and McNeill, 1977). For example, the relative potencies of compounds as agonists of physiological responses mediated by histamine H_2-receptors (but not H_1-receptors) agree well with their relative potencies as activators of the adenylate cyclase preparation. In addition, the inhibition constants for antagonists (eg. cimetidine, metiamide) for the effects of histamine on the chronotropic effects on guinea-pig atria and on stimulation of the adenylate cyclase preparation also agreed (Kanof and Greengard, 1979). However, the adenylate cyclase preparation of Kanof and Greengard (1979) that had all the characteristics of histamine H_2-receptors was also affected by the classical antihistamines (eg. mepyramine, diphenhydramine, promethazine) in a competitive manner. This finding confirmed the earlier report by Johnson and Mizoguchi (1977) who also found that histamine H_1-receptor antagonists acted as competitive inhibitors of histamine-sensitive adenylate cyclase. According to Kanof and Greengard (1979) the histamine H_1-receptor antagonists were much less potent inhibitors of histamine H_2-receptors coupled to adenylate cyclase than as inhibitors of the physio-

logical effects of histamine mediated by H_1-receptors.

The classical antihistamines are known to possess local anaesthetic properties at high concentrations (Dutta, 1949). It is clear from the results of Kanof and Greengard (1979) that under certain conditions (eg. in cell-free preparations of guinea-pig cardiac ventricular muscle) histamine H_1-antagonists can competitively inhibit the effects of histamine on H_2-receptors. Kanof and Greengard (1979) also demonstrated that this effect was dependent on the chemical class of the histamine H_1-antagonist. These authors speculated that the discrepancies in the literature regarding the effects of histamine H_1-antagonists on the myocardial effects of histamine could in part be due to differences in concentrations and types of antagonists used. In view of this suggestion, it would have been interesting to study the effects of other histamine H_1-receptor antagonists in some of the experiments discussed.

It is evident that histamine H_2-receptors are involved in the chronotropic effect of histamine in the kitten right atrium. This result was next verified using a specific histamine H_2-recept agonist, 4-methylhistamine. Using this agonist, a dose-dependent increase in rate was again antagonised by both cimetidine and propranolol (Figures 6 and 7). Again it is apparent that histamine and its analogs produce a positive chronotropic effect both via a direct histamine H_2-receptor stimulation and through an indirect mechanism, namely a release of endogenous catecholamines. As was mentioned in an earlier discussion of the results, high doses of 4-methylhistamines were required to produce a chronotropic effect; this would be expected since Black et al., (1972) have pointed out that this agonist possesses a maximum of only 43.0% of the maximum activity

of histamine on histamine H_2-receptor mediated responses such as in the guinea-pig right atrium.

Using a test dose of histamine, Trendelenburg (1960) was able to record only small changes in force of contraction. However, due to a large variability in the response, Trendelenburg failed to report any inotropic data for histamine in the isolated kitten heart. In the present study, when the inotropic effect of histamine was studied in three electrically paced tissues from the kitten heart, neither a histamine H_1- or H_2-receptor antagonist altered the responses (Figures 3, 4 and 5). Some potentiation of the histamine response was observed in the kitten left atrium and right papillary muscle in the presence of promethazine (Figures 3 and 5). No results for the effect of promethazine in the kitten right ventricle strip are shown, since under these conditions the variability of both the basal tension and the developed tension was extraordinarily high. No immediate explanation for this observation can be provided.

Propranolol significantly reduced the inotropic response to both histamine and 4-methylhistamine in the kitten left atrium, right ventricle strip and right papillary muscle (Figures 6 to 10). Since only propranolol and neither of the histamine receptor-antagonists, altered the inotropic responses of histamine and 4-methylhistamine, it was assumed that the inotropic responses to these amines in the kitten heart were due entirely to indirect mechanisms involving a release of endogenous catecholamines.

Histamine is known to cause a release of catecholamines from the adrenal medalla via histamine H_1-receptor stimulation (Burn and Dale, 1926; Emmelin and Muren, 1949). In the rat uterus, histamine is thought to release catecholamines through mediation by histamine H_2-receptors (Verma

and McNeill, 1976; McNeill and Verma, 1975). If either histamine H_1- or H_2-receptors were involved in the release of catecholamines during the inotropic responses of histamine in the kitten heart, then addition of either promethazine or cimetidine would have reduced the responses to a greater or equal extent than the reduction observed when low doses of propranolol were used. In fact, inotropic responses to 2-methylhistamine and PEA (results not shown) as well as to 4-methylhistamine were also unaffected by the respective blocking agents, but were decreased by the use of propranolol. These results would argue against a receptor-mediated release of catecholamines during the inotropic or chronotropic responses of histamine in the kitten heart.

Owen (1977), in a preliminary study, reported that histamine-induced changes in heart rate and force of contraction in anaesthetised cats could be reduced or abolished by treatment with either low doses of propranolol (1 mg/kg) or a high dose of mepyramine (5 mg/kg). In these animals which were pretreated with mecamylamine to block autonomic ganglia and so prevent reflex increases in heart rate associated with the depressor response to histamine, no direct effect of histamine on the heart was reported. Higher doses of histamine caused an increased heart rate that was antagonised by metiamide. Owen (1977) therefore concluded that histamine caused tachycardia both by a direct interaction with histamine H_2-receptors as well as by a release of catecholamines from chromaffin tissue.

It was previously mentioned in the introduction to this thesis that Klein and Levey (1971) were able to obtain an activation of myocardial adenylate cyclase by histamine in guinea-pig, cat and human heart. These authors reported that the histamine-mediated activation of adenylate

cyclase in all three preparations was abolished by a dose of diphenhydramine (histamine H_1-receptor antagonist) that caused no effect on a similar response produced by noradrenaline in these tissues. In contrast, DL-propranolol antagonised only the noradrenaline effects in these tissues, and not the histamine activation of myocardial adenylate cyclase. While histamine produced a 300% stimulation of adenylate cyclase activity in the guinea-pig heart, under similar conditions histamine produced only a 60% stimulation of adenylate cyclase activity in the cat heart (Klein and Levey, 1971). Interpretation of the results of the present study on the effects of histamine in the isolated kitten heart are at variance with the report by Klein and Levey (1971); but confirms the lack of a histamine-sensitive adenylate cyclase in the kitten heart suggested in recent experiments by Johnson (personal communication).

While studying the nature of the positive inotropic response to histamine in the guinea-pig left atrium, Broadley and Wilson (1978) reported that PEA caused an indirect β_1-adrenoceptor stimulation in addition to its direct histamine H_1-receptor stimulation. The positive inotropic response of the left atrium in this species is known to be mediated via histamine H_1-receptors (Reinhard et al., 1974; Steinburg and Holland, 1975 and Verma and McNeill, 1977). Numerous studies in our laboratory utilise this agent as a specific histamine H_1-agonist (Verma and McNeill, 1977; McNeill and Verma, 1979) in cardiac preparations of the guinea-pig and rabbit. Histamine H_1-receptors, as well as H_2-receptors, are known to mediate the inotropic responses of histamine in the guinea-pig right ventricle strip. It was of interest during this study to reinvestigate the specificity of PEA as a direct receptor stimulant at the histamine H_1-

receptor in both the guinea-pig left atrium and right ventricle strip; in addition, it was also of interest to characterise any chronotropic changes produced by this agonist.

PEA produced a dose-dependent chronotropic effect in the guinea-pig isolated right atrium that was unaltered in the presence of either promethazine or cimetidine (Figure 11). This result was expected because the guinea-pig right atrium is known not to contain histamine H_1-receptors, based on the classical studies of Ash and Schild (1966). In addition, cimetidine would not have been expected to antagonise the effects of PEA at histamine H_1-receptors, since Black et al. (1972) have demonstrated that the histamine H_2-receptor antagonists (at very high concentrations) are non-competitive antagonists at histamine H_1-receptors. Based on the results observed using PEA in the guinea-pig right atrium, it can be stated that the chronotropic effect of this agent is not mediated through either histamine H_1- or H_2-receptors.

Following this result, right atria were next obtained from animals depleted of endogenous catecholamines by pretreatment with reserpine (some tissues were challenged with tyramine to check the pretreatment schedule). In atria that were depleted of catecholamines, PEA produced a modest increase in rate that became significant only at the higher doses (Figure 12). The residual increase in rate (due to a release of catecholamines) was abolished by using the β-adrenoceptor antagonist, propranolol. These results indicate that the rate changes produced by PEA in the guinea-pig right atrium results from a release of endogenous catecholamines, thus causing a stimulation of β-adrenoceptors.

A part of the inotropic response to PEA in the guinea-pig left atrium

and right ventricle strip also appeared to result from indirect β-adrenoceptor stimulation, as suggested by the results obtained in this study (Figures 13-15). The increases in contractile force produced by PEA in both the guinea-pig left atrium and right ventricle strip was unaffected by cimetidine and reduced in the presence of promethazine or propranolol, or following reserpine pretreatment. Since the antagonism by promethazine occurred only at the lower doses of PEA, while the antagonism due to propranolol only at higher doses of PEA, it would appear that in the guinea-pig left atrium (Figure 13) PEA initially stimulates histamine H_1-receptors directly, while at higher doses the agonist causes a non-specific release of catecholamines. Reserpine pretreatment significantly reduced the maximal response observed with PEA, while at the same time producing no change in sensitivity to the direct actions of histamine at H_1-receptors in either the left atrium (Figure 14) or the right ventricle strip (Figure 15) of the guinea-pig heart.

Verma and McNeill (1977) have previously demonstrated that inotropic responses due to 4-methylhistamine in the guinea-pig right ventricle strip were associated with increases in cyclic AMP, while changes in force due to PEA were not preceded by changes in cyclic AMP in the same tissue. In addition, these workers showed that while changes in the maximal increase in force due to 4-methylhistamine took under 30 seconds to develop in the right ventricle, an equal increase in force produced by PEA in the same tissue required over 60 seconds to develop. The slow nature of the response due to indirectly acting doses of PEA is again apparent.

However, it is evident that in the guinea-pig right ventricle strip, a very narrow dose-range exists for the inotropic response of PEA (Figure 15).

This is in agreement with Verma and McNeill (1977), and to be expected in view of the report by Black et al. (1972) stating that the activities of histamine H_1-receptor specific agonists relative to that of histamine are poor. Flynn et al. (1979) in their studies on the roles of histamine H_1- and H_2-receptors in the working heart preparation of the guinea-pig also reported that PEA had little effect on cardiac function (sinus rate, left intraventricular pressure, total cardiac output and external pressure-volume work) unless administered in large doses. Even at these doses, the increases were modest. Based on a comparison of potency values, Flynn et al. (1979) concluded that some of the actions of PEA in their preparation was due to a possible involvement of histamine H_2-receptors.

In addition, Flynn et al. (1979) reported that mepyramine did not antagonise the stimulant effects of PEA on any parameter, while cimetidine antagonised the effects of PEA on all parameters with the exception of sinus rate and coronary flow.

The difference between the results obtained in the present study, and by Verma and McNeill (1977), and those of Flynn et al. (1979) with regard to the involvement of histamine H_2-receptors during the inotropic responses due to PEA is not immediately apparent. A possible explanation could be that while this study and that of Verma and McNeill (1977) utilised the electrically driven guinea-pig right ventricle strip, Flynn et al. (1979) based their results on the left ventricle of an isolated working heart preparation. Therefore, the possibility of a difference between left and right ventricle exists.

It has already been mentioned that the chronotropic response due to PEA was unaffected by either promethazine or cimetidine. This finding is

in agreement with those of Flynn et al., who were also able to abolish the chronotropic effects of PEA only by using propranolol and not by using either cimetidine alone or in combination with mepyramine.

Since the lack of specificity of PEA is apparent, it seemed logical to next investigate whether a similar problem existed with histamine H_2-receptor agonists, eg. 4-methylhistamine. The chronotropic response due to this agonist in the guinea-pig right atrium was unaffected by the β-adrenoceptor antagonist, propranolol, while cimetidine caused a parallel displacement of the dose-response curve characteristic of competitive inhibition at the histamine H_2-receptor. Black et al. (1972) reported that this agonist possessed 0.2% of the activity relative to histamine at the guinea-pig ileum in vitro. Preliminary data (results not shown) indicated that a similar situation existed in the guinea-pig left atrium, where 4-methylhistamine caused no significant increase in contractility. Similar results were reported by Verma and McNeill (1977).

The results of this study using PEA indicate that compared to histamine H_2-receptors, H_1-receptors play a lesser role in the mediation of the cardiac effects of histamine in the guinea-pig. Whether or not similar conclusions are valid in other cardiac systems that predominantly contain histamine H_1-receptors, eg. rabbit heart (McNeill and Verma, 1979) is unclear. However, in the dog heart, which is known to respond to histamine predominantly via histamine H_1-receptors (Powell and Brady, 1976) histamine has been shown to cause its inotropic and chronotropic responses by both a promethazine and a propranolol sensitive mechanism (Flacke et al., 1967). Whether the problem of the histamine H_1-receptor mediated responses outlined is a reflection of a poor affinity or a poor efficacy of the

agonists is still unclear.

The inotropic response of PEA in guinea-pig left atria was also studied in our laboratory by Tenner and McNeill (1978). These authors attempted to characterise the response by altering the environmental conditions of the preparation. It was found that conditions enhancing basal developed force (eg. hypothermia, increased frequency of stimulation and increased extracellular calcium concentration) enhanced the sensitivity of the left atrium to isoprenaline, while at the same time producing an opposite effect on the sensitivity to histamine. In some instances, eg. when the tissue was paced at 4Hz (37^{o}C, 2.2 mM Ca^{2+}) the inotropic response of histamine was not clearly evident. These authors concluded that the positive inotropic effect of histamine could be demonstrated only within a very narrow range of experimental conditions.

Based on the study by Tenner and McNeill (1978), it is apparent that under normal physiological conditions, the inotropic response due to histamine H_1-receptor stimulation is of little importance during the overall cardiac response to histamine (that is, at least in the guinea-pig).

The presence of histamine H_2-receptors in the rat heart was suggested by Korosec and Erjavec (1978). Using both cimetidine and promethazine, the involvement of histamine H_1- and/or H_2-receptors in the cardiac responses of histamine in the rat was excluded in the present study. However, in the presence of propranolol or following reserpine pretreatment, large doses of histamine produced a negative chronotropic effect (Figure 17). At the same time, the inotropic effect of histamine in the rat left atrium was markedly reduced (Figure 18) indicating that the inotropic responses to large doses of histamine in the rat was due entirely to a release of catecholamines.

The negative chronotropic response due to large doses of histamine (in the presence of propranolol or in reserpine pretreated atria) was reversed by using the muscarinic antagonist, atropine (Figure 17). It is clear that in the rat right atrium, large doses of histamine can release both acetylcholine and endogenous catecholamines. It is also abundantly clear that the rat heart possesses no histamine receptors, as claimed by Korosec and Erjavec (1978) and by Satayavivad et al. (1977).

The rat heart is known to be insensitive to histamine (McNeill, unpublished; Bartlet, 1963). Went et al. (1954) noted that histamine first decreases and subsequently increases the force of contraction of the rat heart, while Bartlet (1963) reported that the response of histamine in a perfused rat heart was variable, producing either a negative inotropic response or a weak positive change. It is quite likely that the responses recorded by Went et al. (1954) and those of Bartlet (1963) were due to the simultaneous release of both catecholamines and acetylcholine by large doses of histamine in the rat heart.

CONCLUSION AND SUMMARY

It is clear from the results of this study that a tremendous species difference exists with regard to the direct actions of histamine in the heart. In agreement with other workers (Steinberg and Holland, 1975; Verma and McNeill, 1977) it was also found that the type of receptor involved in the cardiac responses of histamine also depends on the part of the heart examined.

The majority of the cardiac responses of histamine in the isolated kitten heart were found to be due to a release of endogenous catecholamines, with some contribution of histamine H_2-receptors in the chronotropic responses in the right atrium. On the other hand, all the cardiac responses due to histamine in the rat heart were through indirect mechanisms, involving both catecholamines and acetylcholine.

Therefore, the cardiac effects of histamine and its analogs are not always due to direct receptor stimulation, since such agents are also able to produce changes due to indirect receptor stimulation through release of catecholamines.

Finally, the physiological significance of histamine H_1-receptors in the guinea-pig heart is questioned with the finding that direct stimulation of these receptors produced only weak changes in contractility, under conditions which normally exist *in vivo*.

APPENDIX

Summary of the cardiac effects of histamine in different species.

(All -effects and muscarinic effects (M) are due to indirect mechanisms.)

	Right Atria	Left Atria	Right Ventricle	Right Papillary Muscle
Guinea-pig (McNeill and Verma, 1979)	H_2	H_1	H_1 H_2	H_2
Rabbit (McNeill and Verma, 1979)	H_1 H_2	H_1	H_1	H_1
Cat	β H_2	β	β	β
Guinea-pig (using PEA as agonist)	β	β H_1	β H_1	
Rat	β M	β		

REFERENCES

Altura, B.M. and Halevy, S. Cardiovascular Actions of Histamine, in "Handbook of Experimental Pharmacology," vol. 18, Part II, Histamine and Anti-Histaminics (M. Rocha e Silva, ed.) pp. 1-39, Springer-Verlag, New York, 1978.

Arunlakshana, O. and Schild, H.O. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14:48-58, 1959.

Ash, A.S.F. and Schild, H.O. Receptors mediating some actions of histamine. Br. J. Pharmacol. Chemotherap. 27:427-439, 1966.

Bartlet, A.L. The action of histamine on the isolated heart. Br. J. Pharmacol. 21:450-461, 1963.

Black, J.W., Duncan, W.A.M., Durant, G.J., Genellin, C.R. and Parsons, M.E. Definition and antagonism of histamine H_2-receptors. Nature, Lond. 236:385-390, 1972.

Black, J.W., Duncan, W.A.M., Emmett, J.C., Genellin, C.R., Hesselbo, T., Parsons, M.E. and Wyllie, J.H. Metiamide -- an orally active histamine H_2-receptor antagonist. Agents and Actions 3:133-137, 1973.

Bovet, D. and Staub, A.M. Action protectrice des ethers phenoliques au cours de l'intoxication histaminique. C.R. Soc. Biol. (Paris) 124: 547-549, 1937.

Brimblecombe, R.W., Duncan, W.A.M., Durant, G.J., Emmett, J.C., Genellin, C.R. and Parsons, M.E. Cimetidine -- a non-thiourea H_2-receptor antagonist. J. Int. Med. Res. 3:86-91, 1975.

Broadley, K.J. and Wilson, C. The biphatic inotropic response of guinea-pig isolated atria to histamine receptor agonists. Br. J. Pharmacol. 64:387-388 P, 1978.

Burn, J.H. and Dale, H.H. The vasocilator action of histamine and its physiological significance. J. Physiol., London, 61:185-214, 1926.

Chenoweth, M.B. and Koelle, E.S. An isolated heart perfusion system adapted to the determination of non-gaseious metabolites. J. Lab. Clin. Med. 31:600-608, 1946.

Chiba, S. Blocking effect of tripelennamine on histamine-induced positive chronotropic and inotropic responses of the dog atrium. Tohoku J. Exp. Med. 120:299-300, 1976.

Dale, H.H. "Advendures in Physiology." Pergamon Press, London, 1953.

Dale, H.H. and Laidlow, P.P. The physiological action of -iminazol-ethylamine. J. Pharmacol. Exp. Ther. 18:103-110, 1921.

Dean, P.M. Investigation into the mode of the action of histamine on the isolated rabbit heart. Br. J. Pharmacol. Chem. 32:65-77, 1968.

Douglas, W.W. Histamine and antihistamine; 5-hydroxytryptamine and antagonists, in "The Pharmacological Basis of Therapeutics." (L.S. Goodman and A. Gilman, eds.) pp. 590-629, Macmillan, New York, 1975.

Durant, G.J., Genellin, C.R. and Parsons, M.E. Chemical differentiation of histamine H_1- and H_2-receptor antagonists. J. Med. Chem. 18:905-909, 1975.

Dutta, M.K. Some pharmacological properties common to antihistamine compounds. Br. J. Pharmacol. Exp. Chemotherap. 4:281-289, 1949.

Emmelin, N. and Muren, A. Effects of antihistamine compounds on the adrenaline liberation from supradrenals. Acta. Physiol. Scand. 17: 345-355, 1949.

Flacke, W., Atanackovic, D., Gillis, R.A. and Alper, M.H. The actions of histamine on the mammalian heart. J. Pharmacol. Exp. Ther. 155: 271-278, 1967.

Flynn, S.B., Gristwood, R.W. and Owen, D.A.A. Differentiation of the roles of histamine H_1- and H_2-receptors in the mediation of the effects of histamine in the isolated working heart of the guinea-pig. Br. J. Pharmacol. 65:127-137, 1979.

Harvey, S.C. Studies on myocardial histamine. Effects of catecholamine-depleting drugs. Arch. Int. Pharmacodyn. 232:141-149, 1978.

Hood, A.J.C., Smy, J.R. and Weetman, D.F. An indirect sympathomimetric effect of burimamide on kitten isolated atria. Br. J. Pharmacol. 53: 525-529, 1975.

Hughes, M.J. and Coret, I.A. On the specificity of histamine receptors in the heart. Am. J. Physiol. 223:1257-1262, 1972.

Johnson, C.L. and Mizoguchi, H. The interaction of histamine and guanylnucleotides with cardiac adenylate cyclase and its relationship to cardiac contractility. J. Pharmacol. Exp. Ther. 200:174-186, 1977.

Kanof, P.D. and Greengard, P. Pharmacological properties of histamine-sensitive adenylate cyclase from guinea pig cardiac ventricular muscle. Mol. Pharmacol. 15:445-461, 1979.

Kŏch-Weser, J. and Blinks, J.R. The influence of the interval between beats on myocardial contractility. Pharmacol. Rev. 15:601-652, 1963.

Korasěc, L. and Erjavěc, F. The study of inhibition of histamine H_2-receptor in the heart of the rat. Pol. J. Pharmacol. 30:369-376, 1978.

Ledda, F., Fantozzi, A., Mugelli, A., Moroni, F. and Mannaioni, P.F. The antagonism of the positive inotropic effect of histamine and noradrenoline by H_1- and H_2-receptor blocking agents. Agents and Actions 4:193-194, 1974.

Lee, C.-H. and Levi, R. Arrhythmogenic effects of histamine and their potentiation in hyperthyroidism. Fed. Proc. 36:1043- , 1977.

Levi, R. and Capurro, N. Cardiac histamine-ouabain interactions: potentiation by ouabain of the arrhythmogenic effects of histamine. J. Pharmacol. Exp. Ther. 192:113-119, 1975.

Levi, R. and Koye, J.O. Pharmacological characterisation of cardiac histamine receptors: sensitivity to H_1-receptor antagonists. Eur. J. Pharmacol. 27:330-338, 1974.

Levi, R., Allan, G. and Zarecz, J.H. Cardiac histamine receptors. Fed. Proc. 35:1942-1949, 1976.

Lorenz, W. Histamine release in man. Agent Action 5:402-416, 1975.

Mannaioni, P.F. Interaction between histamine and dichloroisoproterenol, hexamethonium perpidine and diphenhydramine in normal and reserpine treated heart preparations. Br. J. Pharmacol. 15:500-505, 1960.

McIntire, F.C. Histamine release by antigen-antibody reactions, in "International Encyclopedia of Pharmacology and Therapeutics." (M. Schachter, sec. ed.) vol. 1, section 74, Histamines and Antihistamines, pp. 45-99, Pergamon Press, Oxford, 1973.

McNeill, J.H. and Muschak, L.D. Histamine effects on cardiac contractility, phosphonylase and adenyl cyclase. J. Molec. Cell. Cardiol. 4:611-624, 1972.

McNeill, J.H. and Verma, S.C. Blockade of cardiac histamine receptors by promethazine. Can. J. Physiol. Pharmacol. 52:23-27, 1974a.

----------. Blockade by burimamide of the effects of histamine and histamine analogs on cardiac contractility, phosphonylase activation and cyclic adenosine monophosphate. J. Pharmacol. Exp. Ther. 188: 180-188, 1974b.

----------. Histamine H_2-receptors in rat uterus. Res. Commun. Chem. Pathol. Pharmacol. 11:639-644, 1975.

----------. Histamine receptors in rabbit heart. Proc. West. Pharmacol. Soc. 21:99-101, 1978.

----------. Cardiac histamine receptors and cyclic AMP: differences between guinea-pig and rabbit heart, in "Histamine Receptors." (ed. T.O. Yellin) pp. 271-284. Spectrum Publications, New York, 1979.

Moroni, F., Ledda, F., Fantozzi, R., Mugelli, A. and Mannaioni, P.F. Specific inhibition of the cardiac effects of histamine on contraction and cyclic AMP by burimamide. Naunyn-Schmiedeberg's Arch. Pharmacol. 280:223-228, 1973.

Owen, D.A.A. Histamine-induced changes in heart rate in anaesthetised cats. Proceedings of the British Pharmacological Society, Br. J. Pharmacol., 285 P, 1977.

Parsons, M.E., Owen, D.A.A., Durant, G.J. and Genellin, C.R. Dimaprit -- S - 3 - (N,N-dimethylamino) propyl isothiourea - a highly specific histamine H_2-receptor agonist - I. Pharmacology. Agents and Actions 7:31-37, 1977.

Reinhardt, D., Wagner, J. and Schumann, H.J. Differentiation of H_1- and H_2-receptors mediating positivie chrono- and inotropic responses to histamine on atrial preparations of the guinea-pig. Agents and Actions 4:217-221, 1974.

Robison, G.A., Butcher, R.W., Øye, I., Morgan, H.E. and Sutherland, E.W. The effect of epinephrine on adenosine 3',5'-phosphate levels in the isolated perfused rat heart. Mol. Pharmacol. 1:168-177, 1965.

Satayavivad, J., Adler, A. and Kirsten, E.B. Influence of methylxanthine derivatives on the positive chronotropic action of histamine in guinea pig and rat atria. Fed. Proc. 36:4055, 1977.

Steinberg, M.I. and Holland, D.R. Separate receptors mediating the positive inotropic and chronotropic effect of histamine in guinea-pig atria. Eur. J. Pharmacol. 34:95-104, 1975.

Tenner, T.E., Jr. and McNeill, J.H. Characterisation of the inotropic response induced by stimulation of -adrenergic and H_1 histaminergic receptors in guinea pig left atria. Can. J. Physiol. and Pharmacol. 56:926-933, 1978.

Trendelenburg, V. The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. J. Pharmacol. Exp. Ther. 130:450-460, 1960.

Verma, S.C. and McNeill, J.H. The effect of histamine, isoproterenol and tyramine on rat uterine cyclic AMP. Res. Commun. Chem. Pathol. Pharmacol. 13:55-64, 1976.

----------. Cardiac histamine receptors and cyclic AMP. Life Sciences 19:1797-1802, 1976.

----------. Cardiac histamine receptors: differences between left and right atria and right ventricle. J. Pharmacol. Exp. Ther. 200: 352-362, 1977.

von Rossum, J.M. and Van den Brink, F.G. Cumulative dose-response curves. I. Introduction to the Technique. Arch. Int. Pharmacodyn. 143:240-246, 1963.

Vugman, I. and Rocha e Silva, M. Biological determinations of histamine in living tissues and body fluids, in "Handbook of Experimental Pharmacology," vol. 18, part 1, Histamine. (M. Rocha e Silva, ed.) pp. 81-115, Springer-Verlag, New York, 1966.

Weinryb, I. and Michel, I.M. Comparison of the effects of histamine and tolazoline on adenylate cyclase activity from guinea pig heart. J. Med. Chem. 18:23-26, 1975.

Went, S., Varga, E., Szues, E. and Feher, O. Eine analyse der "symathomimetischen" wirtung des histamins in isolietten. Saugetierherzpraparaten. Acta. Physiol. Acad. Sci. Lung. 5:121-130, 1954.

Windaus, A. and Vogt, W. Synthese des imidazolylathylamins. Berichte deutsch. chem. Gersellschaft 40:3091-3695, 1907.