Br. J. Pharmac. Chemother. (1968), 32, 65–77.

INVESTIGATION INTO THE MODE OF ACTION OF HISTAMINE ON THE ISOLATED RABBIT HEART

BY

P. M. DEAN*

From the Department of Pharmacology, University of Leeds, Leeds 2

(*Received June* 29, 1967)

Dale and Laidlaw, in 1910, showed that histamine increased the contractions of the isolated rabbit heart. From the perfusate of a heart stimulated by histamine, Went, Varga, Szücz & Fehér (1954) collected a substance which diminished the spontaneous contractions of the rabbit duodenum, relaxed rat uterus and contracted the cat nictitating membrane; they attributed these effects to an adrenaline-like substance released from the heart. Mannaioni (1960) showed that diphenhydramine reduced the stimulant effect of histamine on the rate and force of contraction but did not affect the response of guinea-pig atria to adrenaline. Dichloroisoproterenol abolished the stimulant effect of noradrenaline; it also significantly reduced the response to histamine. There was no decrease in the response to histamine with atria depleted of catecholamines by pretreatment of the animals with reserpine (Trendelenburg, 1960). Mepyramine and pyribenzamine antagonized the stimulant effect of histamine on guinea-pig atria, although the pA_2 value for mepyramine-histamine antagonism—about 5—was lower than that for guinea-pig ileum, which was about 9. From this evidence Trendelenburg concluded that cardiac histamine receptors differ from those found in smooth muscle. In contrast with these results, Bartlet (1963) found that mepyramine and diphenhydramine did not antagonize the action of histamine on the guinea-pig heart.

In the present work an attempt has been made to resolve this conflicting evidence, first by trying to detect whether histamine does release sympathomimetic amines from the rabbit heart, and second by studying the mode of action of histamine on atria in order to investigate the interrelationship between the cardiac histamine and noradrenaline receptors.

METHODS

Perfusion studies

Male New Zealand white rabbits, 2.0–4.0 kg, were used. A rabbit was killed by a blow on the head and the heart removed rapidly and placed in a solution of the following composition: sodium chloride 9 g; potassium chloride 0.4 g; sodium hydrogen carbonate 0.15 g; anhydrous calcium chloride 0.24 g; glucose 4 g; made up to 1 l. with distilled water. This solution was maintained at 29° C and gassed with oxygen.

The heart was perfused according to the method of Langendorff (1895). The perfusate from the heart was superfused at 29° C over various test organs in series, by a method similar to that used by Vane (1964). The test organs used were rabbit duodenum and rat stomach strip.

* Present address: Department of Pharmacology, University of Cambridge, Cambridge.

Where indicated, atropine (5 μg/ml.) and desmethylimipramine were present in the perfusion fluid. 5-Hydroxytryptamine (10 μg/l.) was added to the perfusate which passed over rat stomach strip (Armitage & Vane, 1964). Except where the direct effect of histamine on the test organs was investigated, pyribenzamine (4 μg/ml.) was added to the perfusate to antagonize the stimulatory effect of histamine.

Experiments on atria

The atria were dissected free from the isolated rabbit heart in a solution of the composition previously described and placed in an organ bath containing this solution maintained at 29° C and gassed with oxygen. In order to record the force of contraction the atria were attached by cotton thread to an RCA 5734 mechano-electric transducer placed vertically above the bath. The electrical circuit used was similar to that proposed by Donaldson (1958). The output from the transducer was fed into a Devices DC.2 preamplifier connected to a hot-wire pen recorder. A resting tension of about 0.5 g was applied to the atria.

The force of contraction could be measured directly as $g.cm.sec^{-2}$ by calibrating the record by hanging weights from the tip of the transducer. The atrial rate was obtained by counting the oscillations recorded on the trace during a period of 12 sec.

All drug solutions were made up with distilled water and the volume added to the 100 ml. bath varied between 0.2 ml. and 1 ml. The dose has been expressed as the final concentration of drug (as a salt) in the bath. A 10 min cycle for drug application was used. The drug solution was added to the bath for 2 min, the atria were then washed three times during the next 3 min and then allowed to equilibrate for 5 min before a further dose of drug. The atrial rate and force of contraction were measured during the control period before drug administration and at the time of maximal activity.

In the results the response has been expressed as the increase in the force of contraction, or rate, produced by the stimulant drug. This method has the advantage that there is less variation in the measured response than when the response is expressed as a percentage of the unstimulated level of activity (Trendelenburg, 1960). When dose-response curves were constructed the doses were given in a geometric series with $R=2$, or $R=0.5$. The series where $R=2$ is referred to as the ascending order, and where $R=0.5$ as the descending order. In tests for significance of antagonism, or potentiation, the Student's t test was used.

Drugs

Atropine sulphate (B.D.H.); 1-adrenaline bitartrate (B.D.H.); 1-noradrenaline bitartrate (B.D.H.); histamine acid phosphate (B.D.H.); hexamethonium bromide (Koch Light Laboratories); propranolol hydrochloride (I.C.I.); pronethalol hydrochloride (I.C.I.); nicotine hydrogen tartrate (B.D.H.); pyribenzamine hydrochloride (CIBA); 5-hydroxytryptamine creatinine sulphate (May & Baker); desmethylimipramine (Geigy); acetylcholine iodide (Koch Light Laboratories); aminophylline (Thackeray, Leeds). In the reserpinized rabbits, serpasil (CIBA) 3 mg/kg was injected intraperitoneally 20 and 44 hr before the heart was removed.

RESULTS

Perfusion studies

The only previous evidence for the release of sympathomimetic amines from hearts treated with histamine is that produced by Went *et al.* (1952, 1954). These workers also claimed that histamine exerts a biphasic effect on the isolated rabbit heart. It was necessary, therefore, to differentiate between the direct effect of histamine on the test tissues and other substances which might have been released into the perfusion fluid from hearts treated with histamine. Solution containing pyribenzamine (4 μg/ml.) was added to the perfusate at a rate of 4–6 ml./min.

Before the heart was set up the sensitivity of the test tissues to noradrenaline was determined by injecting noradrenaline (in 1 ml. of perfusion solution) into the aortic cannula. The flow from this cannula was adjusted to about 13 ml./min because this was approximately the output from perfused hearts.

Rabbit duodenum

In the absence of pyribenzamine, histamine first increased the contractions of rabbit duodenum, and then the preparation gradually relaxed below the control levels (Fig. 1a). In the presence of pyribenzamine, however, histamine produced a brief increase in the contractions followed by a prolonged relaxation (Fig. 1b). With noradrenaline there was no initial increase in the contractions and relaxation was produced which seemed to be similar to that produced by histamine in the presence of pyribenzamine (Fig. 1c). As a result of these effects, it would have been difficult to detect whether the perfusate from the heart treated with histamine contained sympathomimetic amines when tested on rabbit duodenum.

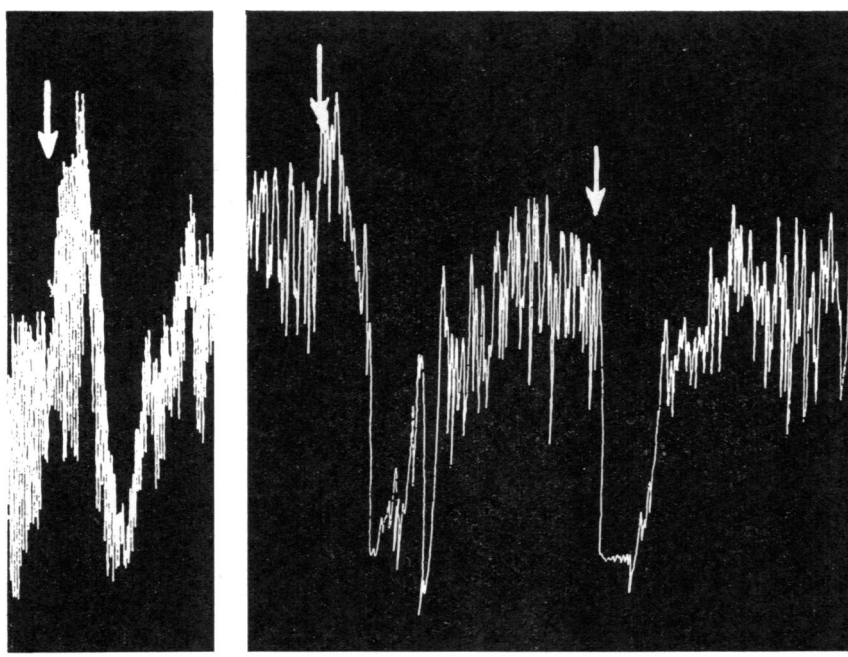

Fig. 1. Responses of rabbit duodenum to histamine and to noradrenaline. a, 20 μg histamine in the absence of pyribenzamine; b, 20 μg histamine in the presence of pyribenzamine; c, 1 μg noradrenaline in the presence of pyribenzamine.

Rat stomach strip

Histamine produced contractions of the rat stomach strip which could be reduced, or eliminated, by pyribenzamine. In the presence of pyribenzamine histamine did not produce relaxation. The sensitivity of this preparation to noradrenaline was 30–100 ng. Thus rat stomach strip treated with pyribenzamine was used as a test organ to detect whether sympathomimetic amines were released from the heart.

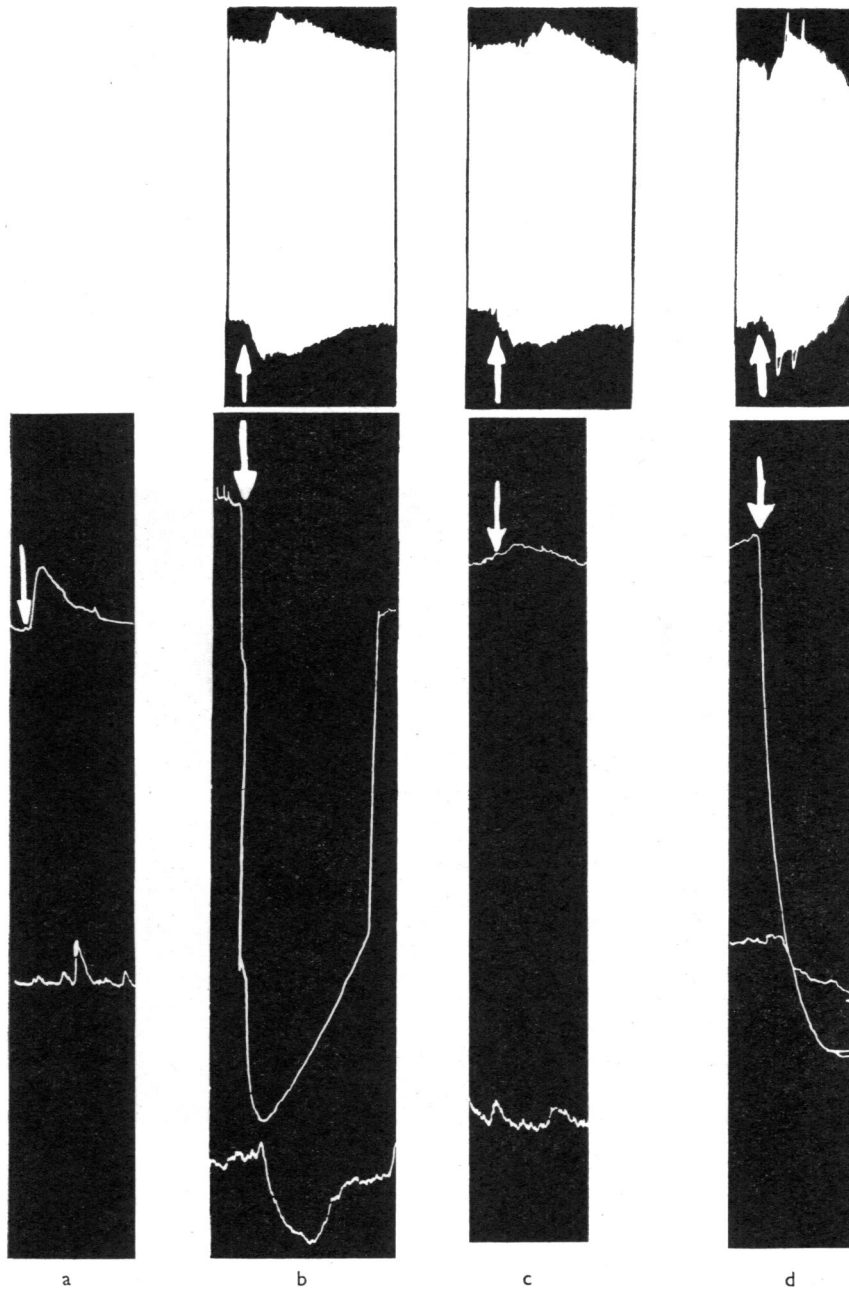

Fig. 2. Effect of perfusates from an isolated rabbit heart on two rat stomach strip preparations in series. Solution perfusing the heart contained atropine (5 μg/ml.). Pyribenzamine was added to the perfusate. Top record: heart beat; bottom record: rat stomach strips. a, effect of 80 μg acetylcholine applied directly to rat stomach strips; b, 80 μg acetylcholine applied to the heart; c, 80 μg histamine applied to the heart; d, 1.5 μg noradrenaline applied to the heart.

When the perfusion fluid contained 5 μg of atropine/ml., 20–80 μg of acetylcholine did not relax rat stomach strip (Fig. 2a). With three atropinized hearts treated with acetylcholine the perfusate relaxed the test preparations (Fig. 2b). For comparison the effect of 1.5 μg of noradrenaline applied to the heart is shown in Fig. 2d. With four hearts it was not possible to detect a substance in the perfusate which would relax rat stomach strip when doses of 20–80 μg histamine were given, although a large inotropic response was produced (Fig. 2c).

In the rat heart, Iversen (1965) observed that desmethylimipramine blocked the uptake of noradrenaline. Using five hearts perfused with 10^{-6}M desmethylimipramine it was not possible to demonstrate the release of noradrenaline when histamine was injected into the aortic cannula. Rat stomach strip retained its sensitivity in the presence of desmethylimipramine. In nine hearts treated with histamine no biphasic response was produced.

Experiments on atria

Effect of hexamethonium

In atropinized atria, 20 μg nicotine had positive inotropic and chronotropic effects; 10 μg histamine acted similarly. After 50 μg hexamethonium for 10 min the response to nicotine was abolished but the effect of 10 μg histamine was unchanged. This result confirms that of Trendelenburg (1960): histamine does not stimulate atrial ganglionic cholinergic receptors.

Dose-response relationships for histamine and for noradrenaline on atria

When a strict 10 min cycle for drug application was used and doses of histamine were given in the ascending order, bell-shaped dose-response curves were produced both for

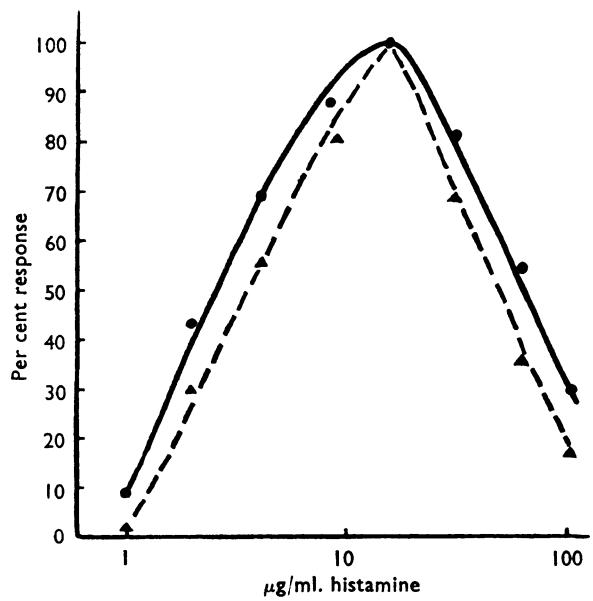

Fig. 3. Ascending order dose-response curves for histamine. ——, Inotropic effect; - - - -, chronotropic effect.

the inotropic and chronotropic effects (Fig. 3). If the effect was measured as a percentage of the maximum response, the inotropic and chronotropic responses could be compared directly. The potency for the inotropic effect was greater than that for the chronotropic effect for the rising part of the dose-response curve. After the maximum had been reached the relationship for the relative potencies was variable. Similar curves were obtained with noradrenaline. Moreover, bell-shaped dose-response curves were obtained with histamine on atria derived from reserpinized rabbits.

If autoinhibition were the cause of the bell-shaped curves, it would be expected from the theories of Ariens, Simonis & Van Rossum (1964) that doses of histamine given in the descending order would also have produced bell-shaped curves. Descending order dose-response curves for histamine and for noradrenaline were, however, not bell-shaped (Fig. 4). Noradrenaline was more potent than histamine and the maximum inotropic effect produced by noradrenaline was about twice that produced by histamine.

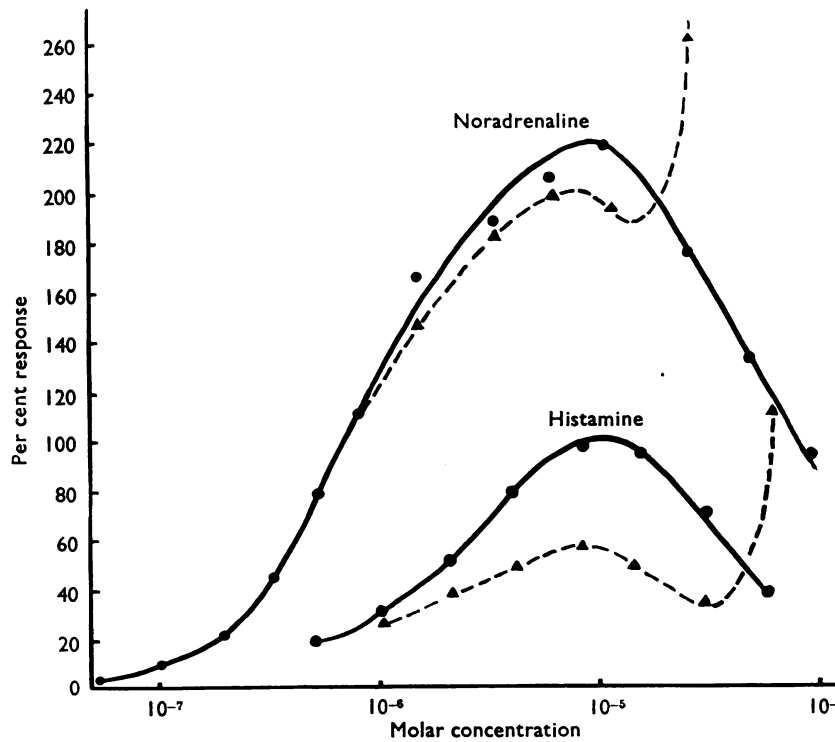

Fig. 4. Inotropic dose-response curves for histamine and for noradrenaline. ——, Doses given in ascending order; - - - -, doses given in descending order.

Effect of β-blocking agents

In order to see how closely the inotropic response of histamine resembled that of noradrenaline, ascending order dose-response curves were constructed in the absence and in the presence of β-blockade. Pronethalol and propranolol were used at concentrations of 5–10 μg/ml. The atria were allowed to equilibrate for 20 min in a solution containing

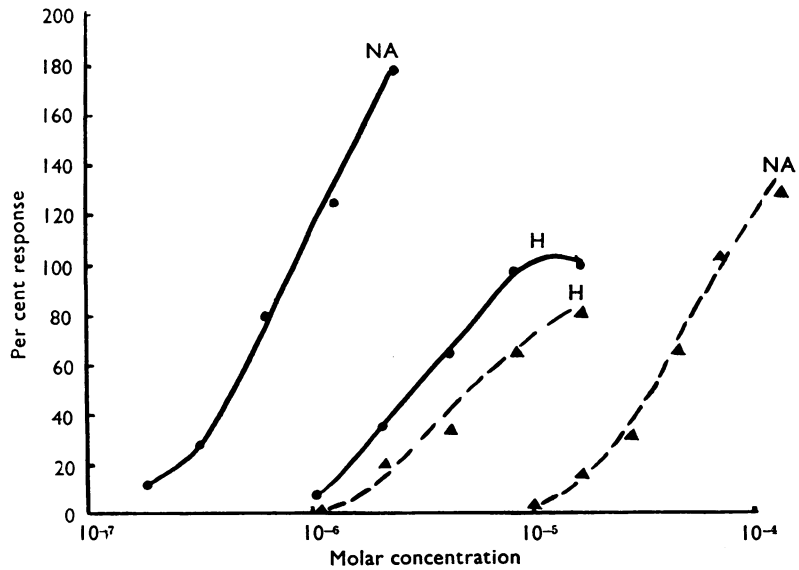

Fig. 5. Antagonism by β-blocking agents of the inotropic response to histamine (H) and to noradrenaline (NA). Ascending order dose-response curves: ——, absence of pronethalol; - - - - -, presence of pronethalol (5 μg/ml.).

the β-blocker before the curves were constructed. The β-blocking agents alone had a negative inotropic effect.

In the presence of propranolol or pronethalol the dose-response curves for histamine were moved slightly to the right (Fig. 5). The antagonism was significant ($P=0.05$). The same effect occurred in atria from reserpinized rabbits. These β-blocking agents are therefore weak antagonists for histamine but are more potent antagonists for noradrenaline.

Effect of pyribenzamine

Dose-response curves for histamine and for noradrenaline were constructed in the absence and in the presence of pyribenzamine 10 μg/ml. The antagonism produced by pyribenzamine towards histamine was significant ($P=0.05$). In two of three experiments the inotropic response to noradrenaline was potentiated by pyribenzamine; this could be interpreted as potentiation by blockade of noradrenaline uptake (Isaac & Goth, 1965).

Effect of aminophylline

Rall & West (1963) showed that aminophylline potentiated the inotropic response to noradrenaline on rabbit atria. In the present study aminophylline 5–50 μg/ml. potentiated and markedly increased the inotropic and chronotropic response to histamine (Fig. 6). The potentiation was significant ($P=0.05$).

Effect of carbachol

It is well known that acetylcholine antagonizes the stimulatory action of noradrenaline on the heart. Ascending order dose-response curves for histamine and for noradrenaline

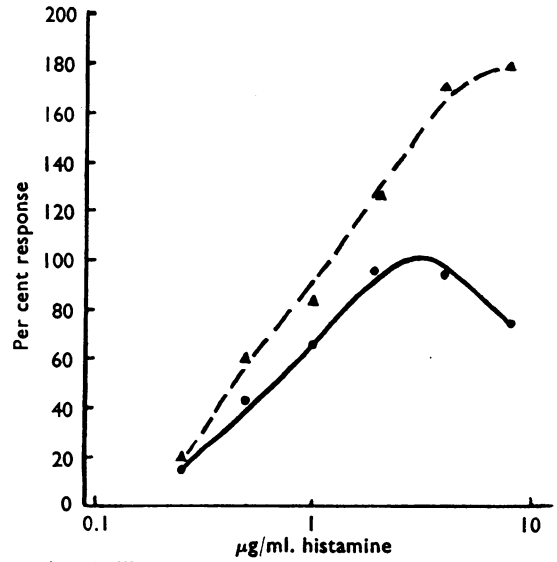

Fig. 6. Potentiation by aminophylline of the inotropic response to histamine. Ascending order dose-response curves constructed in the absence (——), and in the presence (- - - -) of aminophylline (5 μg/ml.).

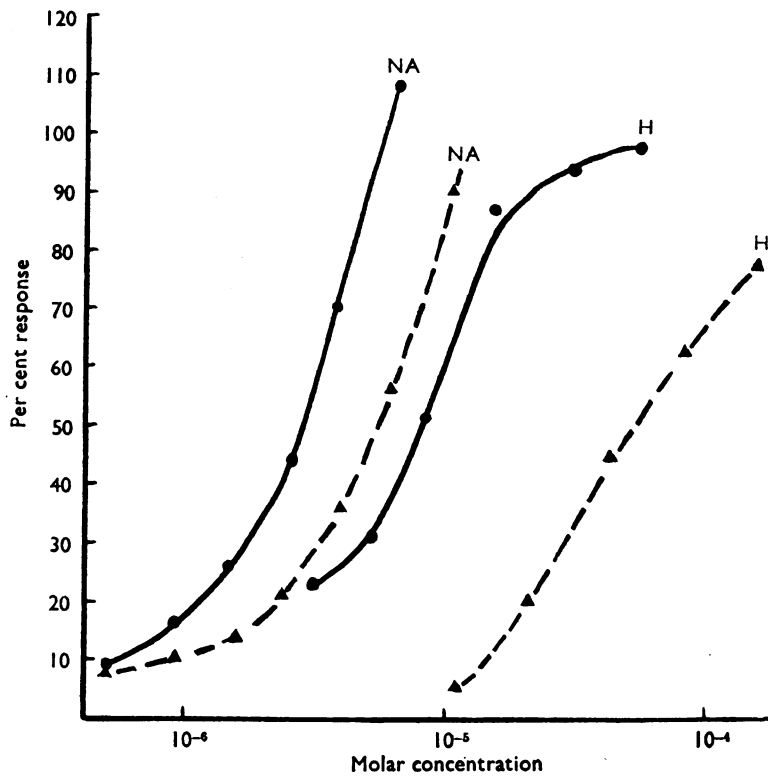

Fig. 7. Antagonism by carbachol of the inotropic response to histamine (H) and noradrenaline (NA). Ascending order dose-response curves. ——, Absence of carbachol; - - - -, presence of carbachol (0.01 μg/ml.).

were moved to the right when constructed in solutions containing carbachol 0.01–0.02 μg/ml. (Fig. 7). The antagonism produced by carbachol on the agonist activity of histamine was significant ($P=0.05$).

Desensitization studies

Using a strict 10 min cycle ascending order dose-response curves for histamine were constructed to the point of complete desensitization. At this point a submaximal dose of histamine was administered every 10 min. It can be seen from Fig. 8 that atria started to recover their sensitivity to histamine after 12 min and had completely recovered after about 50 min.

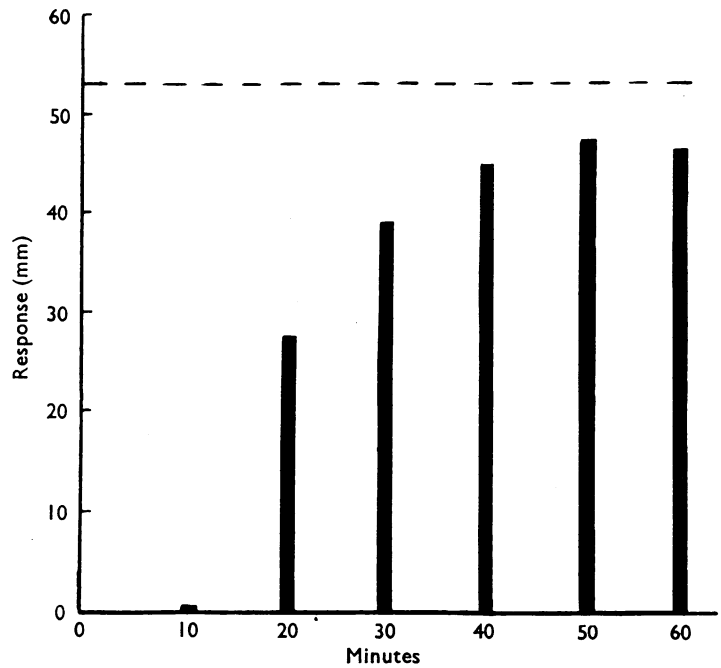

Fig. 8. The dotted line represents the response to the submaximal dose of histamine before desensitization. The columns show the recovery of the response to this dose after desensitization.

Recent theories suggest that receptors are regulatory enzymes which catalyse the metabolism of a preformed substrate (Belleau, 1965). Regulatory molecules, such as a noradrenaline, may modulate the catalytic activity of these enzymes. It has been suggested that substrate depletion accounts for desensitization (Bloom & Goldman, 1966). Thus, if the atrial receptors for histamine and for noradrenaline require the same substrates, cross-desensitization should occur. In order to test whether the hypothetical substrates were the same for the two receptors the effect of pretreatment with histamine on the response to noradrenaline was studied. Control responses to submaximal doses of noradrenaline were obtained. Pretreatment with histamine consisted of 10 μg, 20 μg and 50 μg histamine. Fig. 9a shows the desensitization curves produced by histamine. The effect of noradrenaline after treatment with histamine was measured (Fig. 9b).

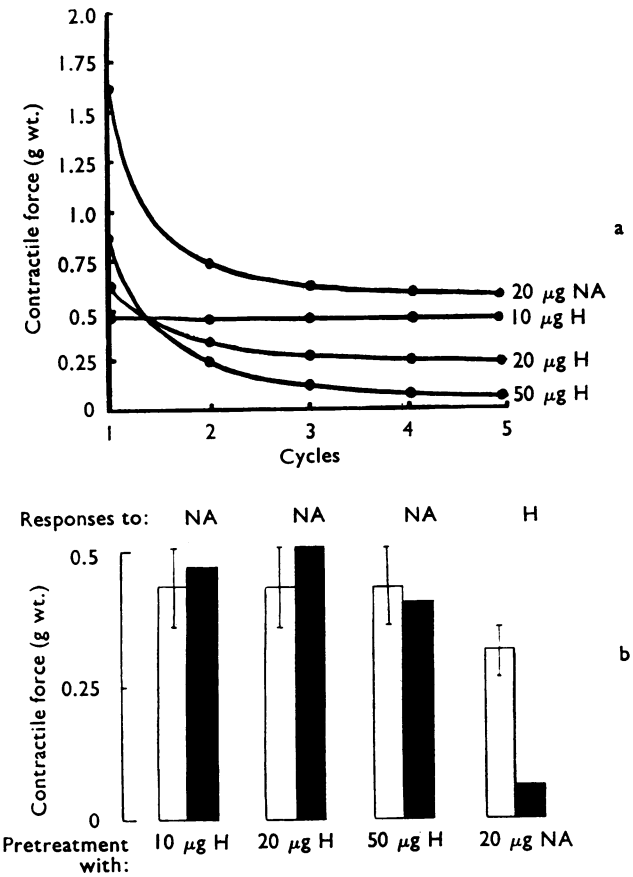

Fig. 9. One-way cross-desensitization between histamine (H) and noradrenaline (NA). a, Desensitization curves for 10 μg, 20 μg and 50 μg histamine and for 20 μg noradrenaline. b, Control responses before pretreatment shown in the clear columns together with the standard deviation. Responses after pretreatment are shown in the black columns.

From Figs. 9a and 9b it can be seen that pretreatment with histamine did not markedly affect the response to noradrenaline. When the reverse process was studied, however, pretreatment with 20 μg noradrenaline for five cycles markedly reduced the response to histamine. That is, one-way cross-desensitization occurred. Cross-desensitization on guinea-pig atria could not be studied, because the dose of histamine or noradrenaline required for the maximum inotropic effect caused a marked depression of basal activity after the drug had been washed out.

DISCUSSION

The relaxation produced by histamine on rabbit duodenum in the presence of pyribenzamine casts doubt on the interpretation of the results obtained by Went *et al.* (1952, 1954). The inability to detect sympathomimetic amines in the perfusates from

hearts treated with histamine supports the evidence produced by Trendelenburg (1960) that histamine stimulates the heart directly and not through the release of sympathomimetic amines.

Because histamine can release noradrenaline from adrenal-medullary-chromaffin cells (Staszewska-Barczac & Vane, 1965), it seems surprising that it was not possible to demonstrate the release of noradrenaline from the heart stimulated by histamine. Perhaps this difference can be accounted for by the fact that in the heart noradrenaline is present predominantly in sympathetic nerve fibres and there is little evidence for the existence of chromaffin cells (Angelekos *et al.*, 1963 ; Angelekos, 1965).

The experiments on rabbit atria have shown some similarities between cardiac stimulatory properties of histamine and noradrenaline. Activation of the cardiac histamine receptor leads to an increase in the rate of energy utilization and pyribenzamine would be expected to block selectively the cardiac histamine receptor. The potentiation of the agonist activity of histamine by aminophylline might be the result of the theophylline component blocking 3′,5′-nucleotide-phosphodiesterase (Butcher & Sutherland, 1962). If this were so, 3′,5′-adenosine monophosphate (3′,5′ AMP) would be implicated in the cardiac agonist activity of histamine. Williamson (1966) has shown that the inotropic response produced by noradrenaline parallels the increase in the tissue concentration of 3′,5′ AMP.

Results with β-blocking agents suggest: that the β-blockers may have a non-specific action in the weak antagonism of histamine which might be caused by their local anaesthetic properties (Black & Stephenson, 1962) ; that β-blocking agents may directly affect the cardiac histamine receptor, although this is unlikely to be competitive antagonism because the maximum inotropic response was sometimes depressed ; that β-blocking agents may reduce the concentration of a substance needed for activation of the histamine receptor system. Robison, Butcher, Oye, Morgan & Sutherland (1965) have shown that pronethalol prevents the increase in the level of 3′,5′ AMP and the inotropic response to noradrenaline. The antagonism towards histamine and the negative inotropic effect produced by carbachol may arise from the inhibitory action of carbachol on adenyl cyclase, because Murad, Chi, Rall & Sutherland (1962) showed that carbachol inhibited by 30% the formation of 3′,5′ AMP in hearts stimlated by noradrenaline.

Depletion of a hypothetical substrate required for a receptor (considered as a regulatory enzyme) has been proposed by Bloom & Goldman (1966) to explain the phenomenon of desensitization. The one-way cross-desensitization between histamine and noradrenaline found in the experiments on atria would suggest, from this hypothesis, that the substrates are different for the histamine and the adrenaline receptors. Alternatively, evidence against this hypothesis was obtained by Rall & Kakiuchi (1966). They showed that in rabbit cerebellum histamine and noradrenaline stimulated adenyl cyclase, both drugs showed desensitization in the production of 3′,5′ AMP but cross-desensitization did not occur.

The similarity between the agonist activities of histamine and noradrenaline on rabbit atria is striking. If histamine does stimulate 3′,5′ AMP formation in the heart, as it does in the cerebellum (Rall & Kakiuchi, 1966), it would be necessary to postulate the existence of a histamine receptor linked to the adenyl cyclase enzyme.

SUMMARY

1. It was not possible to detect the release of noradrenaline from perfused rabbit hearts treated with histamine by passing the perfusate over rat stomach strip.

2. Contractions of isolated rabbit atria were measured by a transducer so that the rate and force of contraction could be recorded simultaneously.

3. Dose-response curves for histamine and for noradrenaline were bell-shaped when the doses were given in the ascending order. The bell-shaped curves were attributed to desensitization.

4. Carbachol, pronethalol and propranolol antagonized the stimulatory effects of histamine and of noradrenaline. Pyribenzamine was found to be a specific antagonist for histamine on atria.

5. Aminophylline potentiated and increased the maximum response to histamine.

6. Pretreatment with noradrenaline reduced the inotropic response to histamine, whereas pretreatment with histamine did not affect the response to noradrenaline.

7. It is suggested that a histamine receptor linked to adenyl cyclase may account for the interrelationships between the histamine and adrenergic receptor systems.

I wish to thank Dr. G. A. Mogey for reading the manuscript. I am grateful to Mr. H. A. Tyrrel (CIBA) for the gift of pyribenzamine and to Dr. C. Maxwell (Geigy) for the gift of desmethylimipramine.

REFERENCES

ANGELEKOS, E. T. (1965). Regional distribution of catecholamines in the dog heart. *Circulation Res.*, **16**, 39–44.

ANGELEKOS, E. T., FUXE, K. & TORCHINA, M. L. (1963). Chemical and histochemical evaluation of the distribution of catecholamines in the rabbit and guinea-pig hearts. *Acta physiol. scand.*, **59**, 184–192.

ARIENS, E. J., SIMONIS, A. M. & VAN ROSSUM, J. M. (1964). Drug-receptor interaction: interaction of one or more drugs with different receptor systems. In *Molecular pharmacology. The mode of action of biologically active compounds.* Vol. 1., ed. ARIENS, E. J. London: Academic Press.

ARMITAGE, A. K. & VANE, J. R. (1964). A sensitive method for the assay of catecholamines. *Br. J. Pharmac. Chemother.*, **22**, 204–210.

BARTLET, A. L. (1963). The action of histamine on the isolated heart. *Br. J. Pharmac. Chemother.*, **21**, 450–461.

BELLEAU, B. (1965). Conformational perturbation in relation to the regulation of enzyme and receptor behaviour. *Adv. Drug Res.*, **2**, 89–126.

BLACK, J. W. & STEPHENSON, J. S. (1962). Pharmacology of a new adrenergic blocking compound Pronethalol. *Lancet, ii*, 311–314.

BLOOM, B. M. & GOLDMAN, I. M. (1966). The nature of catecholamine-adenine mononucleotide interactions in adrenergic mechanisms. *Adv. Drug Res.*, **3**, 121–171.

BUTCHER, R. W. & SUTHERLAND, E. W. (1962). Adenosine 3′,5′-monophosphate in biological materials. 1. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and the use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine. *J. biol. Chem.*, **237**, 1244–1250.

DALE, H. H. & LAIDLAW, P. P. (1910). The physiological action of β-iminazolethylamine. *J. Physiol., Lond.*, **41**, 318–344.

DONALDSON, P. E. K. (1958). *Electronic apparatus for biological research.* London: Butterworth.

ISAAC, L. & GOTH, A. (1965). Interaction of antihistamines with norepinephrine uptake. A cocaine-like effect. *Life Sci.*, **4**, 1899–1904.

IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by drugs. *Adv. Drug Res.*, **2**, 5–23.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Saugetierherzen. *Pflügers Arch. ges. Physiol.*, **61**, 291–332.

MANNAIONI, P. F. (1960). Interaction between histamine and dichloroisoproterenol, hexamethonium, pempidine, and diphenhydramine in normal and reserpine-treated heart preparations. *Br. J. Pharmac. Chemother.*, **15**, 500–505.

MURAD, F., CHI, Y. M., RALL, T. W. & SUTHERLAND, E. W. (1962). Adenyl cyclase 111. The effect of catecholamines and choline esters on the formation of adenosine 3′,5′ phosphate by preparations from cardiac muscle and liver. *J. biol. Chem.*, **237**, 1233–1238.

RALL, T. W. & KAKIUCHI, S. (1966). The influence of certain neurohormones and drugs on the accumulation of cyclic 3′,5′ AMP in brain tissue. In *Molecular basis of some aspects of mental activity*, ed. WALLAS, O., vol. 1. London: Academic Press.

RALL, T. W. & WEST, T. C. (1963). The potentiation of cardiac inotropic responses to norepinephrine by theophylline. *J. Pharmac.*, **139**, 269–274.

ROBISON, G. A., BUTCHER, R. W., OYE, I., MORGAN, H. E. & SUTHERLAND, E. W. (1965). The effect of epinephrine on adenosine 3′,5′ phosphate levels in the isolated perfused rat heart. *Molec. Pharmac.*, **1**, 168–177

STASZEWSKA-BARCZAC, J. & VANE, J. R. (1965). The release of catecholamines from the adrenal medulla by histamine. *Br. J. Pharmac. Chemother.*, **25**, 728–742.

TRENDELENBURG, U. (1960). The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J. Pnarmac.*, **130**, 450–460.

VANE, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360–373.

WENT, S., VARGA, E., SZÜCS, E. & FEHÉR, O. (1952). Ubereine " sympathomimetischen " Wirkung des Histamins. *Arch. exp. Path. Pharmak.*, **215**, 129–132.

WENT, S., VARGA, E., SZÜCS, E. & FEHÉR, O. (1954). Analyse der " sympathomimetischen " Wirkung des Histamins an isolierten Saugetierhertzpraparaten. *Acta physiol. Acad. Sci. hung.*, **5**, 121–130.

WILLIAMSON, J. R. (1966). Kinetic studies of epinephrine effects in the perfused rat heart. *Pharmac. Rev.*, **18**, 205–210.