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MODIFICATION OF CARDIOVASCULAR RESPONSES TO HISTAMINE BY DITHIOTHREITOL

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1 Histamine produced dose-dependent contractile responses on both isolated perfused ear arteries and aortic strips of the rabbit. These responses were blocked by mepyramine and potentiated by both metiamide and dithiothreitol.

2 In the presence of maximum potentiation by metiamide, dithiothreitol still potentiated the contractile response to histamine of both preparations.

3 In the presence of mepyramine, histamine produced dose-dependent reductions in the contractile response to noradrenaline. This vasodilator action of histamine was abolished by metiamide but was unaffected by dithiothreitol.

4 The vasodilator action of histamine on the human isolated perfused temporal artery and the positive inotropic effect of histamine on the isolated spontaneously beating atria of the rabbit were blocked by metiamide but unaffected by dithiothreitol.

5 It is concluded that the rabbit aorta, like the ear artery, contains both H_1 and H_2 histamine receptors and that dithiothreitol potentiates cardiovascular responses mediated by H_1-receptors but not by H_2-receptors.

Introduction

Finding that dithiothreitol (DTT, Cleland's reagent), an agent which reduces disulphide linkages to sulphydryl groups, potentiates the constrictor response of the rabbit aorta to histamine, Fleisch, Krzan & Titus (1973) concluded that disulphide bridges occupied prominent positions either in histamine receptors or along the excitation-contraction pathway.

However, it is known that responses to histamine are mediated by two types of receptor (Black, Duncan, Durant, Ganellin & Parsons, 1972). In the rabbit, the biphasic effect which histamine has on arterial pressue is a balance between a pressor effect mediated by H_1-receptors and a depressor effect mediated by H_2-receptors (Parsons & Owen, 1973; Carroll, Glover & Latt, 1974). Further, both H_1 and H_2-receptors have been demonstrated in the same isolated artery; thus the response of the rabbit ear artery is a balance between a predominant constrictor action mediated by H_1-receptors and a vasodilator action mediated by H_2-receptors (Glover, Carroll & Latt, 1973; Parsons & Owen, 1973).

The present experiments were therefore designed to study the actions of DTT on cardiovascular responses mediated by both types of receptor. A brief account of some of these results has been published (Carroll & Glover, 1974).

Methods

Isolated segments of rabbit ear and human temporal arteries were cannulated, and perfused at constant flow with Krebs bicarbonate solution of the following composition (mmol/l): NaCl 118, $NaHCO_3$ 25, NaH_2PO_4 1.33, KCl 4.7, $CaCl_2$ 2.7, $MgCl_2$ 1.44 and glucose 5.5, aerated with 5% CO_2 in O_2. Human temporal arteries were obtained at autopsy within 6–12 h after death. The flow rate through the arteries was kept constant in the range 5–10 ml/min, so perfusion pressures in the absence of any drugs were 20–30 mmHg for the rabbit ear and 10–40 mmHg for the human temporal vessels. Care was taken to ensure that the cannula contributed negligible resistance. Perfusion pressure was measured with a Statham P23AC pressure transducer and recorded on a Grass polygraph. Drugs were injected in volumes of 0.05–0.2 ml through a rubber connection close to the cannula or added directly to the perfusate.

Helically cut segments of rabbit aorta were suspended in a 15 ml organ bath at an initial tension of 1 gram. Krebs bicarbonate solution was continually pumped through the bath at a rate of 25 ml/min and drugs were either injected in volumes of 0.05–0.5 ml into the perfusion stream or added directly to the perfusate. Contractions were measured with a Grass

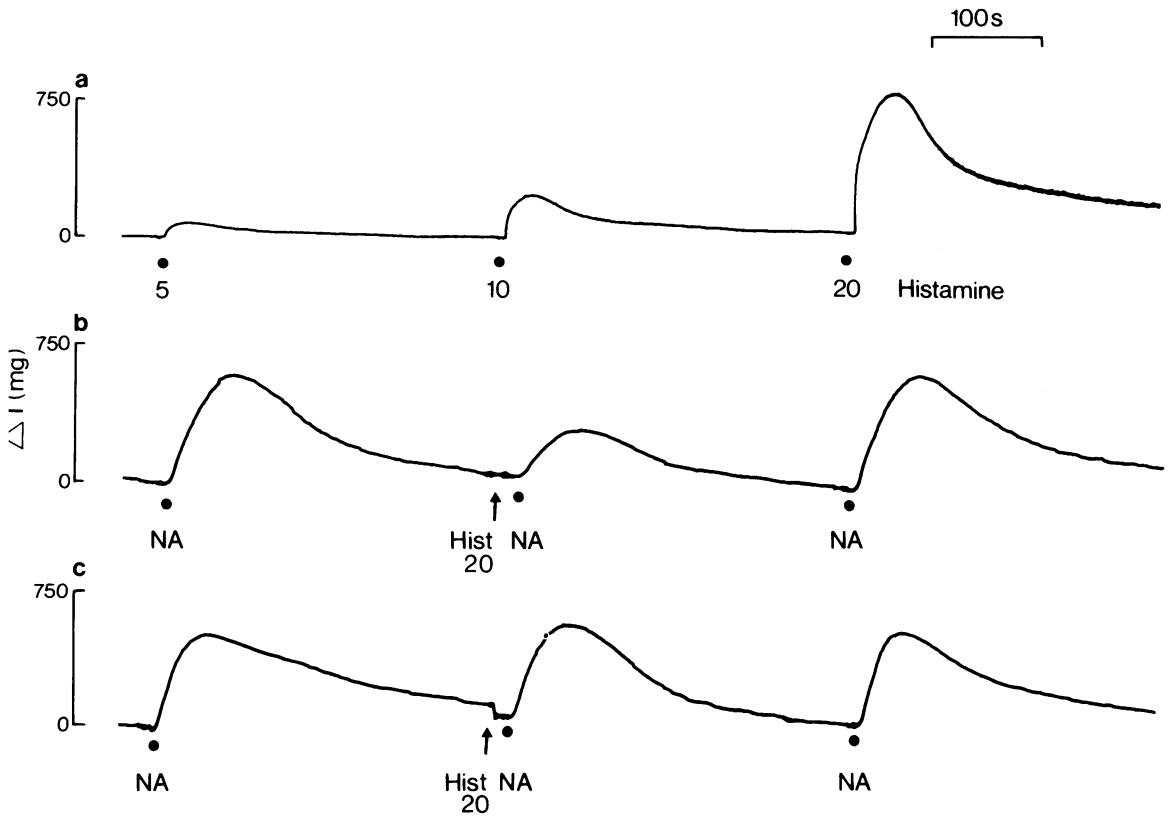

Figure 1 Rabbit aortic strip. (a) Contractile responses to histamine (at dots) in normal Krebs solution. Doses are shown in μg. (b) In the presence of mepyramine, 1 μg/ml. Histamine (Hist), 20 μg had no contractile effect but reduced the otherwise constant contractile response to noradrenaline (NA), 0.25 μg. (c) In the presence of mepyramine, 1 μg/ml and metiamide, 1 μg/ml. Histamine (Hist), 20 μg had no effect on the response to noradrenaline (NA), 0.25 μg.

FT03 force-displacement transducer and recorded on a Grass polygraph.

In experiments in which vasoconstrictor responses to histamine were studied three or four doses which were found to cause responses on the linear part of the dose-response curve were used. Each dose was repeated 3 times and the mean response used to plot a dose-response curve; changes in sensitivity were expressed by comparing equi-active dose ratios.

Vasodilator responses were studied by the method of Carroll & Glover (1973). Mepyramine was added to the perfusate if necessary to abolish the constrictor (H_1) action of histamine. A dose of noradrenaline was chosen which gave a reproducible vasoconstrictor response (rise in perfusion pressure or increase in tension). Histamine was injected at a constant time (in the range 10–30 s) before such an injection, and a vasodilator response to histamine was expressed as the percentage fall in the otherwise constant vasoconstrictor response.

Spontaneously beating rabbit atria were suspended in a 20 ml organ bath containing Krebs bicarbonate solution. Contractions were measured with a Grass FT03 force-displacement transducer and recorded on a Grass polygraph. Drugs were added directly to the bathing solution.

All drugs were dissolved in 0.9% w/v NaCl solution containing ascorbic acid 50 μg/ml and all experiments were carried out at 37°C.

Drugs

The following drugs were used: dithiothreitol (Calbiochem), histamine diphosphate (Fluka, A.G.), mepyramine maleate (May & Baker), metiamide (Smith, Kline & French) and noradrenaline acid tartrate (Winthrop). The amounts stated in the text refer to these compounds with the exception of noradrenaline where the amount stated refers to the base.

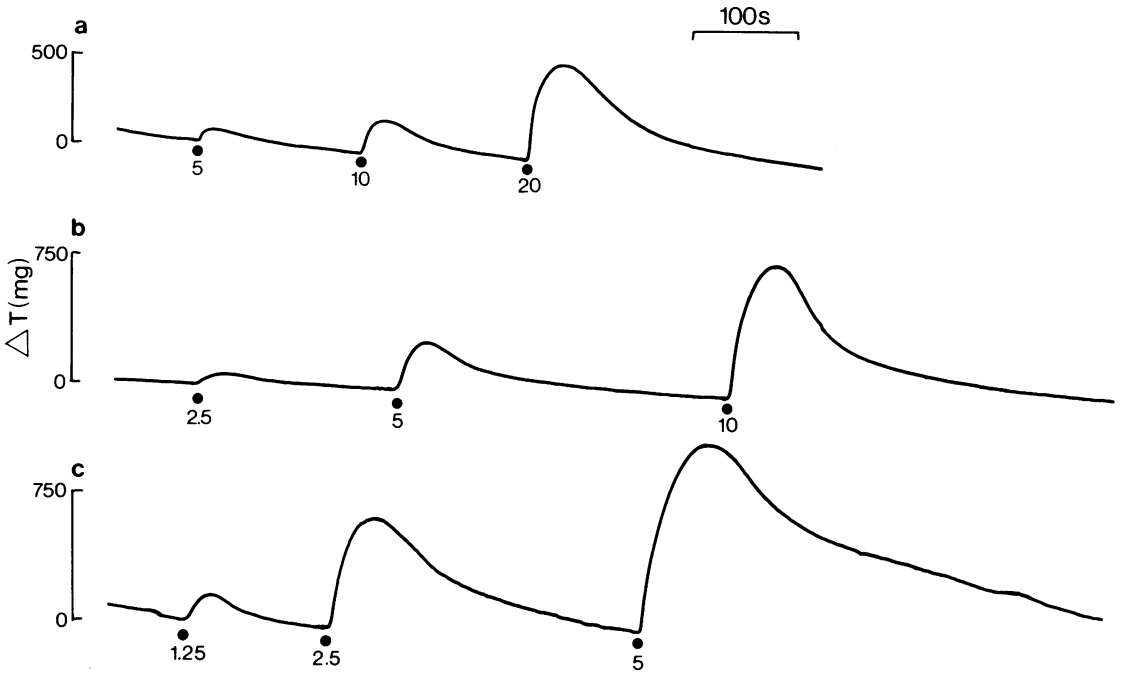

Figure 2 Rabbit aortic strip. Contractile responses to histamine (at dots). Doses are shown in μg. (a) In normal Krebs solution; (b) in the presence of metiamide, 10 μg/ml; (c) in the presence of dithiothreitol, 154 μg/ml.

Results

Histamine receptors in aortic strips

When injected in doses of 1 to 100 μg into the perfusion stream, histamine produced brief, reproducible dose-related contractions of twelve aortic strips (Figures 1a and 2). This action of histamine was mediated by H_1-receptors since the contractile responses to these doses were abolished in the presence of mepyramine (10 ng to 1 μg/ml).

In the presence of mepyramine, histamine had no effect on the tension of the aortic strips. However, when injected before a dose of noradrenaline, histamine produced dose-dependent reductions in the otherwise constant contractile response. This vasodilator action of histamine was abolished by metiamide and can therefore be attributed to H_2-receptor activity. Figure 1b and c shows records from a typical experiment and 5 dose-response curves are illustrated in Figure 3. In eight similar experiments histamine (10 μg), in the absence of mepyramine, caused a tension increase of 603 ± 56 mg (mean ± s.e.) and, in the presence of mepyramine (1 μg/ml), a reduction of $46 \pm 9\%$ in the contractile response to noradrenaline. The dose of noradrenaline, while constant in any one experiment, was in the range 0.1 to 2.5 μg and resulted in tension changes of 630 ± 45 mg.

In the rabbit ear artery the constrictor response to histamine is potentiated by metiamide and hence represents a balance between a predominant constrictor action mediated by mepyramine-sensitive H_1-receptors and a vasodilator action mediated by metiamide-sensitive H_2-receptors (Glover *et al.*, 1973). In the present experiments, metiamide (10 μg/ml) caused a 2.7 ± 1.3 fold potentiation of the responses of five aortic strips to histamine, as calculated from the shifts to the left of the histamine dose-response curves. The effect of metiamide on the responses of one of these preparations to histamine is illustrated in Figure 2 and a typical dose-response curve is shown in Figure 4.

Effects of dithiothreitol on H_1-responses to histamine

(a) *Aortic strip.* As shown by Fleisch *et al.* (1973), the contractile responses of rabbit aortic strips to histamine were potentiated by DTT. A typical record is shown in Figure 2. In seven experiments DTT (154 μg/ml i.e. 1 mM) potentiated the response to histamine 6.5 ± 2.1 times as indicated by the shifts to the left of the histamine dose-response curves.

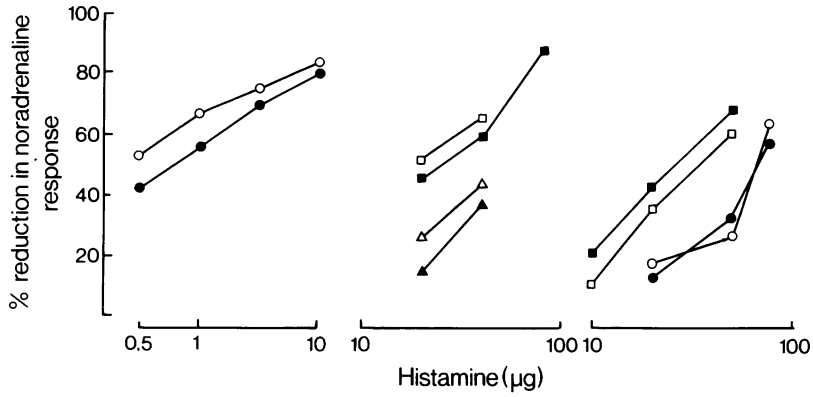

Figure 3 Vasodilator action of histamine in rabbit aorta: effect of dithiothreitol. In the presence of mepyramine, histamine was injected before a dose of noradrenaline and the histamine response expressed as the percentage reduction in the otherwise constant contractile response to noradrenaline. Paired dose-response curves obtained in five experiments. Closed symbols—control responses, open symbols—in presence of DTT 154 μg/ml. Each point is the mean of three observations. In no case was the s.e. mean greater than 5.

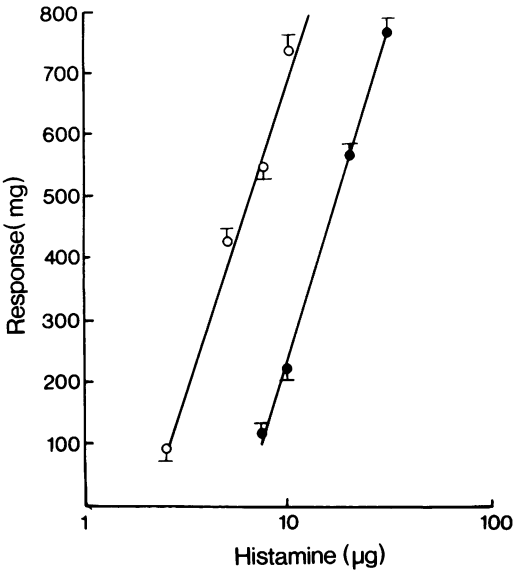

Figure 4 Effect of metiamide on the constrictor response of the rabbit aortic strip to histamine. (●) Control, (○) metiamide 10 μg/ml. Each point is the mean of three observations and vertical bars indicate s.e. mean.

(b) *Ear arteries.* DTT (154 μg/ml) had no effect on the tension of aortic strips, but in three out of eight experiments caused small increases (5–15 mmHg) in the perfusion pressure of the rabbit ear arteries. In every case DTT caused a marked potentiation of the responses of the ear arteries to histamine. The onset of potentiation was rapid and the responses remained stable for 60–90 min although there was generally some deterioration after this time. Dose-response curves were obtained to histamine on the eight arteries before and 30 min after the addition of DTT (154 μg/ml). The addition of DTT produced a shift to the left of the histamine dose-response curves and, as indicated by these shifts, a 79.0 ± 29.1 times potentiation of the responses to histamine.

(c) *In the presence of metiamide.* Metiamide causes potentiation of histamine vasoconstrictor responses by blocking H_2-receptors. DTT, however, still caused marked potentiation of the constrictor responses of both the aorta and the ear artery in the presence of a maximal degree of H_2-receptor blockade with metiamide. In this series of experiments the concentration of metiamide was progressively increased until a point was reached where doubling the concentration produced no further potentiation of the response to histamine. The concentration of metiamide required to achieve this was 40 to 100 μg/ml. Under these conditions the responses of five ear arteries were potentiated 5.1 ± 1.7 times and the responses of two aortic strips 2.1 and 2.4 times. However, with this maximum blockade of the H_2-receptors the addition of DTT (154 μg/ml) potentiated the responses of the five ear arteries to histamine a further 25.1 ± 5.9 times and the responses of the two aortic strips a further 3.2 and 3.7 times. The results obtained from one of the ear arteries are shown in Figure 5.

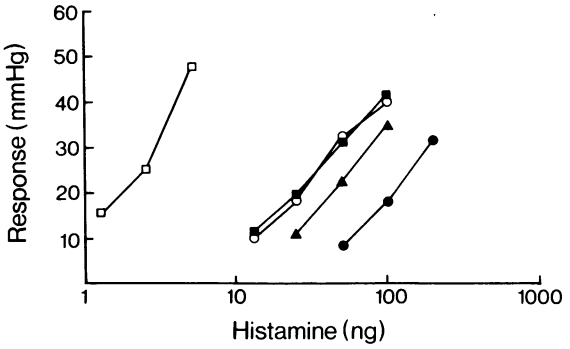

Figure 5 Effect of metiamide and metiamide plus dithiothreitol (DTT) on the constrictor response of the rabbit ear artery to histamine. (●) Normal Krebs, (▲) metiamide 10 μg/ml, (○) metiamide 20 μg/ml, (■) metiamide 40 μg/ml and (□) metiamide 40 μg/ml plus DTT 154 μg/ml. Each point is the mean of three observations. In no case was the s.e. mean greater than 3 mmHg.

Effects of dithiothreitol on H_2-responses to histamine

(a) *Aorta and ear artery.* In the presence of mepyramine (10 ng to 1 μg/ml) the constrictor response of both vessels to histamine was abolished and a vasodilator action revealed. In each of ten ear arteries and five aortic strips histamine (1 to 50 μg) caused reproducible reductions in the responses to noradrenaline. This action of histamine was unaffected by DTT (154 μg/ml) but was abolished by metiamide (1 to 10 μg/ml). A typical record from one aortic strip is illustrated in Figure 6, and the effect of DTT on the responses of both vessels summarized in Figures 3 and 7.

Following the addition of DTT to the perfusate the dose of noradrenaline was adjusted where necessary to maintain the vasoconstrictor response at the pre-DTT level. The ratio of noradrenaline doses used therefore provided an index of any change in sensitivity to noradrenaline caused by DTT. The results are summarized in Table 1a and b.

In three ear arteries DTT had no effect on the response to noradrenaline but in 7 it was potentiated 1.5–4.0 times (mean of 10 experiments $= 2.2 \pm 0.37$).

In contrast, the responses of all 5 aortic strips to noradrenaline were reduced by DTT, and it was necessary to increase the dose of noradrenaline in each case to maintain a constant constrictor response (mean of 5 experiments $= 8.5 \pm 2.7$).

(b) *Human temporal artery.* Histamine (0.1 to 25 μg) had no constrictor action on this preparation and, in the absence of mepyramine, produced dose-dependent reduction in the constrictor responses to noradrenaline. In each of five experiments this action of histamine was unaffected by DTT (154 μg/ml) (Figure 8) but was abolished by metiamide (1 to 10 μg/ml).

DTT had no consistent effect on the responses of these arteries to noradrenaline. Table 1c shows that in 1 experiment the response was unchanged, in 2 it was slightly increased (2 times) and in 2 it was slightly reduced (0.6 and 0.8 times).

(c) *Rabbit atria.* In 6 spontaneously beating rabbit atria preparations histamine (2.5 μg/ml for 1 min) produced an increase in the force of contraction (mean maximum $71.8 \pm 9.4\%$). This action of histamine was unchanged ($71.6 \pm 9.9\%$) in the presence of DTT (154 μg/ml) but was greatly reduced ($11.2 \pm 3.2\%$) by metiamide (5 μg/ml). A typical experiment is illustrated in Figure 9.

Discussion

The results show that the rabbit aorta, like the ear artery (Glover *et al.*, 1973), contains both H_1 and H_2 histamine receptors. Thus, although histamine normally has a contractile or vasoconstrictor action on these isolated vessels, a metiamide-sensitive vasodilator action is revealed when the H_1-receptors are blocked with mepyramine. In order to demonstrate vasodilator responses in isolated artery preparations it is usually necessary first to raise their tone by vasoconstrictor stimulation (de la Lande & Rand, 1965; Starr & West, 1966). Alternatively, a vasodilator stimulus may be applied before a vasoconstrictor stimulus, and the vasodilator response recorded as a reduction in the otherwise constant vasoconstrictor response (Carroll & Glover, 1973). This was the method used to demonstrate H_2-receptor activity in rabbit ear and human temporal arteries (Glover *et al.*, 1973), and we have now applied it to rabbit aortic strips. Histamine normally contracted these strips; however, after mepyramine it had no effect on the resting tension but caused a dose-related reduction in the response to noradrenaline. This action of histamine was abolished by metiamide and hence can be attributed to stimulation of H_2-receptors. In addition, in both the rabbit ear artery and aorta the normal response to histamine is a balance between a predominant constrictor action mediated by H_1-receptors and a vasodilator action mediated by H_2-receptors, since it is abolished by mepyramine and potentiated by metiamide.

Our results confirm the finding that DTT potentiates the contractile response of the rabbit aorta to histamine (Fleisch *et al.*, 1973; Fleisch, Krzan & Titus, 1974), and we have extended this observation to the rabbit ear artery. In our aortic strip experiments, 3–4 graded doses of histamine were injected into the

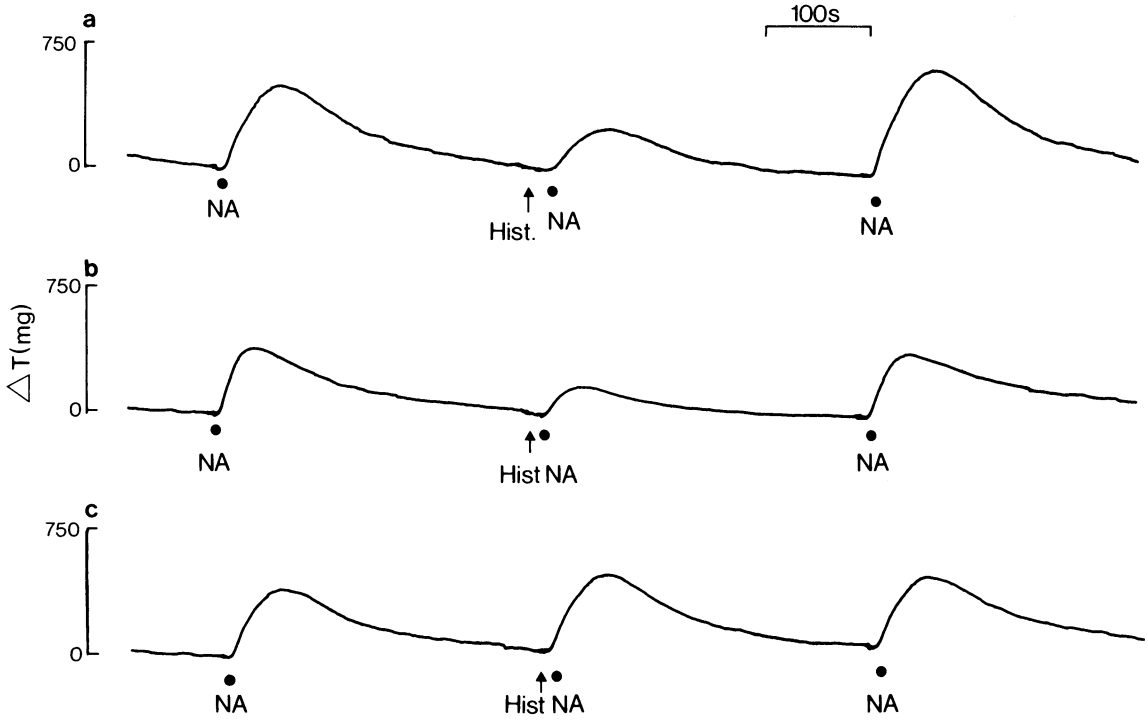

Figure 6 Rabbit aortic strip. All records were obtained in the presence of mepyramine, 1 μg/ml. (a) Histamine (Hist), 25 μg had no contractile effect but reduced the contractile response to noradrenaline (NA), 0.1 μg. (b) In the presence of dithiothreitol (DTT), 154 μg/ml this effect of histamine was unchanged. (c) In the presence of metiamide, 1 μg/ml this effect of histamine was abolished. Note that in the presence of DTT and metiamide it was necessary to increase the dose of noradrenaline (NA) to 0.5 μg to keep the contractile response constant.

Krebs solution which flowed rapidly through the organ bath, in contrast to the cumulative additions used by Fleisch *et al.* (1973; 1974). The responses were therefore brief, thus allowing 3 points on the straight part of the dose-response curve to be obtained rapidly. We did not use doses which would elicit a maximal response, but the method has the advantage that the desensitization which follows cumulative additions and a maximal response to histamine does not occur, and the responses were reproducible. In the absence of a maximal response to the ED_{50} (concentration of agonist required to elicit 50% of the maximal response) cannot be calculated, but DTT caused a shift of the dose-response curve to the left with a dose-ratio of 6.5 ± 2.1. This compares well with the ED_{50} ratios reported by Fleisch *et al.* in 1973 and 1974 of 10 and just over 7 respectively. DTT caused an even greater potentiation of the responses of the ear artery to histamine. It is not possible to obtain maximum responses and full dose-response curves using the perfused artery method, but in these preparations we found that DTT caused a marked shift to the left of the dose-response curve with a dose-ratio of 79 ± 29.

Fleisch *et al.* (1973) concluded that the enhancement of the constrictor response of the rabbit aorta to histamine produced by DTT was due to an increase in the activity of the histamine receptor system. Subsequently (1974) they found that, in contrast, DTT depressed the response of the guinea-pig aorta to histamine, and suggested that this could be explained by the existence of different histamine H_1-receptor sub-types. Our finding that the rabbit aorta and ear artery contain both H_1 and H_2 histamine receptors, and that the contractile response to histamine was increased when the H_2-receptors were blocked with metiamide raised another possibility, that is, that DTT caused potentiations not by increasing the activity of H_1-receptors, but by decreasing the activity of H_2-receptors.

However, our results show that DTT still caused marked potentiations of the constrictor response to histamine in the presence of the maximal degree of H_2-receptor blockade produced by metiamide. It seems reasonable to conclude that DTT does cause an increase in the activity of H_1-receptor systems in these rabbit arteries. This finding does not exclude the possibility of a concurrent action, either blockade or

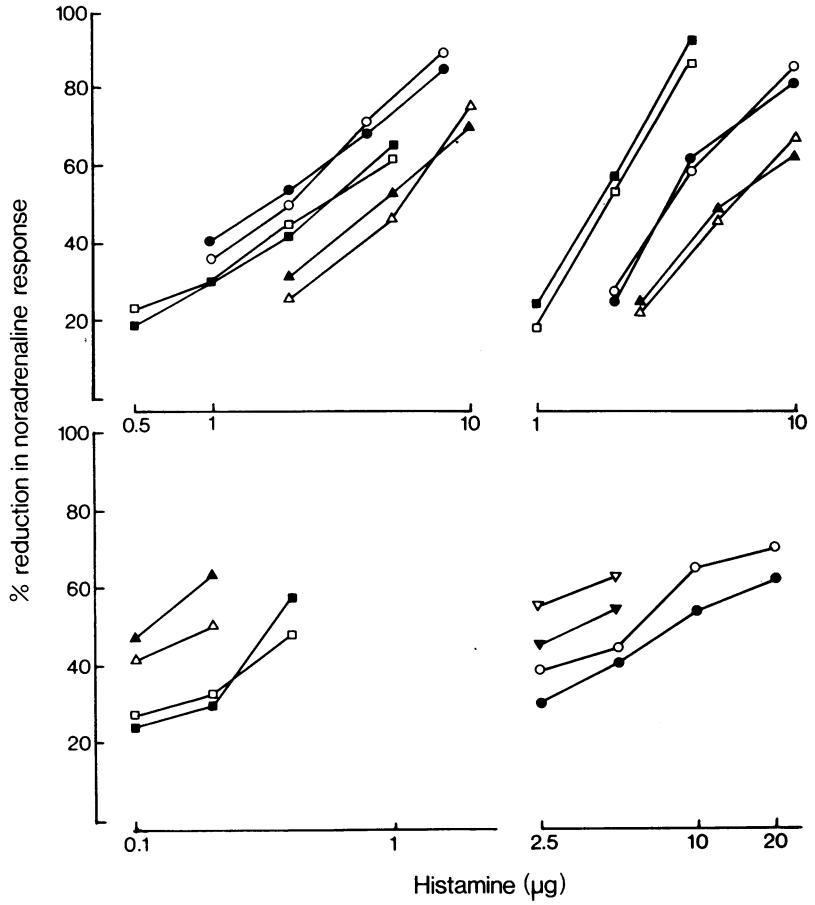

Figure 7 Vasodilator action of histamine in rabbit ear artery: effect of dithiothreitol (DTT). Responses expressed as in Figure 3. Paired dose-response curves obtained in ten experiments. Closed symbols—control; open symbols—DTT 154 μg/ml. Each point is the mean of three observations. In no case was the s.e. mean greater than 5.

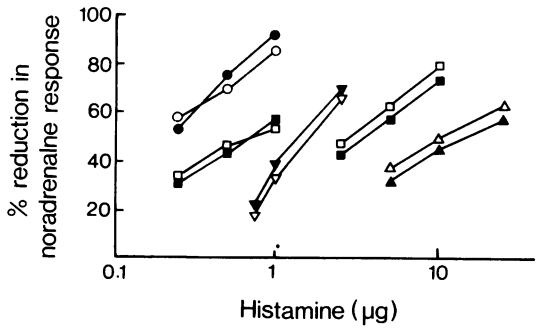

Figure 8 Vasodilator action of histamine in human temporal artery: effect of dithiothreitol (DTT). Responses expressed as in Figure 3. Paired dose-response curves obtained in five experiments. Closed symbols—control; open symbols—DTT 154 μg/ml. Each point is the mean of three observations. In no case was the s.e. mean greater than 5.

potentiation of H_2-receptor systems. However, after H_1-receptor blockade with mepyramine, the H_2-receptor effect, revealed as a depression of the constrictor response to noradrenaline, was unaffected by DTT. It is unlikely that mepyramine was modifying a possible effect of DTT on the H_2-receptors in these vessels, unless there are sub-types of H_2-receptors, since the responses of the human temporal artery were unaffected by DTT. In the temporal artery, histamine has no vasoconstrictor action and it was not necessary to use mepyramine to reveal the H_2-response. Further, the positive inotropic action of histamine in the isolated rabbit atria was unaffected by DTT. It can therefore be concluded that these cardiovascular responses mediated by H_2-receptors are unaffected by DTT.

Fleisch *et al.* (1973) showed that the potentiating action of DTT was specific for histamine contractile responses in the rabbit aorta since DTT caused a

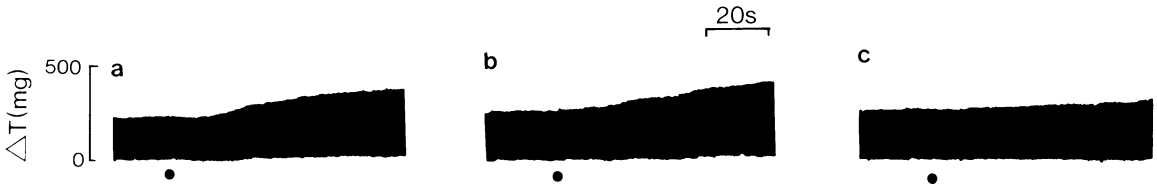

Figure 9 Spontaneously beating rabbit atria. Responses to histamine (at dots), 2.5 μg/ml. (a) In normal Krebs solution; (b) in the presence of dithiothreitol 154 μg/ml; (c) in the presence of metiamide 5 μg/ml.

Table 1 Change in sensitivity to noradrenaline caused by dithiothreitol (DTT) in ear artery and aorta of the rabbit and in human temporal artery

(a) *Rabbit ear artery*

Control		*DTT*		*Ratio control/DTT*	
Dose (ng)	*Response (mmHg)*	*Dose (ng)*	*Response (mmHg)*	*Dose*	*Response*
2	25	2	23	1.0	1.09
4	24	4	20	1.0	1.2
5	19	5	21	1.0	0.9
7.5	30	5	28	1.5	1.07
4	27	2	31	2.0	0.87
20	24	10	27	2.0	0.89
2.5	29	1	31	2.5	0.94
3	26	1	25	3.0	1.08
4	33	1	35	4.0	0.94
20	30	5	33	4.0	0.91
			Mean ± s.e.	2.2 ± 0.37	0.99 ± 0.04

(b) *Rabbit aorta*

Control		*DTT*		*Ratio control/DTT*	
Dose (μg)	*Response (mg)*	*Dose (μg)*	*Response (mg)*	*Dose*	*Response*
0.3	690	1	665	0.3	1.04
0.25	630	1	595	0.25	1.06
0.1	510	0.5	410	0.2	1.24
0.1	440	1.5	420	0.07	1.05
0.1	905	1.5	780	0.07	1.16
			Mean ± s.e.	0.18 ± 0.05	1.11 ± 0.04

(c) *Human temporal artery*

Control		*DTT*		*Ratio control/DTT*	
Dose (ng)	*Response (mmHg)*	*Dose (ng)*	*Response (mmHg)*	*Dose*	*Response*
100	24	50	22	2.0	1.09
250	22	400	25	0.6	0.88
25	23	30	24	0.8	0.96
50	29	50	27	1.0	1.07
500	28	250	32	2.0	0.88
			Mean ± s.e.	1.28 ± 0.30	0.98 ± 0.05

slight reduction in the maximal response to KCl, increased the values of ED_{50} for noradrenaline and 5-hydroxytryptamine approximately 4.5 fold and completely abolished the response to angiotensin. We have also found that DTT caused slight depression of the contractile response to noradrenaline in the rabbit aorta. However, we have not studied the effect of DTT on the dose-response curves of noradrenaline and other vasoconstrictor drugs in the rabbit ear artery, but our results show that whereas DTT caused a 79 ± 29 fold potentiation of the constrictor response to histamine, it caused only slight potentiation (2.2 ± 0.37) of the response to noradrenaline. It seems reasonable to conclude that the marked potentiation of the histamine constrictor response caused by DTT is not due to a non-specific action on the vascular smooth muscle. Whether or not there are responses mediated by receptors other than adrenoceptors which are potentiated by DTT, we can conclude that DTT modifies H_1 but not H_2-receptor systems in the rabbit aorta and ear artery, and to this extent is highly specific.

Enhancement of the histamine response in the rabbit aorta is not due to inhibition of histamine-metabolizing enzymes, since histaminase activity is negligible in this tissue and histamine-*N*-methyl-transferase activity was inhibited only 10% by DTT (Fleisch *et al.*, 1973). Our finding that the H_2-responses are not potentiated by DTT supports this conclusion and in addition makes it unlikely that the potentiation could be due to an inhibition of histamine uptake in these tissues.

It is well known that DTT reduces disulphide linkages to sulphydryl groups, and Fleisch *et al.* (1973) concluded that disulphide bridges must occupy prominent positions either in histamine receptors or along the subsequent excitation-contraction pathway. Our results show that this conclusion can be applied to certain cardiovascular H_1 but not H_2 histamine receptor responses in the rabbit.

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