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Selective enhancement of histamine H_1-receptor responses in guinea-pig ileal smooth muscle by 1,4-dithiothreitol

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1 1,4-Dithiothreitol (DTT; 1 mM, 30 min preincubation) produced a small, non-specific potentiation of spasmogenic activity in longitudinal muscle strips of guinea-pig small intestine.

2 A direct comparison of contractile responses elicited by histamine and a range of H_1- and non-H_1-receptor agonists indicated that DTT produced a significantly greater potentiation of H_1-receptor responses.

3 This apparently selective increase in tissue sensitivity to histamine H_1-receptor agonists did not appear to be a consequence of the inhibition of histamine N-methyl transferase or diamine oxidase activity. Potentiation of the responses to histamine by DTT was still observed in the presence of SKF 91488 (10 μM) and aminoguanidine (1μM).

4 The potentiation elicited by DTT was readily reversed by the sulphydryl oxidizing agent dithiobis-(2-nitrobenzoic acid) (DTNB). This suggests that the mechanism of action of DTT involves the reduction of disulphide bonds.

5 Exposure of ileal smooth muscle to DTT following desensitization with histamine ($100 \times EC_{50}$ [– DTT]) resulted in a 6.9 ± 0.7 fold shift of the concentration-response curve to lower agonist concentrations. Conversely, following potentiation of the response to histamine with DTT, exposure of the tissue to desensitizing concentrations of histamine resulted in a dextral shift of the dose-response curve (dose ratio = 39.5 ± 1.2) to higher agonist concentrations.

6 The results of this study suggest that DTT may be a useful tool with which to investigate histamine H_1-receptor mechanisms in ileal smooth muscle.

Introduction

1,4-Dithiothreitol (DTT), an agent which reduces disulphide bonds to sulphydryl groups, has been widely used in receptor studies to establish the presence and functional importance of disulphide bonds in receptor structure and the pathway linking receptor occupancy to functional response (Rang & Ritter, 1971; Karlin, 1974; Lucas *et al.*, 1978; Hazum *et al.*, 1979; Suen *et al.*, 1980; Chang *et al.*, 1982; Massague & Czech, 1982; Bleehan *et al.*, 1983; Carman-Krzan, 1983; Schwartz & Kellar, 1983). DTT treatment of rabbit aortic strips appears to potentiate selectively contractile responses mediated via histamine H_1-receptors (Fleisch *et al.*, 1973; 1974; Carroll & Glover, 1977). A similar selective increase in sensitivity to histamine has been reported in guinea-pig ileal smooth muscle (Glover, 1979). However, more recent studies suggest that the action of DTT on histamine H_1-receptor mediated responses in guinea-pig ileal smooth muscle is a consequence of a non-specific enhancement of spasmogenic activity in this tissue (Watson & Iversen, 1982; Fontaine *et al.*,1984). These observations have prompted us to examine in more detail the nature of the effect of DTT on contractile responses elicited by histamine in the longitudinal smooth muscle of guinea-pig small intestine. A preliminary account of this work has been presented to the British Pharmacological Society (Donaldson & Hill, 1985a).

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Methods

Organ bath studies

Hartley strain guinea-pigs of either sex (200–400 g) were killed by cervical dislocation and decapitation.

Longitudinal smooth muscle strips of ileal smooth muscle were prepared essentially as described by Rang (1964). Muscle strips were suspended in 10 ml of Krebs-Henseleit solution (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.5; pH 7.4 gassed with O_2/CO_2 (95:5) at 37°C in a conventional organ bath. The muscle strips were equilibrated for 60 min before being exposed to agonists, during which time the bathing fluid was changed at 15 min intervals to prevent the accumulation of metabolic products. Contractions to agonists were recorded isotonically. Agonists were in contact with the tissue for 15–25 s and applied at 2 min intervals. Longer intervals between agonist applications were sometimes required following inhibition of histamine metabolizing enzymes. Sequential concentration-response curves were obtained, before and after treatment with test agents. Antagonists, enzyme inhibitors, DTT or 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were added to the reservoir solution and allowed to equilibrate with the tissue for at least 30 min before subsequent concentration-response curves were determined in the continued presence of the test agent. In desensitization experiments a concentration equivalent to one hundred times the EC_{50} value for histamine was added to the tissue bath. After 45 min the histamine was removed from the organ bath and the normal resting tension re-established before determination of agonist dose-response curves.

Data analysis

Concentration-response curves obtained in the presence and absence of DTT were either drawn by inspection or fitted to a Hill equation using the programme ALLFIT (Delean *et al.*, 1978) as described previously (Donaldson & Hill, 1985b). The equation fitted was:

$$\% \text{ of maximal response} = \frac{E_{MAX} \times D^n}{D^n + (EC_{50})^n}$$

where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the concentration of agonist giving half maximal stimulation and E_{MAX} is the maximal stimulation. Affinity constants for mepyramine were obtained from the parallel shift of the log concentration-response curves to agonists obtained in the presence or absence of DTT using the relationship:

$$\text{Dose-ratio} = A \times K_A + 1$$

where A is the concentration of mepyramine, K_A is the affinity constant of mepyramine and the dose-ratio is the ratio of the concentration of agonist necessary to give a specified response in the presence of mepyramine to the concentration of agonist required for the same response in the absence of antagonist. Where the data were adequate (i.e. in experiments in which three or more concentrations of mepyramine were used) the dose-ratios obtained were used to determine Schild slopes (m) by unweighted linear regression of the Schild equation (Arunlakshana & Schild, 1959):

$$\log (\text{dose-ratio} - 1) = m \times \log A + \log K_A$$

The potentiation by DTT of the response to a given agonist was quantified in terms of the ratio of the EC_{50} values obtained in the presence and absence of 1 mM DTT. Thus,

$$\text{potentiation} = \frac{EC_{50}[-\text{DTT}]}{EC_{50}[+\text{DTT}]}$$

where $EC_{50}[-\text{DTT}]$ and $EC_{50}[+\text{DTT}]$ were obtained in the absence and presence of DTT respectively. Differences in the extent of the potentiation achieved with histamine and the test agonist were analysed using a paired *t* test and the Wilcoxon signed rank test. The extent of the potentiation of the response to an individual agonist was tested for significance using the non-parametric Wilcoxon signed rank test on the logarithm of the EC_{50} ratio. This was effectively a test of the difference between the log EC_{50} values obtained in the absence and presence of 1 mM DTT. Chi-squared analysis of the logarithmic and linear distributions of the EC_{50} ratios for histamine in 55 experiments suggested that the logarithmic, but not the linear, distribution deviated from the normal distribution.

Drugs

Histamine dihydrochloride was obtained from BDH and mepyramine maleate, acetylcholine chloride, aminoguanidine, 5-hydroxytryptamine creatine sulphate, 1,4-dithiothreitol and 5,5'-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma. Gifts of N^{α}-methylhistamine, N^{α},N^{α}-dimethylhistamine, 2-pyridylethylamine (2-(2-aminoethyl)pyridine, 2-thiazolylethylamine (2-(2-aminoethyl)thiazole) and SKF 91488 (s-[2-n,n-dimethyl amino) butyl] isothiourea) from Smith Kline and French are gratefully acknowledged. All histamine analogues were in the form of the dihydrochloride salt.

Results

Alterations in histamine H_1-receptor activity by 1,4-dithiothreitol

DTT produced a marked potentiation of the histamine-induced contractile response of longitudinal muscle strips of guinea-pig ileum (Figure 1). This effect was normally accompanied by an increase in

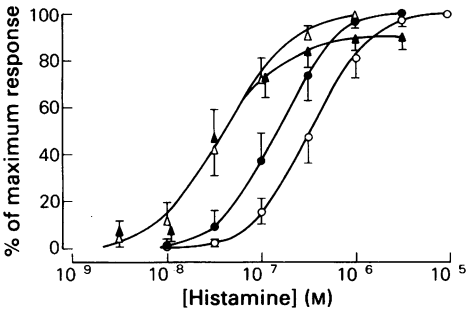

Figure 1 The effect of 1,4-dithiothreitol (DTT) (●) 10^{-5} M, (△) 10^{-3} M and (▲) 3×10^{-3} M on histamine-induced contractile activity in longitudinal smooth muscle strips of guinea-pig ileum. (○) Control. Data are expressed as a percentage of the maximal response to histamine obtained in each experiment. Points represent the mean from 4–5 experiments. Vertical lines show s.e.mean. DTT was added to the reservoir solution in increasing concentrations and allowed to equilibrate with the tissue for 30 min (1st concentration of DTT), or 15 min for subsequent concentrations of DTT, before determination of histamine concentration-response curves. Data were also obtained with 10^{-4} M DTT but not included here for the sake of clarity. This latter concentration of DTT produced a 3.4 ± 1.1 ($n = 5$) fold sinistral shift of the histamine concentration-response curve.

spontaneous activity as observed previously by Watson & Iversen (1982), but no change in baseline was observed. At low concentrations of DTT, there was a parallel shift of the histamine concentration-response curve to lower agonist concentrations (Figure 1). A maximal potentiation was obtained with a concentration of 1 mM and further increases in the concentration of DTT resulted in a depression of the maximal response. For this reason all subsequent experiments were performed with a concentration of 1 mM DTT.

The onset of potentiation was rapid and responses were well maintained. A maximal effect was normally achieved within 15 min, although a preincubation time of 30 min was chosen for most studies. After 150 min some deterioration of the maximal response was occasionally observed and experiments were not normally extended beyond this period of incubation with DTT.

DTT (1 mM, 30 min preincubation) produced a large parallel shift of the histamine concentration-response curve to lower agonist concentrations. Analysis of the fitted maxima and Hill coefficients (see Methods), determined in 18 experiments from histamine dose-response curves, confirmed that DTT had no significant effect on these parameters. The extent of the parallel shift of the histamine dose-response curve was subsequently quantified in terms of the ratio of the EC_{50} values obtained in the absence and presence of DTT (1 mM) as described under Methods. In 55 experiments the mean potentiation of the response to histamine obtained with DTT was 11.0 ± 0.8.

A comparison of the effects of DTT on responses elicited by alternate doses of histamine and acetyl-

Table 1 Comparison of the effect of 1,4-dithiothreitol (DTT) on contractile responses to histamine and a range of other spasmogens in guinea-pig ileum

Agonist	*Relative H_1-receptor potency* (histamine = 100)	*Potentiation* (EC_{50}[− DTT]/EC_{50}[+ DTT]) *of response to:* *Agonist*	*Histamine*	(n)
Histamine analogues				
2-Pyridylethylamine	5.3 ± 0.2	6.7 ± 1.9	7.6 ± 1.9	6
2-Thiazolylethylamine	29.5 ± 2.7	6.5 ± 1.0	6.6 ± 1.3	6
$N^{\alpha}N^{\alpha}$-dimethylhistamine	67.2 ± 12.9	10.8 ± 1.8	9.8 ± 1.1	6
N^{α}-methylhistamine	104.8 ± 26.0	10.5 ± 5.8	11.6 ± 4.4	3
Non H_1-receptor spasmogens				
5-Hydroxytryptamine	—	2.1 ± 0.3*	7.5 ± 1.2#	6
Acetylcholine	—	1.8 ± 0.4*	14.5 ± 1.1#	6
KCl	—	1.5 ± 0.3*	14.0 ± 2.8#	6

Values represent mean ± s.e.mean. The extent of the potentiation by DTT (1 mM) is given by the ratio of EC_{50} values determined, in the absence and presence of DTT, for agonist or histamine measured in the same strip of longitudinal smooth muscle. n represents the number of paired determinations (agonist and histamine).
*$P < 0.05$ (Wilcoxon signed rank test) EC_{50} in the presence of DTT compared to the EC_{50} of the control curve.
#$P < 0.01$ (paired t test) or $P < 0.05$ (Wilcoxon signed rank test) with respect to the increase in sensitivity to agonist measured in the same smooth muscle strip.

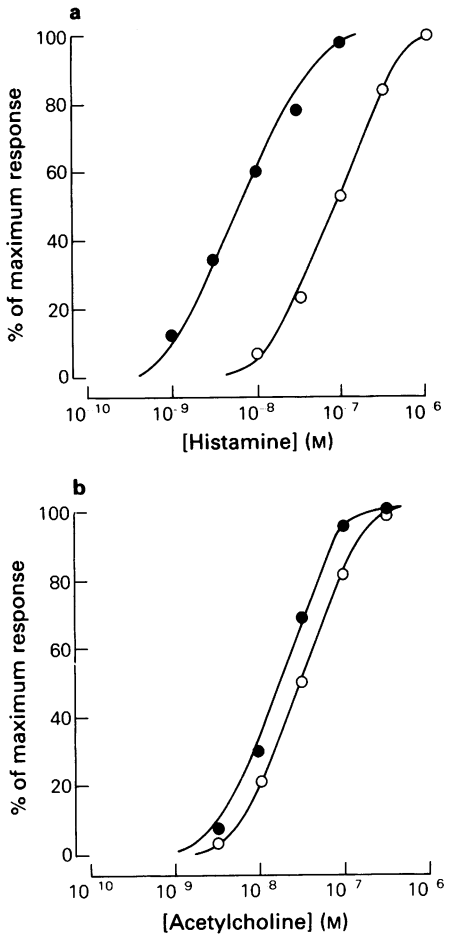

Figure 2 Concentration-response curves for histamine (a) and acetylcholine (b) obtained, in the presence (●) and absence (○) of 1,4-dithiothreitol (DTT), 1 mM, in the same strip of ileal smooth muscle. Data are expressed as a percentage of the maximal response obtained with histamine. Dose-response curves for acetylcholine and histamine were obtained essentially simultaneously by alternate dosing of the tissue with the two agonists. DTT 1 mM was added to the reservoir solution and allowed to equilibrate with the tissue for 30 min before redetermination of agonist dose-response curves in the continued presence of DTT. Similar results were obtained in five other experiments (Table 1).

choline in the same strips of longitudinal smooth muscle (Figure 2) revealed an apparently selective effect of DTT on histamine-elicited responses (Table 1). A similar protocol to that described in the legend to Figure 2 was employed to compare the alteration in histamine receptor activity induced by DTT with its effect on contractile responses elicited by a number of H_1- and non-H_1-receptor smooth muscle spasmogens. The results from these studies are summarised in Table 1. Incubation with DTT resulted in a small but significant ($P < 0.05$) sinistral shift of the concentration-response curves for the non-H_1-receptor spasmogens, acetylcholine, KCl and 5-hydroxytryptamine to lower agonist concentrations (Table 1). This small non-specific potentiation of spasmogenic activity, however, was in marked contrast to the significantly larger effect of DTT on histamine sensitivity in the same smooth muscle preparation (Table 1). The extent of the potentiation of tissue responses to H_1-selective (2-thiazolylethylamine and 2-pyridylethylamine) and non-selective (N^{α},N^{α}-dimethylhistamine and N^{α}-methylhistamine) histamine analogues was very similar to that determined for histamine in the same strip of ileal smooth muscle.

A limited number of experiments were also conducted using the above protocol, to compare the effect of DTT on responses elicited by histamine and acetylcholine, in intact segments of guinea-pig whole ileum. In these experiments, DTT produced a significantly greater potentiation of the contractile response to histamine ($P < 0.001$; paired t test, $n = 6$; potentiation = 9.0 ± 0.9) than that of acetylcholine in the same segments of whole ileum (potentiation = 2.9 ± 0.6).

To determine whether DTT had an influence on the interaction of competitive antagonists with the ileal H_1-receptor, some studies were performed with the selective H_1-receptor antagonist, mepyramine. The effect of mepyramine on histamine concentration-response curves, determined in the presence and absence of DTT, was investigated in adjacent strips of longitudinal smooth muscle taken from the same animal. In the absence of DTT, mepyramine shifted the dose-response curve to higher agonist concentrations consistent with competitive antagonism (Table 2). Competitive antagonism was also demonstrable in the presence of DTT and no significant difference in affinity constant or Schild slope was observed between the two treatments (Table 2). Similar results were obtained when the H_1-selective 2-thiazolylethylamine was used as agonist. In this latter case, K_A values for mepyramine of $3.2 \pm 0.3 \times 10^9$ and $1.2 \pm 0.4 \times 10^9\ M^{-1}$ were obtained in the presence and absence of DTT respectively ($n = 3$, in each case).

Reversal by dithiobis-(2-nitrobenzoic acid) (DTNB)

The effect of DTT (1 mM) on the contractile response of ileal smooth muscle elicited by H_1-receptor stimulation was only slowly reversible (no reversal was detected within 3 h) following removal of the disulphide reducing agent. Addition of the sulphydryl oxidizing agent DTNB (1 mM), however, readily rever-

Table 2 Influence of 1,4-dithiothreitol (DTT) (1 mM) on the characteristics of H_1-receptor antagonism by mepyramine in guinea-pig ileal smooth muscle

	Affinity constant (K_A) *or Schild slope* (m)	
	− DTT	+ DTT
K_A	$7.4 \pm 1.2 \times 10^8\ M^{-1}$ (7)	$1.5 \pm 0.3 \times 10^9\ M^{-1}$ (7)
m	1.01 ± 0.15 (4)	0.91 ± 0.11 (4)

Values represent mean ± s.e.mean. Affinity constants or Schild slopes, obtained in the presence or absence of DTT (1 mM), were determined in adjacent strips of longitudinal smooth muscle taken from the same animal. The number of animals is given in parentheses. In four of the experiments, where three or more antagonist concentrations were used, Schild slopes (m) were determined as described under Methods. For the experiments with DTT, the concentration-response curve for histamine was shifted to the left with DTT (1 mM) before the antagonist affinity of mepyramine was determined in the continued presence of DTT.

sed the effect of DTT (Figure 3). In parallel studies (Figure 4) DTNB alone had no significant effect on the histamine concentration-response curve. Furthermore, addition of equivalent concentrations (1 mM) of DTT and DTNB to the incubation medium was also without effect (Figure 4). When DTNB was washed out and replaced by DTT alone, potentiation of the histamine response was then observed.

Enzyme inhibitors

It is possible that the apparently selective effect of DTT on histamine H_1-responses is attributable to an interference with histamine metabolism in the longitudinal smooth muscle of guinea-pig ileum. However, pretreatment of ileal smooth muscle strips with the histamine-N-methyl transferase inhibitor, SKF 91488 (10 μM; Beavan & Shaff, 1979) or the diamine oxidase inhibitor, aminoguanidine (1 μM; Schuler, 1952), at concentrations which selectively inhibit at least 90% of the activity of the respective enzymes, failed to alter the tissue responsiveness to either histamine ($n = 4$, for each inhibitor) or the H_1-selective agonist 2-pyridylethylamine ($n = 3$, for each inhibitor). Increasing the concentration of aminoguanidine to 10^{-3} M which also blocks histamine uptake into smooth muscle cells (Zilletti *et al.*, 1978) similarly had no effect on the responses elicited by

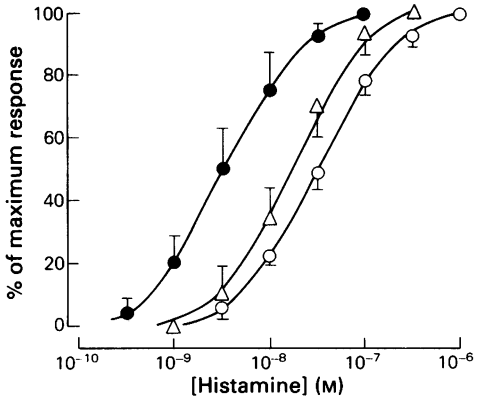

Figure 3 Reversal of the effects of 1,4-dithiothreitol (DTT) by the sulphydryl oxidizing agent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). (○) Control; (●) 1 mM DTT, 30 min preincubation; (△) 1 mM DTNB, 30 min preincubation. Concentration-response curves for histamine were determined in the continued presence of the test agent. DTNB was added to the tissue preparation immediately following washout of the DTT containing Krebs medium. Data are expressed as a percentage of the maximal response obtained with histamine under control conditions. Each point represents the mean from three experiments; vertical lines show s.e.means.

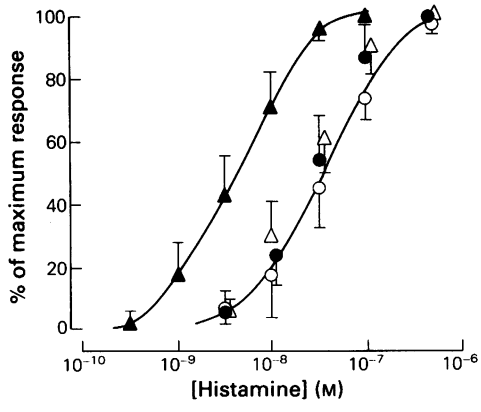

Figure 4 Influence of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 1,4-dithiothreitol (DTT) on histamine-induced contractile activity in guinea-pig ileal smooth muscle. Concentration-response curves for histamine were obtained in the following order: (○) control; (●) DTNB (1 mM); (△) DTT + DTNB (both 1 mM) and finally (▲) DTT alone (1 mM) following washout of DTNB. Results are expressed as a percentage of the maximal response obtained with histamine under control conditions. Each point represents the mean from three experiments; vertical lines show s.e.means.

these two agonists. In the presence of both inhibitors a small sinistral shift of the histamine concentration-response curve was observed (1.4 fold; Figure 5a), but subsequent addition of DTT to the incubation medium resulted in a further potentiation of histamine-mediated responses, observed as an 11.2 fold shift of the dose-response curve to lower agonist concentrations. In contrast, simultaneous blockade of histamine-N-methyltransferase and diamine oxidase had no effect on the potency of 2-pyridylethylamine (Figure 5b), measured in the same experiments. This histamine analogue has been previously reported to be resistant to metabolism by diamine oxidase (Arunlakshana *et al.*, 1954). In experiments in which the effect of DTT was determined in the continued presence of enzyme blockade, the extent of the potentiation achieved with either histamine or 2-pyridylethylamine as agonist (in the same muscle strip) was very similar ($EC_{50}[-DTT]/EC_{50}[+DTT] = 9.7 \pm 3.0$ and 9.7 ± 2.8 for the two agonists respectively; $n = 3$).

Desensitization

In view of the report by Siegel & Triggle (1980) that DTT prevents histamine-induced desensitization in guinea-pig ileum, we have investigated the possibility that this mechanism may explain the effects observed above. Exposure of guinea-pig ileal smooth muscle to a concentration of histamine equivalent to $100 \times EC_{50}[-DTT]$ (histamine) for 45 min resulted in a marked desensitization of the tissue as indicated by a dextral shift of the concentration-response curve to higher agonist concentrations (Figure 6). The mean value for the desensitized dose-ratio obtained in three such experiments was 19.3 ± 0.7. Subsequent exposure (30 min) of the tissue to DTT in the presence of the desensitizing concentration of histamine resulted in a parallel shift to the left of the concentration-response curve (Figure 6), producing a mean shift of 6.9 ± 0.7 fold ($n = 3$; expressed as a ratio of the two EC_{50} values obtained before, and 30 min after, treatment with DTT). It was necessary to include the desensitizing dose of histamine during incubation with DTT since any subsequent potentiation of the response might have been attributable to natural recovery from desensitization rather than the influence of DTT. That DTT does not prevent desensitization was demonstrated in a series of experiments in which tissues were initially exposed to DTT and then treated with a desensitizing dose ($100 \times EC_{50}[-DTT]$) of histamine in the continued presence of DTT. Following potentiation (EC_{50} ratio $= 19.1 \pm 6.5, n = 3$) of the response to histamine with DTT, exposure of the tissue to desensitizing concentrations of histamine resulted in a dextral shift of the dose-response curve (dose-ratio $= 39.5 \pm 1.2$, $n = 3$) (Figure 7) to higher agonist concentrations.

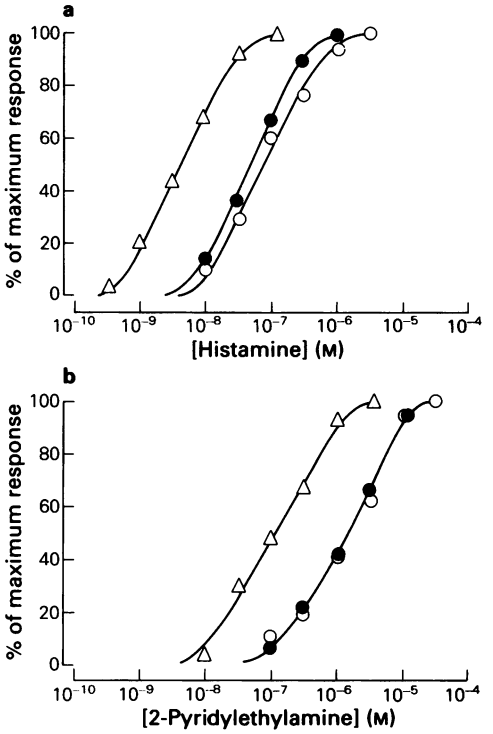

Figure 5 The effect of inhibition of histamine-*N*-methyltransferase and diamine oxidase on tissue sensitivity to histamine (a) and the H_1-selective agonist 2-pyridylethylamine (b) in the same strip of ileal smooth muscle. (○) Control; (●) SKF 91488 (10 μM) + aminoguanidine (1 μM); (△) 1,4-dithiothreitol (1 mM) + SKF 91488 (10 μM) + aminoguanidine (1 μM). Data are expressed as a percentage of the maximal response obtained with histamine under control conditions. Similar results were obtained in three other experiments.

Discussion

The results presented here, in which a direct comparison has been made between the effect of DTT on histamine-induced contractile activity and its effect on responses elicited by a number of other spasmogens in the same strip of ileal smooth muscle provide strong evidence that DTT has a selective effect on H_1-receptor mediated responses. This confirms previous reports of a selective enhancement of H_1-receptor activity in rabbit aorta (Fleisch *et al.*, 1973; 1974; Carroll & Glover, 1977), colon (Glover, 1979) and guinea-pig ileum (Glover, 1979).

In guinea-pig ileal smooth muscle, DTT produced a marked increase in tissue sensitivity to histamine. Furthermore, DTT increased the potency of a range of different H_1-receptor agonists (relative potency rang-

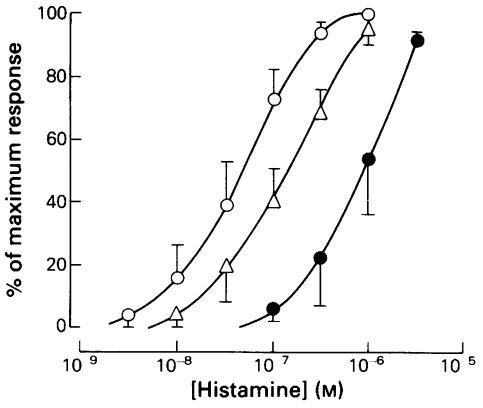

Figure 6 The effect of 1,4-dithiothreitol (DTT) on histamine-induced desensitization in guinea-pig ileal smooth muscle. Concentration-response curves for histamine were obtained: (○) under control conditions; (●) following 45 min exposure to a concentration of histamine equivalent to 100 × EC_{50}[− DTT] (~ 3 × 10^{-6} M) or (△) after subsequent exposure of the tissue to DTT (1 mM, 30 min preincubation) in the presence of the desensitizing concentration of histamine. Data are expressed as a percentage of the maximal response to histamine. Each point represents the mean of three experiments; vertical lines show s.e.means.

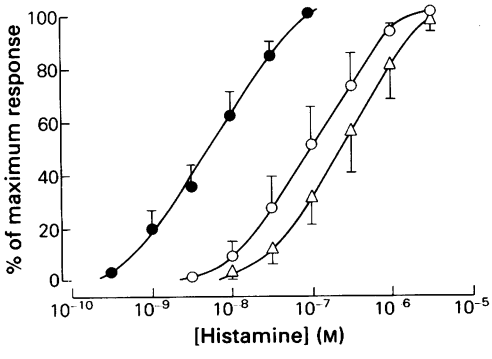

Figure 7 Desensitization of histamine-induced contractions of guinea-pig ileal smooth muscle following treatment with 1,4-dithiothreitol (DTT) (1 mM). (○) Control; (●) DTT (1 mM), 30 min preincubation; (△) desensitization following exposure (45 min) of the muscle strip to a concentration of histamine equivalent to 100 × EC_{50} [− DTT] (~ 3 × 10^{-6} M) in the presence of DTT. Data are expressed as a percentage of the maximal response to histamine. Each point represents the mean of three experiments; vertical lines show s.e.means.

ing from 5 to 105; histamine = 100) to the same extent as that of histamine. In contrast, the effect of this reducing agent on contractions elicited by acetylcholine, 5-hydroxytryptamine and KCl was minimal in comparison to the larger potentiation of histamine-mediated responses. This small increase in sensitivity to non-H_1-receptor stimulants was, however, significant. It seems likely that this non-specific effect is due to an interaction of DTT with either the smooth muscle membrane or the contractile apparatus and may be associated with the increase in spontaneous activity observed in the present study and that of Watson & Iversen (1982).

The evidence, presented here, for the selectivity of action of DTT on histamine H_1-receptors in guinea-pig ileum conflicts with the conclusions drawn in the studies of Watson & Iversen (1982) and Fontaine *et al.* (1984). It is unlikely that this discrepancy is due to the different tissue preparations used since we have obtained similar results in the two preparations (i.e. longitudinal smooth muscle and whole ileum). It should be pointed out, however, that the study of Fontaine *et al.* (1984) used a lower concentration of DTT (2.6 × 10^{-4} M) and employed single fixed submaximal concentrations of the different agonists. Thus, demonstration of a greater enhancement of histamine-mediated contractions by DTT may have been compromised under this experimental design. Furthermore, examination of the data presented by Watson & Iversen (1982) does show that although DTT (1 mM) potentiated the response to all agonists tested (confirmed in our study) there was a greater increase in sensitivity to histamine. Thus the shift in EC_{50} value to the left (log units) reported by these workers was 0.64 ± 0.08 (4.4 fold) for histamine and 0.31 ± 0.041 (2 fold) and 0.24 ± 0.04 (1.7 fold) for acetylcholine and KCl respectively. The experimental approach adopted in the present study, in making a direct comparison of the effect of DTT on responses elicited by histamine and a second spasmogen in the same strip of smooth muscle, has allowed us to demonstrate the significance of this observation. The importance of such an experimental approach is emphasised by the variability of the effect of DTT on histamine responses observed in different muscle strips (in 55 experiments the extent of the potentiation ranged from 3 to 32 fold).

Studies using the sulphydryl oxidizing agent DTNB suggested that the increase in tissue sensitivity to histamine is due to the ability of DTT to reduce disulphide bonds rather than another property of the molecule. DTNB alone had no effect on tissue responsiveness to histamine, but was able to reverse fully the potentiating effect of DTT. Furthermore, addition of DTT to ileal smooth muscle in the presence of an equivalent concentration of DTNB was without significant effect on the contractile responses elicited by

histamine. This suggests that tissue disulphide bonds are protected by the presence of an excess of disulphide bonds in DTNB. Thus a sulphydryl-disulphide exchange occurs between DTT and DTNB rather than between DTT and tissue disulphide bonds. That DTT probably acts by reducing disulphide bonds is substantiated by the findings of Fleisch *et al.* (1974) who noted that oxidized DTT was without significant effect in rabbit aorta.

The location of the disulphide bonds is as yet unknown. They may be situated at the level of the receptor or somewhere along the chain of events linking receptor occupancy by agonist, with stimulation of the contractile apparatus. Alternatively the potentiation of the histamine response may result from inhibition of histamine metabolism. However this explanation seems unlikely, since following blockade of both diamine oxidase and histamine-N-methyl transferase, with aminoguanidine and SKF 91488 respectively, only a small increase in histamine sensitivity was observed (3.8 ± 1.9 fold shift of the dose-response curve, $n = 5$) whereas subsequent addition of DTT to the tissue preparation produced a further 9.3 ± 2.2 fold potentiation of histamine responses ($n = 4$). Furthermore the response to the H_1-agonist 2-pyridylethylamine was not modified by enzyme inhibition but was potentiated by DTT to the same extent as histamine. Fleisch *et al.* (1973) have similarly discounted enzyme inhibition as a major determinant of the effect of DTT in rabbit aorta.

An additional way in which DTT may affect tissue responsiveness has been suggested by Siegel & Triggle (1980). They have reported that DTT prevents histamine-induced desensitization in guinea-pig ileum. Interestingly, DTT has also been shown to prevent the agonist-induced clustering of opiate receptors in neuroblastoma cells, which may be part of a desensitization mechanism (Hazum *et al.*, 1979). In the present study, however, DTT clearly did not prevent desensitization to histamine. This suggests that the observations of Siegel & Triggle (1980) are a result of the separate and opposing effects of DTT and desensitizing concentrations of histamine, rather than a consequence of interference with the process of desensitization by DTT.

It seems most likely that the enhancement of H_1-receptor activity by DTT in guinea-pig ileum is due to the reduction of disulphide bonds within the receptor protein or at some point along the receptor-effector pathway. The direct comparison of spasmogen responsiveness suggests that this point needs to be proximal to the stage at which the receptor-effector pathway is shared by responses elicited by acetylcholine, 5-hydroxytryptamine and KCl. In summary, the results of this study suggest that DTT may prove to be a useful tool with which to investigate histamine H_1-receptor mechanisms in ileal smooth muscle.

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