1,4-Dithiothreitol-induced changes in histamine H₁-agonist efficacy and affinity in the longitudinal smooth muscle of guinea-pig ileum

J. Donaldson & S.J. Hill¹

Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH.

- 1 The effect of 1,4-dithiothreitol (DTT) on histamine H₁-receptor agonist affinity and efficacy has been investigated in longitudinal muscle strips of guinea-pig ileum.
- 2 Exposure of ileal smooth muscle to DTT significantly increased the maximal responses to the partial agonists SKF 71473 and DE-2PEA, indicative of an increase in agonist efficacy. This effect was paralleled by a small decrease in EC₅₀ values. In contrast, DTT produced a parallel displacement of the concentration-response curve to the full agonist histamine in the same muscle strips.
- 3 Studies in which phenoxybenzamine and benzylcholine mustard were used to reduce the maximum response to histamine suggested that DTT altered both agonist affinity and efficacy. The affinity constant for histamine, calculated by the method of Furchgott & Bursztyn (1967), increased by 2.7 fold in the presence of DTT. Furthermore, agonist efficacy also appeared to increase in the presence of DTT since the maximum response to histamine following phenoxybenzamine treatment increased on application of DTT.
- 4 [³H]-mepyramine binding studies confirmed that DTT increased agonist affinity. DTT produced a significant parallel shift to the left of the displacement curves for histamine, 2-methylhistamine, 2-pyridylethylamine and 2-thiazolyethylamine.
- 5 The results of this study therefore suggest that DTT potentiates H₁-receptor-mediated contractile activity in guinea-pig ileum by increasing both agonist efficacy and affinity.

Introduction

The disulphide bond reducing agent 1,4-dithiothreitol (DTT) has been shown to potentiate histamine H₁-receptor-mediated contractile activity in both visceral (Donaldson & Hill, 1985a; 1986a) and vascular smooth muscle (Fleisch *et al.*, 1973; 1974; Carroll & Glover, 1977). This effect appears to be specific to the H₁-receptor since a similar potentiation of agonist activity is not observed with other smooth muscle spasmogens such as acetylcholine, 5-hydroxytryptamine and KCl (Fleisch *et al.*, 1973; Donaldson & Hill, 1985a; 1986a). The selective enhancement of H₁-receptor activity in rabbit colon and guinea-pig ileum does not, however, appear to be associated with an interference with histamine metabolism (Fleisch *et al.*, 1973; Donaldson & Hill, 1986a). These data suggest that the site of action of DTT is at the level of the H₁-receptor or at some point along the receptor-effector pathway proximal to the stage at which it is shared by

responses elicited by other smooth muscle spasmogens.

In guinea-pig brain slices, DTT has been shown to potentiate both the H₁-receptor mediated accumulation of inositol phosphates and the H₁-mediated augmentation of adenosine-stimulated cyclic AMP accumulation (Donaldson & Hill, 1986b). Studies with partial agonists in slices of guinea-pig cerebellum and cerebral cortex suggest that this potentiating effect of DTT is associated, in part, with an increase in H₁-agonist efficacy (Donaldson & Hill, 1986b). Furthermore, ligand binding studies with [³H]-mepyramine in homogenates of guinea-pig cerebral cortex and cerebellum indicate that DTT may also modify the binding affinity of agonists for the H₁-receptor in brain (Donaldson & Hill, 1986c).

The present series of experiments was undertaken to determine whether the potentiation of H₁-receptor mediated contractile activity in the longitudinal smooth muscle of guinea-pig ileum is related to

¹ Author for correspondence.

changes in agonist affinity, efficacy or a combination of these two agonist determinants.

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 guinea-pig ileum were prepared essentially as described by Rang (1964). Muscle strips were suspended in 10 ml Krebs-Henseleit solution (mM): NaCl 118, KCl 4.7, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25 and glucose 5.5; pH 7.4 gassed with O₂/CO₂ (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. Concentration-response curves for histamine and a partial agonist 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.

Experiments with irreversible antagonists

Benzilylcholine mustard was allowed to cyclise as a 0.1 mM solution in 10 mM Na-K phosphate buffer (7.6 mM Na₂HPO₄, 2.4 mM KH₂PO₄) pH 7.4, at room temperature for 60 min. The yield of the aziridinium ion under these conditions was expected to be greater than 90% (Burgen *et al.*, 1974). Phenoxybenzamine was cyclised in 0.9% saline for 60 min at room temperature at a concentration of 0.1 mM. Longitudinal smooth muscle strips of guinea-pig ileum were incubated in phenoxybenzamine (5 µM) for 30 min or benzilylcholine mustard (5 µM) for 40 min. Following treatment with the irreversible antagonist, muscle strips were washed with Krebs solution containing 1 mM sodium thiosulphate at 15 min intervals for 60 min before subsequent dose-response curves were determined. This agent reacts with any free aziridinium ions to form a biologically inert Bunte salt (Graham & Lewis, 1954).

Measurement of [³H]-mepyramine binding

Longitudinal smooth muscle strips from the ileum of two guinea-pigs were prepared in Krebs-Henseleit medium gassed with O₂/CO₂ (95:5), pH 7.4. Muscle

strips were initially cut into small pieces before homogenization in 5 volumes of 50 mM Na-K phosphate buffer (37.8 mM Na₂HPO₄, 12.2 mM KH₂PO₄), pH 7.4, with a Polytron blender (setting 5, 3 × 20 s periods at 2 min intervals). The homogenate was then centrifuged at 18,000 g for 10 min at 4°C. The pellet was washed by resuspension in phosphate buffer and recentrifuged at 18,000 g for 10 min. The resulting pellet was finally resuspended in 5 ml phosphate buffer and kept on ice prior to measurement of [³H]-mepyramine binding. The protein concentration of the final suspension was measured by the method of Lowry *et al.* (1951).

Aliquots of the freshly prepared membranes (40 µl, 0.3 to 0.5 mg protein) were added to 0.95 ml of 50 mM phosphate buffer, pH 7.4, and incubated at 37°C for 20 min in the presence or absence of DTT (1 mM). [³H]-mepyramine and, where appropriate, competing ligand were then added in 10 µl of phosphate buffer and the incubation continued for a further 35 min. Incubations were terminated by the addition of 4 ml of ice-cold phosphate buffer containing 1 µM non-radioactive mepyramine (Daum *et al.*, 1982) and filtered immediately through Whatman GF/B glass fibre filters. The filters were washed twice with 4 ml of ice-cold buffer containing 1 µM mepyramine and tritium was determined by liquid scintillation counting. The level of non-specific binding was defined as that insensitive to inhibition by 2 µM promethazine.

Data analysis

Concentration-response curves from individual experiments, 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:

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

where D is the agonist concentration, n is the Hill coefficient, EC₅₀ is the concentration of agonist giving half maximal stimulation and E_{max} is the maximal stimulation. Differences in the maximal stimulation (E_{max}) obtained with partial agonists, in the presence and absence of DTT, were analysed by means of a paired *t* test and the Wilcoxon signed rank test. Changes in EC₅₀ value were tested for significance as described previously (Donaldson & Hill, 1986a). Affinity constants for histamine were determined in contractile studies according to Furchgott & Bursztyn (1967) following partial alkylation of the H₁-receptor population with phenoxybenzamine. Curves of the specific binding of [³H]-mepyramine at different concentrations of the ³H-ligand were fitted using

ALLFIT. The equation was:

$$\text{Specific binding of } [^3\text{H}]\text{-mepyramine} = \frac{B_{\max} \times A^n}{A^n + (1/K_A)^n}$$

where $B_{\max}$ is the maximal specific binding site capacity (pmol g⁻¹ protein), A is the concentration of [³H]-mepyramine, K_A is the equilibrium affinity constant of [³H]-mepyramine and n is the Hill coefficient. Curves of inhibition of [³H]-mepyramine binding were also fitted to a Hill equation using ALLFIT. The actual equation fitted was:

$$\% \text{ inhibition of specific } [^3\text{H}]\text{-mepyramine binding} = \frac{100 \times I^n}{I^n + (IC_{50})^n}$$

where I is the inhibitor concentration, n is the Hill coefficient and IC_{50} is the concentration of non-radioactive inhibitor producing 50% inhibition of the specific binding of 1 nM [³H]-mepyramine. Each point was weighted according to the reciprocal of the variance associated with it. ALLFIT was used to test for differences in the binding parameters (IC_{50} or n), obtained in the presence and absence of DTT, by inspecting the effect on the residual variance of forcing them to be equal (Delean *et al.*, 1978). Such a constraint (parameter sharing) normally increased the residual variance in addition to the number of degrees of freedom (number of points minus the number of estimated parameters). The appropriateness of a particular constraint was then evaluated by analysis of the residual variances using the following statistical test (Munson & Rodbard, 1980):

$$F = \frac{(R_2 - R_1)/(df_2 - df_1)}{R_1/df_1}$$

where R_1 and R_2 are the residual sums of squares and df_1 and df_2 are the degrees of freedom associated with the original fit and the analysis with shared parameters respectively. F represents the variance ratio with $(df_2 - df_1)$ and df_1 degrees of freedom.

The affinity constants for inhibitors of [³H]-mepyramine binding were calculated from the IC_{50} values using the relationship: $K_A = ([M] \times K_M + 1)/IC_{50}$ where $[M]$ is the concentration of [³H]-mepyramine and K_M is its affinity constant, $1.4 \times 10^9 \text{ M}^{-1}$ (see results). In the special case of non-radioactive mepyramine this expression simplifies to $K_A = 1/(IC_{50} \cdot [M])$, assuming that the substitution of one atom of tritium for hydrogen has no effect on the affinity constant.

Drugs

[³H]-mepyramine (26 Ci mmol⁻¹) was purchased from the Radiochemical Centre, Amersham. Histamine dihydrochloride was obtained from BDH and mepyramine maleate and 1,4-dithiothreitol were obtained from Sigma. Gifts of 2-methylhistamine, 2-thiazolylethylamine (2-(2-aminoethyl)-thiazole), 2-pyridylethylamine (2-(2-aminoethyl)-pyridine), phenoxymethylamine, SKF 71473 (2-(2-aminoethyl)-imidazo- [1,2- α] pyridine) (all in hydrochloride form; Smith Kline & French), benzilylcholine mustard (Dr J.M. Young, University of Cambridge) and DE-2PEA (N,N-diethyl-2-(2-pyridyl)-ethylamine; Prof. D.A. Cook, University of Alberta, Canada) are gratefully acknowledged. Nuclear magnetic resonance (n.m.r.) analysis indicated that DE-2PEA was (2-pyridyl) rather than the (1-pyridyl) suggested originally (Kenakin & Cook, 1980).

Results

Contractile studies with partial agonists

DTT (1 mM, 30 min preincubation) produced a large parallel shift of the histamine concentration-response curve to lower agonist concentrations in longitudinal

Figure 1 Influence of 1,4-dithiothreitol (DTT) on the contractile activity elicited by SKF 71473 and histamine in guinea-pig ileal smooth muscle. Concentration-response curves for both histamine (○, ●) and SKF 71473 (△, ▲) were obtained in each longitudinal muscle strip in the presence (filled symbols) and absence (open symbols) of 1 mM DTT. Data are expressed as a percentage of the maximal response obtained with histamine in the absence of DTT. Each point represents the mean of six experiments; vertical lines show s.e. means.

Table 1 Effect of 1,4-dithiothreitol (DTT, 1 mM) on the concentration-response parameters for SKF 71473 and N, N-diethyl-2-pyridylethylamine (DE-2PEA)

Agonist	Maximum response (%) [†]		EC_{50} [−DTT]/ EC_{50} [+DTT]	
	Control	DTT (1 mM)	Agonist	Histamine (n)
SKF 71473	58.3 ± 7.6	98.2 ± 1.2*	3.7 ± 0.9**	7.6 ± 0.8** (6)
DE-2PEA	22.7 ± 6.4	71.3 ± 8.0*	5.7 ± 2.1**	10.1 ± 2.8** (6)

Values represent mean ± s.e.mean. ([†])The extent of the maximum response is expressed relative to the maximum response obtained with histamine (in the absence of DTT) in the same strip of ileal smooth muscle. *n* represents the number of paired determinations (agonist and histamine).

**P* < 0.01 paired *t* test; *P* < 0.05, Wilcoxon signed rank test.

***P* < 0.05 Wilcoxon signed rank test; EC_{50} in the presence of DTT compared to the EC_{50} of the control curve.

smooth muscle strips of guinea-pig ileum (Figure 1). This confirms our previous findings with DTT in this tissue (Donaldson & Hill, 1986a).

SKF 71473 (Durant *et al.*, 1973) acted as a partial H_1 -receptor agonist in ileal smooth muscle producing a maximal response which was $58.3 \pm 7.6\%$ ($n = 6$) of the maximal response elicited by histamine in the same smooth muscle strips (Table 1; Figure 1). This analogue was also less potent than histamine in these experiments. The EC_{50} for SKF 71473 was $6.3 \pm 1.7 \mu\text{M}$ while that for histamine was $0.5 \pm 0.1 \mu\text{M}$ ($n = 6$ in each case). Incubation of muscle strips with DTT (1 mM) resulted in a significant increase in the maximal response to SKF 71473 from 58% to a value close to 100% of the maximal response to histamine (Table 1; Figure 1). Thus, DTT-treatment effectively increased the efficacy of SKF 71473 and converted it from a partial agonist into a full agonist. The increase in maximum was accompanied by a significant decrease in the EC_{50} value (Table 1).

N,N-diethyl-2-pyridylethylamine (DE-2PEA, Ken-

Figure 2 Effect of 1,4-dithiothreitol (DTT) on contractile responses to histamine in guinea-pig ileum following partial alkylation of the H_1 -receptor population with phenoxylbenzamine. (a) Concentration-response curves for histamine were determined in the following order: (●) control; (○) phenoxylbenzamine (5 μM, 30 min); (Δ) DTT (1 mM). (b) Control experiments in which following determination of the concentration-response curve to histamine after phenoxylbenzamine treatment (5 μM, 30 min; ○), the tissue was allowed to recover in normal Krebs-medium (i.e. without the addition of DTT) for a further 30 min before determination of a third dose-response curve for histamine (▲). (●) Control dose-response curve obtained prior to phenoxylbenzamine treatment. Data are expressed as a percentage of the maximal response to histamine prior to phenoxylbenzamine treatment. Each point represents the mean from seven (a) or four (b) experiments; vertical lines show s.e.mean.

Table 2 Effect of 1,4-dithiothreitol DTT (1 mM) on the dose-response parameters of the histamine-induced contractile response, following treatment of ileal muscle strips with phenoxybenzamine or benzilylcholine mustard

Alkylating agent	Maximum response (%) [†]		$EC_{50} [-DTT]/EC_{50} [+DTT]$	(n)
	- DTT	+ DTT		
Phenoxybenzamine	59.9 ± 5.9	85.7 ± 4.4*	4.1 ± 0.7	(7)
Benzilylcholine mustard	44.5 ± 5.5	65.8 ± 5.6††	5.7 ± 1.3	(4)

Values represent mean ± s.e.mean. [†]The extent of the maximal contractile response is expressed relative to the maximum response produced by histamine before exposure to phenoxybenzamine (5 μ M, 30 min) or benzilylcholine mustard (5 μ M, 40 min). The number of experiments is showed in parenthesis.

* $P < 0.01$ (paired t test) or $P < 0.02$ (Wilcoxon signed rank test); †† $P < 0.01$ (paired t test).

akin & Cook, 1980) was also a partial H_1 -receptor agonist in this tissue. In our hands DE-2PEA was much weaker than SKF 71473, producing a maximal response which represented 23% of the maximum response to histamine, and an EC_{50} value of $25 \pm 8 \mu$ M (Table 1). In the presence of 1 mM DTT, there was a large increase in the size of the maximal response and a small decrease in the EC_{50} value (Table 1) similar to that found with SKF 71473.

Experiments with irreversible H_1 -receptor antagonists

The ability of the irreversible H_1 -antagonists phenoxybenzamine (Kenakin & Cook, 1976) and benzilylcholine mustard (Reasbeck & Young, 1975) to eliminate the spare receptor reserve and reduce the maximum response to full agonists provided an opportunity to investigate whether DTT potentiates histamine-induced contractile activity by increasing agonist affinity or efficacy.

Exposure of ileal strips to phenoxybenzamine (5 μ M, 30 min) resulted in a dextral shift of the histamine dose-response curve to higher agonist concentrations and a reduction in the maximal response (Figure 2). Following incubation with DTT (1 mM) there was a significant increase in the maximal response (Table 2) and a significant decrease ($P < 0.02$) in the EC_{50} value (Table 2; Figure 2a). In four experiments the histamine dose-response curve was redetermined after a 30 min incubation period in the absence of DTT. No change in the maximal response or location parameter was observed under these conditions (Figure 2b). Similar results were obtained when benzilylcholine mustard (5 μ M, 40 min) was used as the alkylating agent (Table 2).

The data obtained with phenoxybenzamine were used to calculate an affinity constant for histamine, in the absence of DTT, as described under methods (Table 3). In six experiments, muscle strips were treated with DTT (1 mM) before exposure to phenoxybenzamine (5 μ M, 30 min). DTT potentiated the histamine response, producing a 10.2 ± 0.9 sinistral shift of the concentration-response curve. Subsequent

treatment with phenoxybenzamine, in the continued presence of DTT, resulted in a dextral shift of the dose-response curve (70.5 ± 9.8 fold, $n = 6$) and a decrease in the maximal response to $49.3 \pm 8.2\%$ ($n = 6$) of the maximum obtained before treatment with the alkylating agent. Affinity constant determinations indicated that there was a significant increase in the affinity of histamine induced by DTT, when compared to the data obtained using the same alkylating agent in the absence of DTT (Table 3).

Effect of 1,4-dithiothreitol on [3H]-mepyramine binding

DTT had no significant effect on the total or non-specific binding (determined in the presence of 2 μ M

Table 3 Influence of 1,4-dithiothreitol (DTT) (1 mM) on the binding affinity of histamine for the H_1 -receptor in guinea-pig ileum.

Affinity constant (K_A , M^{-1}) for histamine deduced from studies of:		
	Contraction	[3H]-mepyramine binding
- DTT	$1.8 \pm 0.2 \times 10^5$ (7)	$5.7 \pm 1.0 \times 10^4$ (4)
+ DTT	$4.9 \pm 1.1 \times 10^5$ (6)	$12.5 \pm 0.4 \times 10^5$ (4)

Values represent mean ± s.e.mean. Affinity constants were determined either from contractile studies of muscle strips using phenoxybenzamine (5 μ M, 30 min) or from inhibition of the binding of 1 nM [3H]-mepyramine in homogenates of ileal smooth muscle as described under Methods. In binding experiments, affinity constants of histamine were measured in the presence and absence of DTT in each experiment. Determinations of these two affinities (+ or - DTT) from contractile studies were made in separate experiments. The number of experiments is given in parentheses.

* $P < 0.01$, unpaired t test and Mann Whitney U test.

†† $P < 0.01$, paired t test.

Figure 3 Effect of 1,4-dithiothreitol (DTT) on the binding of [³H]-mepyramine to a membrane preparation of guinea-pig ileal smooth muscle. (a) The binding of [³H]-mepyramine in the presence (O) and absence (●) of DTT (1 mM). The upper curve in (a) shows the total binding of [³H]-mepyramine and the lower curve represents the binding obtained in the presence of 2 μM promethazine. Values represent mean of five replicate determinations in a single experiment with s.e. mean shown by vertical bars. Where no error bars are shown the s.e. mean was within the size of the symbol. (b) Promethazine-sensitive binding of [³H]-mepyramine obtained in the presence (O) and absence (●) of DTT (1 mM). The curve drawn is the best-fit line to the control data obtained by non-linear regression using ALLFIT.

promethazine) of [³H]-mepyramine to homogenates of guinea-pig ileal smooth muscle, over the concentration range 0.5 to 10 nM (Figure 3a). The mean affinity constants obtained for [³H]-mepyramine in three such experiments were 1.45 ± 0.08 and $1.39 \pm 0.12 \times 10^9 \text{ M}^{-1}$, in the absence and presence of DTT respectively. There was similarly no significant

Figure 4 Effect of 1 mM 1,4-dithiothreitol (DTT) (O) on the inhibition of the specific binding of 1 nM [³H]-mepyramine produced by histamine (●) in ileal smooth muscle membranes. Values represent the combined mean of five replicate determinations made in each of four separate experiments with s.e. mean shown by vertical bars. In each experiment non-specific binding was defined using 2 μM promethazine. Curves were fitted to the data points using the non-linear regression programme ALLFIT, as described under Methods.

difference in the magnitude of the specific binding site capacity determined in the presence ($102.3 \pm 10.6 \text{ pmol g}^{-1} \text{ protein}$, $n = 3$) and absence ($100.3 \pm 9.9 \text{ pmol g}^{-1} \text{ protein}$, $n = 3$) of DTT (Figure 3b).

The lack of effect of DTT on the affinity of mepyramine in ileum was confirmed in studies of the inhibition of the specific binding of 1 nM [³H]-mepyramine by non-radioactive mepyramine. The affinity constants (derived from the IC_{50} values) for mepyramine in the presence and absence of DTT (1 mM) were $6.3 \pm 0.8 \times 10^8 \text{ M}^{-1}$ and $5.6 \pm 0.7 \times 10^8 \text{ M}^{-1}$ respectively. The slope of the mepyramine competition curve was not affected by DTT and remained close to unity (1.15 ± 0.07 and 1.10 ± 0.06 in the absence and presence of DTT respectively). This is the value expected for a simple and reversible interaction with a single class of binding sites.

Table 4 Effect of 1, 4-dithiothreitol (DTT, 1 mM) on the inhibition of specific [³H]-mepyramine binding by H₁-receptor agonists in guinea-pig ileal membranes

Agonist	Hill coefficient (n)		IC ₅₀ (μM)		IC ₅₀ ratio (A/B)
	[– DTT]	[+ DTT]	[– DTT] (A)	[+ DTT] (B)	
Histamine	0.87 ± 0.10	0.85 ± 0.09	60 ± 9	18 ± 3*	3.3 ± 0.7
2-Methylhistamine	1.13 ± 0.14	0.87 ± 0.10	172 ± 24	48 ± 6*	3.6 ± 0.7
2-Pyridylethylamine	1.07 ± 0.19	0.98 ± 0.13	228 ± 43	40 ± 6*	5.7 ± 1.4
2-Thiazolyethylamine	0.74 ± 0.08	0.70 ± 0.06	246 ± 33	35 ± 5*	6.9 ± 1.3

Values (mean ± s.e.mean) for the Hill coefficient (*n*) and IC₅₀ value were obtained from the combined data obtained in four (histamine) or three (other agonists) experiments, using ALLFIT. In each experiment a direct comparison was made between control (– DTT) and DTT (1 mM)-treated membranes.

**P* < 0.005 compared to control values (analysis of variance according to De Lean *et al.*, 1978).

Incubation of ileal smooth muscle membranes with DTT (1 mM) resulted in a small, but significant, parallel shift of the histamine displacement curve to lower agonist concentrations (Figure 4, Table 4). The apparent affinity constant deduced for histamine (after correction for competition with [³H]-mepyramine assuming a simple equilibrium with a single class of binding sites) was significantly increased following incubation with DTT and the values obtained agreed reasonably well with those determined from contractile studies (Table 3). Treatment of ileal membranes with DTT produced a similar parallel leftward shift of the inhibition curves obtained for three other H₁-receptor agonists, namely 2-methylhistamine, 2-pyridylethylamine and 2-thiazolyethylamine (Table 4).

Discussion

The results presented here confirm our previous observation that treatment of ileal smooth muscle strips with DTT potentiates H₁-receptor-mediated contractile activity in this tissue (Donaldson & Hill, 1986a) and suggest that this effect is due to an increase in both agonist affinity and efficacy.

A direct comparison of the H₁-receptor agonist properties of SKF 71473 or DE-2PEA with histamine in the same muscle strips showed that both of these analogues produced submaximal tissue responses (relative to histamine), indicative of partial agonism. Similar findings have been made previously by Durant *et al.* (1973) for SKF 71473 and by Kenakin & Cook (1980) for DE-2PEA. In the presence of DTT the maximal responses elicited by these two partial agonists were significantly increased. The maximal stimulation elicited by DE-2PEA was increased from 23% to 71%, while that produced by SKF 71473 was changed from 58% to 98% of the maximal response to histamine in the same muscle strips. These data

suggest that the efficiency of the receptor-effector coupling mechanism is increased in the presence of DTT. For SKF 71473 this change is sufficiently large effectively to transform it into a full agonist. Similar effects of DTT on partial agonist activity have been observed in slices of guinea-pig cerebral cortex and cerebellum (Donaldson & Hill, 1986b).

A change in agonist efficacy was also observed with the full agonist histamine in experiments in which the concentration of functional H₁-receptors was reduced with the irreversible antagonists phenoxybenzamine (Kenakin & Cook, 1975) and benzilylcholine (Reasbeck & Young, 1973). By reducing the receptor concentration, it is possible to reduce the efficiency of the receptor-effector system to such an extent that a maximal tissue contractile response is no longer attainable. Under these conditions the maximal response to histamine was reduced to 60% and 45% of that obtained before application of the alkylating agent, with 5 μM phenoxybenzamine (30 min) and 5 μM benzilylcholine mustard (40 min) respectively. Following the addition of DTT to the incubation medium the maximum size of these responses was significantly increased. This change in the extent of the maximum response could be due to a dissociation of the complex between the receptor and the irreversible antagonist (perhaps due to the unstable nature of the covalent bond) in the period following the scavenging of the free aziridinium ions with thiosulphate. However, the fact that no recovery from the effects of phenoxybenzamine was observed in control experiments in the absence of DTT makes this explanation unlikely. Furthermore, the similarity between the effects of DTT on the response to partial agonists (Table 1) and histamine (following treatment with an irreversible antagonist; Table 2) is striking.

Analysis of the effects of phenoxybenzamine on the response to histamine before and after prior treatment with DTT indicates that the effect of DTT on agonist efficacy may be accompanied by an increase in binding

affinity. Thus, the calculated affinity constant for histamine increased by a factor of 2.7 from $1.8 \times 10^5 \text{ M}^{-1}$ to $4.9 \times 10^5 \text{ M}^{-1}$ in the presence of DTT. However, there are difficulties in interpreting these values since the underlying assumption that the antagonist acts directly and irreversibly with only the receptor recognition site (Furchgott & Bursztyn, 1967) may not be entirely justified in the case of phenoxybenzamine. Kenakin & Cook (1975) have suggested that phenoxybenzamine may act allosterically to inhibit contractile activity on a site outside of the histamine receptor itself, in addition to producing alkylation of the receptor macromolecule. Studies in mouse neuroblastoma cells have indicated that this allosteric site might be a calcium channel (El-Fakahany & Richelson, 1981). This finding is supported by evidence obtained in rabbit aortic strips which shows that phenoxybenzamine can interfere with ^{45}Ca influx into smooth muscle cells (Shibata & Carrier, 1967). This complex interaction of phenoxybenzamine with the H_1 -receptor clearly complicates the estimation of agonist affinity constants, particularly since the possibility remains that the apparent effect of DTT on agonist affinity could be contributed to by an interaction between phenoxybenzamine and DTT at the level of the effector system (i.e. the calcium channel). However, even with these caveats, the similarity of the effect of DTT on the apparent affinity of histamine for the H_1 -receptor deduced from contractile studies and from inhibition of [^3H]-mepyramine binding is notable.

Treatment of membrane preparations of ileal smooth muscle with DTT altered agonist, but not antagonist, binding to a high affinity [^3H]-mepyramine binding sites in guinea-pig ileum. DTT did not modify the total or non-specific binding of [^3H]-mepyramine over the concentration range employed (0.5 to 10.0 nM ^3H -ligand). DTT was similarly without effect on the IC_{50} values or slope parameters determined from competition experiments involving non-radioactive mepyramine. These binding data confirm our previous observation in functional studies that DTT does not affect the affinity of mepyramine for the H_1 -receptor (Donaldson & Hill, 1986a). DTT did, however, produce a significant reduction in the IC_{50} values obtained for histamine and the H_1 -receptor agonists, 2-methylhistamine, 2-pyridylethylamine and 2-

thiazolyethylamine. However, in contrast to previous binding data obtained in guinea-pig cerebellum and cerebral cortex (Donaldson & Hill, 1986c) the effect of DTT on agonist IC_{50} values was not accompanied by a change in slope parameter (Hill coefficient). With the exception of the data obtained with 2-thiazolyethylamine, the slopes obtained for H_1 -receptor agonists were not significantly different from unity; the value expected for a simple equilibrium with a single class of binding sites. Thus, at least for histamine, 2-methylhistamine and 2-pyridylethylamine the data obtained with DTT are consistent with a simple change in agonist affinity similar to that observed in bovine aortic membranes (Carmen-Krzan, 1984). The values obtained for the affinity constant of histamine from binding studies are somewhat lower than those deduced from contractile studies, although as noted above there are problems in the calculation of these constants from functional studies. However, previous studies have shown that IC_{50} values for histamine obtained from binding studies in 50 mM Na-K phosphate buffer (used in this study) are in general higher than those measured in more physiological (Krebs-phosphate) buffers (Hill & Young, 1981).

The results of this study suggest that DTT potentiates H_1 -receptor-mediated contractile activity in guinea-pig ileum by increasing both agonist affinity and efficacy. It remains to be established whether these two effects are a consequence of common or different mechanisms. For example, one possibility is that DTT may increase the coupling between the receptor and a transducer molecule (e.g. a GTP binding protein) leading to both an increase in affinity for agonists and an increase in agonist efficacy. Alternatively, the increase in efficacy may be unrelated to the change in agonist affinity and arise from an effect of DTT on a more distal site in the chain of events linking receptor occupancy to the final contractile response. The selectivity of action of DTT on contractile responses to histamine, but not other smooth muscle spasmogens (Donaldson & Hill, 1986a), does however suggest that any separate effect of DTT on agonist efficacy is at an early stage in the stimulus-contraction process.

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