Previous workers [13, 14] have attributed the decreases observed in glucuronidation of *o*-aminophenol and testosterone by liver homogenates and liver slices from diabetic animals to a deficiency of UDP-glucuronic acid. It is possible that both mechanisms may contribute to the decreased glucuronidation of *p*-nitrophenol observed in the whole liver.*

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Department of Pharmacology
University Medical Buildings
Foresterhill
Aberdeen AB9 2ZD, U.K.

M. HELEN MORRISON
GABRIELLE M. HAWKSWORTH†

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† Author to whom correspondence should be addressed.

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1,4-Dithiothreitol non-specifically potentiates spasmogen actions on the guinea-pig ileum

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1,4-Dithiothreitol (DTT)* is commonly used to prevent the oxidation of biologically active materials (e.g. [1]) and to reduce disulphide bonds to sulfhydryl groups (e.g. [2]). This latter action has received application in the cleavage of a disulphide bond located on the nicotinic cholinoceptor at muscle end-plates, enabling the development of active site directed affinity labels [2]. This cleavage results in a decrease in the potency of acetylcholine, which on the frog rectus abdominus muscle is manifested as a 4–5 fold parallel shift to the right in the dose–response curve [3]. The disulphide bond can be reformed through treatment with dithio bis(2-nitrobenzoic acid) (DTNB) [2].

The action of DTT on other receptor systems has also received attention. Thus, it has been reported that treatment of the guinea-pig trachea with DTT increased the sensitivity of the tissue to all agonists tested, while in contrast, DTT treatment of the rabbit aorta produced a selective increase in sensitivity to histamine [4]. Similarly, Glover [5] observed a 4–6 fold increase in sensitivity to histamine in guinea-pig ileum and rabbit colon following treatment with DTT, but found no change in the potency of acetylcholine. Jordan and Owen [6] recently reported that DTT increased the depolarising potency of substance P on frog spinal cord *in vitro*. However, it is unclear whether these observations represent an action of DTT on the receptor recognition site analagous to that at the nicotinic receptor. Therefore, the present study was undertaken to characterise further the action of DTT, using the guinea-pig ileum as the model.

Male or female guinea-pigs (250–400 g) were stunned by a blow to the head and decapitated. The terminal ileum was dissected out and suspended in a 2 ml organ bath containing a modified Krebs bicarbonate solution (127 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 10 mM glucose), gassed with a 95% O_2/5% CO_2 mixture and maintained at a temperature of 37°. Contractions were recorded isotonically under a resting tension of 800 mg, and a 3 min drug cycle was employed with a 30 sec contact time. Following an initial 30 min equilibration period, the tissue was thrice challenged with a maximally effective dose of acetylcholine (1 mM) and left for a further 15 min. A dose–response curve to the agonist in question was then determined, followed by a 10 min incubation with DTT (1 mM). A second dose–response curve was then determined, and the tissue afterwards treated with DTNB (1 mM) for 30 min. Subsequently, a third dose–response curve was determined. Shifts in the curves were analysed using the EC_{50} value as the point of reference.

Stock solutions of agonists were made up in distilled water and stored at −20°. Dilutions were made on the day of the experiment in Krebs bicarbonate solution, which for the peptides had 0.1% bovine serum albumin added to

* Abbreviations used: DTT, 1,4-dithiothreitol; DTNB, dithio bis(2-nitrobenzoic acid).

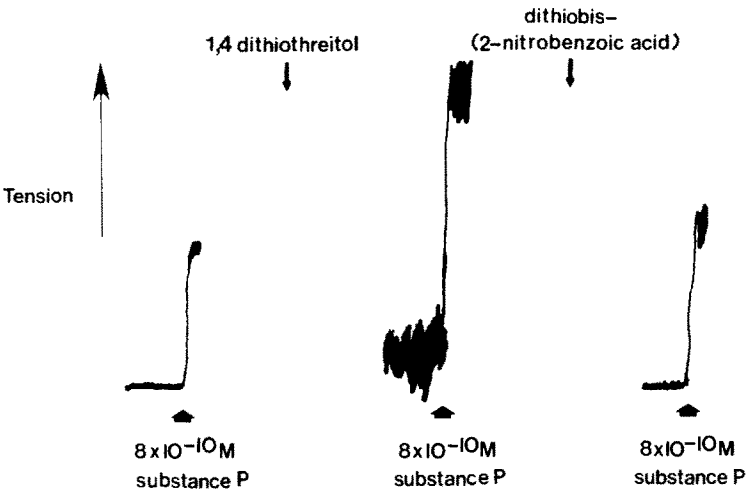

Fig. 1. A sample trace illustrating the effect of treatment with 1,4-dithiothreitol (1 mM) for 10 min on the response of the guinea-pig ileum to substance P (0.8 nM), and the reversal of the potentiation by treatment with dithio bis-(2-nitrobenzoic acid) (1 mM) for 30 min. Note the increase in spontaneous activity induced by 1,4-dithiothreitol and its reversal by dithio-(2-nitrobenzoic acid).

prevent their adsorbance to glass. Drugs were added to the organ bath in 20–100 μl volumes.

The following compounds were used: 1,4-dithiothreitol, dithio bis(2-nitrobenzoic acid), atropine sulphate, acetylcholine chloride and histamine disphosphate obtained from Sigma Chemical Co. (Poole, U.K.); substance P, eledoisin, physalaemin and bradykinin from Peninsula Lab. Inc. (San Carlos, CA) and phenoxybenzamine hydrochloride, kindly donated by Smith, Kline and French (Welwyn Garden City, U.K.).

Incubation with DTT (1 mM) for 10 min was found to produce a steady contraction, which was slow in onset and levelled at approximately 10–20% of the maximal response to acetylcholine. This contraction was also accompanied by an increase in spontaneous activity which remained on washout (Fig. 1). Treatment with DTT (1 mM) was found to increase the potency of all agonists tested, manifested as a parallel shift to the left in their dose–response curves (see Table 1, Fig. 2). Similar results were observed when a higher concentration of DTT was used (10 mM), or when a longer incubation period was tried (30 min); lower concentrations of DTT were not investigated. Other workers have obtained similar results (P. A. Gulliver, personal communication). The extent of the shifts in the response curves to histamine, acetylcholine, substance P and potassium chloride were compared using a one-way analysis of variance [7] and found to be significantly different ($P < 0.001$).

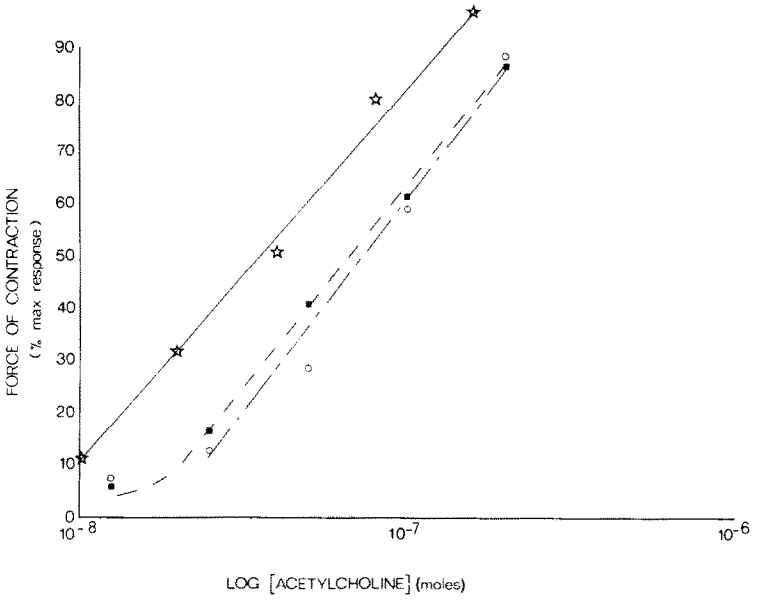

Fig. 2. Dose–response curves to acetylcholine on the untreated guinea-pig (○——○), the 1,4-dithiothreitol (1 mM, 10 min) treated ileum (★——★), and the dithio bis(2-nitrobenzoic acid) (1 mM, 30 min) treated ileum (■---■).

Table 1. Effect of exposing the guinea-pig ileum to 1,4-dithiothreitol (1 mM) for 10 min on the action of spasmogens

Compound	n	Shift in EC_{50} value to the left (log units)	S.E.	P
Histamine	6	0.640	0.081	<0.001
Acetylcholine	6	0.313	0.040	<0.001
KCl	6	0.238	0.038	<0.005
Substance P	6	0.341	0.036	<0.001
Eledoisin	3	0.338	0.059	
Physalaemin	2	0.190		
Bradykinin	2	0.164		

Results are expressed as log mean shift to the left in the dose–response curve using the EC_{50} as the point of reference. For eledoisin the experiment was carried out in the presence of 1×10^{-6} M atropine as the spasmogenic action of eledoisin is partially atropine sensitive [8]. Student's t-test was used to investigate whether the shifts in EC_{50} following DTT treatment were statistically significant.

The shift in the dose–response curve to histamine (4.4 fold) was approximately the same as that reported by Glover [5]. However, DTT was also found to produce a similar shift in the dose–response curve to acetylcholine, an observation not found by Glover [5]. There is no obvious explanation for this discrepancy.

The present study has demonstrated that DTT is able to increase the potency of a range of agonists on the guinea-pig ileum, an observation similar to that made on other tissues (see Introduction). The site and mechanism of this potentiation is unclear but is likely to result from the reduction of disulphide bonds in view of the reversal of potentiation by DTNB, and the observation made by previous workers that oxidised DTT is inactive [4, 5]. The location of the disulphide bonds is unknown but seems likely to be on the membrane surface rather than intracellular, as DTT has a rapid onset of action yet is predominantly charged as physiological pH. Perhaps the reduction of the disulphide bond facilitates ion channel opening perhaps by increasing membrane fluidity, receptor–ionophore coupling, etc. It is unlikely to be due to an action at the level of the receptor as suggested previously [4] in view of the potentiation of potassium chloride effects. Therefore, the present results suggest that caution should be adopted in interpreting the results obtained with DTT. For example, DTT has been used to prevent the oxidation of substance P in release studies [1], but it may be that a non-specific action of DTT modifies the amount of substance P released.

In summary, it appears that DTT causes a non-specific potentiation of the spasmogenic action of a number of agonists on the guinea-pig ileum. Similar observations have been made on other tissues, suggesting that disulphide bonds are of ubiquitous occurrence on cell membranes. Therefore, caution should be exercised in the use of DTT in experimental work.

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MRC Neurochemical Pharmacology Unit
Medical Research Council Centre
Medical School, Hills Road
Cambridge CB2 2QH, U.K.

STEPHEN P. WATSON*
LESLIE L. IVERSEN

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* Author to whom correspondence should be addressed.

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Lack of a gonadal or adrenal androgenic mechanism for the hypertrichosis produced by diazoxide, phenytoin and minoxidil

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Diazoxide, phenytoin and minoxidil are among a number of drugs of differing chemical structures which have been observed to sometimes cause hypertrichosis [1–8], occasionally of a severe degree. Their mechanism of action in this regard has not been elucidated. In view of the importance of androgens in hair follicle development, this possible influence was explored in the present studies.

The drugs were tested in three ways: (1) potency in stimulating testosterone secretion *in vitro* in rat testis alone, and in combination with human chorionic gonadotropin (hCG), (2) potency in stimulating adrenal androgen secretion *in vivo* in castrated dogs alone, and in combination with α^{1-24}-corticotropin (cosyntropin), and (3) ability to displace tritium-labeled testosterone from rat prostate cytosol androgen receptor.

Materials and methods

In the *in vitro* testis studies, 0.1, 1.0 and 10.0 μg of each drug were tested alone, and in combination with 0, 1 and 10 mU of hCG (Ayerst Pharmaceuticals, New York, NY), using an acute preparation of collagenase-dispersed [9, 10] rat testis cells from sexually mature Sprague–Dawley rats