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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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

of an average weight of 360 g, as modified from the method of Moger [11]. Incubations were run for 90 min at 37°, at which time testosterone was extracted for radioimmunoassay in 15 vol. of diethyl ether. Tritiated testosterone label was obtained from the New England Nuclear Corp. (Boston, MA), and an antiserum raised in New Zealand rabbits against 5-androsten-3β-ol-7,17 dione-7-CMO-bovine serum albumin (No. A8331), obtained from Steraloids, Inc. (Wilton, NH), was used at a dilution of 1:6500. Significant cross-reactions with this antiserum include: dihydrotestosterone (9%), androstenedione (0.8%), and dehydroepiandrosterone (0.015%). The testosterone radioimmunoassay was performed in 0.02 M phosphate-buffered saline, pH 7.4, with 0.1% gelatin added, at 4°. A 1:10 dextran–charcoal mixture was used for the separation of bound from free label. The level of sensitivity of the assay was 10 pg of testosterone. hCG was used as a positive control. The collagenase-dispersed testicular cell suspension was sensitive to 0.5 mU of hCG per incubation, which represented 14 mg of testis (wet weight).

For the *in vivo* studies, three adult male castrated dogs weighing 20–24 kg each served as experimental subjects and their own controls. Eighteen hours before an experiment, the dog was injected with 4 mg of dexamethasone intramuscularly (Hexadrol; Organon, NY). Immediately before an experiment the animal was lightly sedated with an intravenous infusion of 5% sodium thiopental, and two 18-gauge catheters were inserted in the fore- and hindlegs.

A combination infusion–withdrawal pump was connected to the two intravenous lines so that blood was withdrawn and normal saline was infused at the rate of 0.7 ml/min. Dexamethasone was also infused at the rate of 0.25 mg/hr. Blood samples were obtained every 10 min and placed in heparinized tubes. Plasma from each experimental run was frozen, and all samples from each run were assayed at the same time.

After a baseline period, the substance to be tested (diazoxide 50 mg, phenytoin 125 mg, or minoxidil 5 mg) was infused intravenously over a 3-min period. To test for a cooperative action with ACTH, a small amount of cosyntropin ($\alpha^{1\text{-}24}$-corticotropin; Cortrosyn; Organon, NJ), adequate to double the baseline level of cortisol, was given (0.5 mU) by intravenous bolus. The previous quantities of the substance to be tested were then given again with 0.5 mU of cosyntropin. Cortisol, A* and DHA were determined using an automatic analyzer (Auto-Assay, Inc., Salt Lake City, UT) after an initial neutral alumina column purification step for A and DHA. Antiserum for DHA was supplied by Auto Assay, Inc., and antiserum against A was produced as previously described [12]. The level of sensitivity of both assays was 10 pg.

Phenytoin (Dilantin) was manufactured by Parke-Davis (Detroit, MI) for intravenous use, diazoxide (Hyperstat) was manufactured by Schering (Kenilworth, NJ) for intravenous use, and Minoxidil (The Upjohn Co., Kalamazoo, MI) was extracted with ethanol from capsules and was purified using a Millipore Swinnex-25 type HA filter of 0.45 μm pore size.

For the rat prostate cytosol androgen receptor studies, sexually mature Sprague–Dawley rats were castrated under ether anesthesia, and were killed 36 hr later by cervical dislocation to maximize prostatic androgen binding [13]. The ventral prostates were removed and placed in 10 mM Tris–HCl buffer (pH 7.4 at 4°), 1.5 mM EDTA, 1 mM dithiothreitol, and 10% glycerol [14, 15]. The tissues were homogenized and, then, centrifuged for 1 hr at 100,000 *g* while at 4°. The supernatant fraction was used for the assays, which were performed for 2 hr at 4° [13]. Tritium-labeled testosterone was added to each assay tube along with 0.1, 1.0 or 10 μg of each drug to be tested. Bound and free testosterone were separated with a 1:10 dextran–charcoal mixture.

Results

Rat testis in vitro. The concentrations of phenytoin, diazoxide and minoxidil (0.1, 1.0 and 10.0 μg per incubation volume of 0.3 ml) produced no stimulation of testosterone production either alone or synergistically in combination with hCG.

Dog adrenal in vivo. Small increases in the concentrations of A and DHA occurred in response to $\alpha^{1\text{-}24}$-corticotropin, and responses of similar magnitudes were seen after an infusion of $\alpha^{1\text{-}24}$-corticotropin and each of the three substances tested. In contrast, no increase in steroid concentration was seen after each test substance alone, and there was no additive effect of any drug demonstrated in combination with $\alpha^{1\text{-}24}$-corticotropin. An example of the experimental protocol and results with minoxidil is shown in Fig. 1. Similar results were obtained with phenytoin and diazoxide.

Rat prostate cytosol androgen receptor. The concentrations of phenytoin, diazoxide and minoxidil used here (0.1, 1.0 and 10.0 μg per incubation volume of 0.3 ml) did not demonstrate any displacement of the tritiated testosterone label (Fig. 2).

Discussion

Androgens are an important influence on hair growth and probably determine the rate of hair growth throughout the hair cycle in the hair follicle. With respect to drug influences, histological examination has shown that diazoxide, which can cause hypertrichosis, stimulates an increased percentage of hairs to enter the anagen, or active growth, phase [2, 3]. Growth of vellus (lanugo) hair is stimulated to a greater extent than scalp hair by diazoxide, and the stimulation persists for several weeks to months. Phenytoin and minoxidil are also associated with hypertrichosis [5–8].

In hypertrichosis associated with diazoxide therapy, the excretion rates of 17-hydroxysteroids, 17-ketosteroids and testosterone glucuronide have been normal [16], but indi-

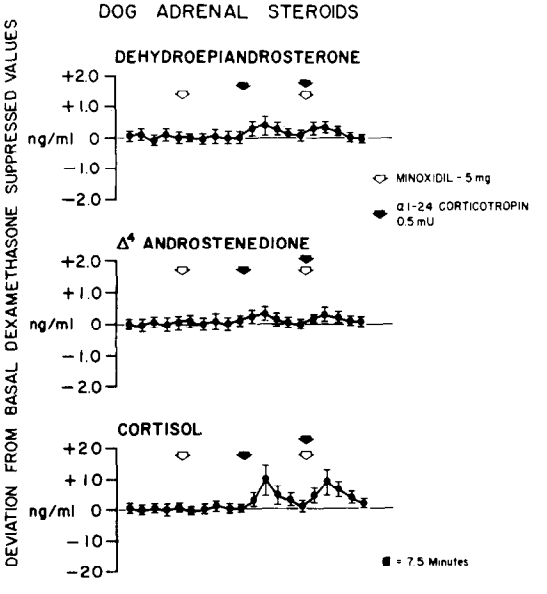

Fig. 1. Deviations of serum concentrations of DHA, A and cortisol from dexamethasone-suppressed values in response to minoxidil and $\alpha^{1\text{-}24}$-corticotropin.

* Abbreviations and common names: androstenedione (A): 4-androstene-3, 17-dione; and dehydroepiandrosterone (DHA): 3β-hydroxy-5-androsten-17-one.

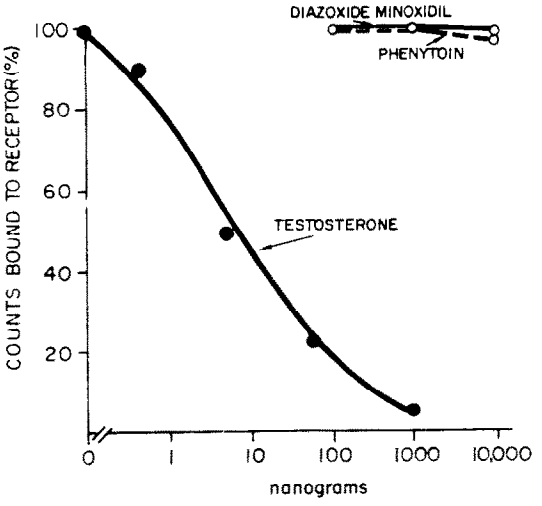

Fig. 2. Lack of displacement of labeled testosterone from rat prostate cytosol androgen receptor by phenytoin, diazoxide and minoxidil.

vidual urinary adrenal androgens, serum androgens, or androgen receptor binding were not studied. The possible interaction of phenytoin and minoxidil with androgen secretion or action has not been studied. In the present studies, phenytoin, diazoxide and minoxidil did not stimulate testosterone secretion, adrenal androgen secretion, or displacement of testosterone from the rat prostate cytosol androgen receptor.

It is not clear why the three drugs tested should occasionally stimulate profuse hair growth. An androgenic mechanism that would not be detected by the above methods is a drug-induced alteration of peripheral steroid metabolism by the hair follicle itself. Alternatively, a species difference in the side effects of these three drugs may exist between dogs, rats and humans. However, androgens, while the best understood, are not the only influences on hair growth, and the hypertrichosis induced by these drugs may be accomplished by a non-androgenic mechanism.

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