Effect of ethane 1,2-dimethane sulphonate on the cyclic AMP response in rat testis to stimulation by HCG

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Ethane 1,2-dimethane sulphonate (EDS: $\text{MeSO}_2 \cdot \text{OC}_2\text{H}_4\text{O} \cdot \text{SO}_2\text{Me}$) is a non-steroidal antifertility agent (Cooper & Jackson, 1970). In the rat a single i.p. dose of 75 mg/kg inhibits the production of testosterone by the testis (Bu'Lock & Jackson, 1971/72), the effect being maximal by 7 days after injection and completely overcome in 6–9 weeks (Bu'Lock & Jackson, 1975). While testicular steroidogenesis is depressed, serum and pituitary levels of LH are raised (Morris & Jackson, 1975), suggesting that failure of the testis to produce testosterone is accompanied by, if not actually due to, inability to respond to LH in the normal manner by increasing testosterone formation.

Small quantities of LH or HCG (< 1 ng) stimulate the production and release of cyclic adenosine 3',5'-monophosphate (cAMP) and of testosterone by the testis *in vitro* (Catt, Watanabe & Dufau, 1972; Cooke, van Beurden, Rommerts & van der Molen, 1972). In the present paper, we investigated the effect of EDS treatment on cAMP release by testicular tissue in response to added HCG, using the method described by Dufau, Watanabe & Catt (1973), who showed that release of cAMP into the incubation medium is much greater than the increase in tissue cAMP levels.

Male American Wistar rats (350–400 g) were killed by decapitation from 4 to 96 hr after administration of a single i.p. injection of EDS (75 mg/kg), made up in dimethyl sulphoxide (DMSO: Hopkin & Williams, Romford, England):water (1:3 v/v) or suspended in tricaprylin (trioctanoin: Fluorochem, Dinting Vale, Derbyshire, England). Control animals received 0.7–0.8 ml vehicle alone. The incubation procedure was similar to that of Dufau *et al.* (1973). Testes were removed, weighed, decapsulated and preincubated for 1 hr in 1.4 ml Krebs–Ringer–bicarbonate (KRB) buffer, containing 1 mg glucose/ml. After addition of 1 i.u. HCG (Sigma; or Pregnyl: Organon) in 0.1 ml phosphate-buffered saline, containing 1 mg bovine serum albumin/ml and 0.5 ml 20 mM-theophylline (in KRB) incubations were continued for a further 2 hr. All incubations were at 33°C in 95% O_2 :5% CO_2 with shaking. After 2 hr, cAMP was measured in samples (25–100 μl) of the incubation medium by the radioisotope dilution assay with adenosine-3',5' monophosphate binding protein (Gilman, 1970), using kits supplied by Boehringer-Mannheim (Cat. No. 15290). Satisfactory removal of interfering materials from the incubation samples was achieved by precipitation with ethanol (4 vols); after centrifugation the supernatant, combined with one ethanol washing, was freeze-dried and stored at –20°C until assayed.

The results are shown in Table 1. The values for un-injected animals (0 hr) are comparable with those reported by Dufau *et al.* (1973), i.e. 0–10 and 400–500 pmol cAMP/testis before and after stimulation by HCG. Some difficulty was encountered in obtaining satisfactory vehicle-only controls. Testes from rats injected i.p. with aqueous DMSO 12 or 24 hr previously gave very variable values for basal and stimulated cAMP release, although by 48 hr a normal picture was again obtained. DMSO has been the usual injection vehicle for EDS (Cooper & Jackson, 1970) and it was employed in the present study for the 48 and 96 hr data since these control values were satisfactory. Tricaprylin was used as the vehicle for the shorter time periods; basal and HCG-stimulated cAMP release were within the normal range of 4 and 24 hr after injection, and below normal, but reproducibly so, after 12 hr. The results were expressed as pmol/testis rather than as pmol/g wet weight of tissue, since the various treatments produced slight changes in testicular weight (Table 1).

Despite the admitted imperfection of this procedure, the results in Table 1 clearly demonstrate that within 48 hr of EDS injection *in vivo* the capacity of testicular tissue to release cAMP when stimulated by HCG was almost abolished. The trend was also apparent at 4 hr after EDS treatment.

Table 1. Effects of ethane 1,2-dimethane sulphonate (EDS) *in vivo* on the release of cAMP (pmol/testis) from rat testicular tissue stimulated by HCG *in vitro*

	Period of EDS stimulation <i>in vivo</i> (hr)					
	0	4	12	24	48	96
Vehicle	0 (control)	Tricaprylin	Tricaprylin	Tricaprylin	DMSO	DMSO
Testicular weight (g)						
Vehicle only	1.80 ± 0.04	1.85 ± 0.11	1.72 ± 0.03	1.87 ± 0.09	1.78 ± 0.08	1.86 ± 0.03
Vehicle + EDS		1.84 ± 0.06	1.65 ± 0.03**	1.87 ± 0.05	2.00 ± 0.12	1.86 ± 0.03
Release of cAMP						
Vehicle only	10	10	10	10	10	10
Vehicle + HCG	520 ± 80	560 ± 90	260 ± 10	415 ± 130	290 ± 140	460 ± 90
Vehicle + HCG + EDS		310 ± 150	74 ± 10	150 ± 50	14 ± 10	24 ± 20
Effect of drug						
% (HCG + vehicle)		56	28***	36**	2**	4**

Values are means ± S.E.M. from a minimum of 5 experiments.

Significantly different from controls, ** $P < 0.01$; *** $P < 0.001$ (unpaired *t* test).

The fall in HCG response occurs much earlier than the decline in steroidogenesis following a similar dose of EDS; testosterone formation has fallen to only half its normal rate by 60 hr after EDS injection (Bu'Lock & Jackson, 1975). If these two processes are related, their differing rates may, at least in part, be due to the time required for the existing enzymes of steroid transformation to decay. They could also reflect the fact that the particular LH receptor sites controlling steroidogenesis have a much higher affinity for the hormone than do most of the sites which respond by producing cAMP (Moyle & Ramachandran, 1973).

Correlation of our observations with the effects of EDS in the intact animal is difficult, since although the HCG-stimulated release of cAMP from the interstitial tissue is a well-established phenomenon, cAMP is normally thought of as an intracellular mediator. Nevertheless, our results show that EDS interferes with the ability of testicular tissue to respond to LH, normally one of the most important stimulators of the testis, because of the failure to produce the cAMP mediator. The effect might be due to an interaction of EDS with the receptor sites of the interstitial cells, or to changes in the properties of the Leydig cell membrane similar to those following hypophysectomy, which also leads to loss of HCG-binding capacity (Catt, Tsuruhara, Mendelson, Ketelflegers & Dufau, 1974).

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