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SHORT COMMUNICATION

TEMPORAL CHANGES IN SERUM ANDROGEN AFTER TEMPORARY IMPAIRMENT OF LEYDIG CELL FUNCTION BY ETHANE-1,2,-DIMETHANE SULPHONATE

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SUMMARY

The administration of ethane-1,2-dimethane sulphonate (EDS) 75 mg/Kg to adult male rats produced a decrease in serum androgen level for up to 7 weeks. The maximum suppression of serum androgen, comparable to castration, was seen 24 h after administration followed by a gradual return to control levels at 7 weeks. Castration-like changes occurred in the ventral prostate and epididymis associated with testicular weight loss. Testis and ventral prostate weight but not epididymal weight had recovered by week 7.

INTRODUCTION

Ethane-1,2-dimethane sulphonate (EDS) a simple diester of methane sulphonic acid is a non steroidal antifertility agent [1]. The antifertility activity of other members of this series of drugs such as butane-1,2-dimethane sulphonate (myleran, busulphan) and methylene dimethane sulphonate can be attributed to their alkylating actions upon the rapidly proliferating spermatogenic epithilium [2]. However, the action of EDS appears to be unique in that the drug is relatively non-toxic and experiments have suggested that a major part of its action can be attributed to a direct action upon the Leydig cells. Following EDS, infertility is accompanied by a temporary involution of the seminal vesicles and ventral prostate [2] which can be protected by simultaneous administration of androgen or human chorionic gonadotrophin (H.C.G.) [3]. Testicular steroidogenesis *in vitro* and the ability of testicular incubates to produce cyclic AMP after stimulation by HCG are also impaired [4, 5]. After EDS the negative feedback signal upon the hypothalamo-pituitary axis is removed as indicated by elevated serum LH [6]. In spite of these effects induced by EDS which suggest Leydig cell impairment the temporal changes in serum androgen and their relationship to the size of the secondary sexual organs has not been documented. This paper will present these changes.

MATERIALS AND METHODS

Adult male Wistar rats 350–400 g at the time of injection were used. EDS was administered as a single intraperitoneal (i.p.) dose (75 mg/kg, 14.1 mg/ml.) in dimethylsulphoxide–water (1:3. V/V). As a control a single i.p. injection of the drug vehicle was injected. Adrenalectomised and castrated rats maintained upon 0.9% w/v sodium chloride for 3 weeks were also prepared. Five drug-treated and five control rats were housed together until the required time after treatment; 1 and 4 days, 1, 2, 3, 5 and 7 weeks. The rats were killed by stunning and decapitation. The blood was collected, serum prepared and then stored at −15°C until assayed. Body tissues were dissected and weighed.

Radioimmunoassay of serum androgen

The procedure for the radioimmunoassay of serum androgen(s) was similar to that described for testosterone by Etches and Cunningham[7]. The antiserum used against testosterone (550/3) was kindly provided by Dr. B. J. A. Furr. The antiserum cross reacted with 5α-dihydrotestosterone-66% and 5-androstene-3β,17β-diol-27% when compared to testosterone-100%. Five hundred or 100 μl of serum was extracted once with 10 vol. of analar diethylether and the extract used without further purification. The results are therefore to be considered in terms of testosterone which was used to construct the standard curve. This procedure extracted approximately 72% of added radioactive testosterone. All serum samples were assay in duplicate and recoveries performed upon each sample. Bound was separated from free testosterone by dextran coated charcoal absorption and estimated using liquid scintillation counting. Control and treated groups were always assayed within the same radioimmunoassay and the results were expressed as a percentage of that control value. The within assay coefficient of variation was 15%. For a more extensive description and evaluation of this assay see Etches and Cunningham[7].

Results are expressed as mean ± S.E.M. and statistical analysis of the results was carried out using the Mann Whitney U test (one-tailed).

RESULTS

The temporal changes in serum androgen and ventral prostate, testis and epididymal weights are presented in Fig. 1. EDS treatment did not significantly alter total body weight. Serum androgen in the untreated rats (4.94 ± 0.52 ng testosterone equivalents per ml, $n = 35$) was dramatically lowered by EDS. One day after a single dose of EDS the concentration of androgen in the serum fell ($P < 0.01$) to 19% of control values and were only slightly greater than the androgen concentration found in the adrenalectomised, castrated rat (0.44 ± 0.07, $n = 6$). Thereafter the level of androgen began to recover achieving control values seven weeks after treatment.

The androgen dependant organ the ventral prostate progressively lost weight after EDS reaching a nadir 2 weeks after dosing ($P < 0.01$) much later than that seen for serum androgen but recovered to the control values at a similar time, 7 weeks. Parallel changes were observed for the seminal vesicles results not presented here. The testis did

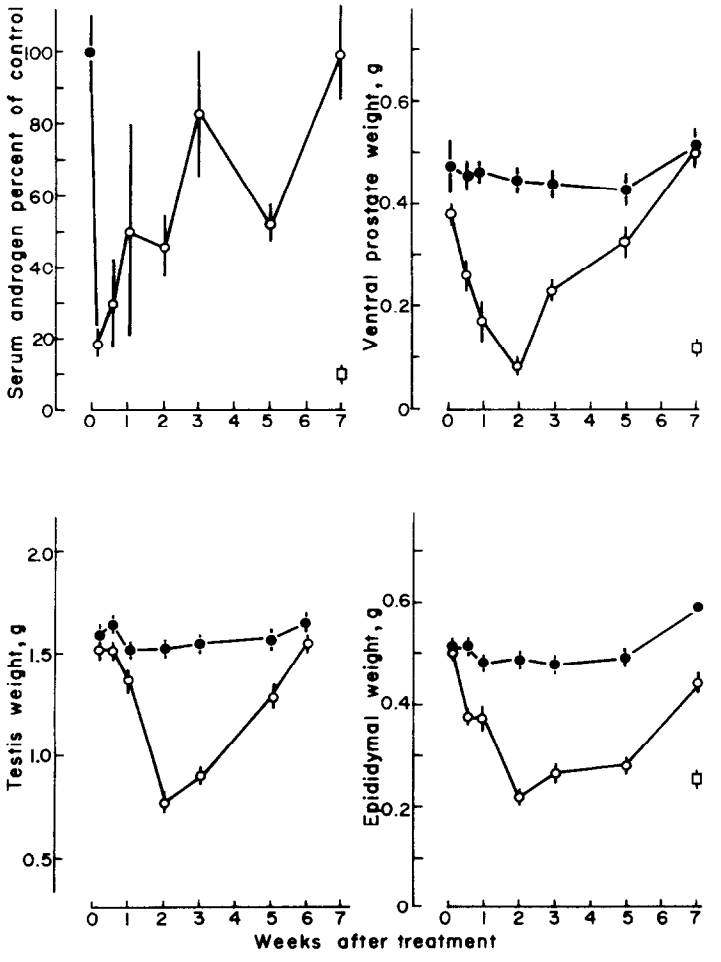

Fig. 1. Temporal changes in serum androgen expressed as a percentage of untreated rat value, ventral prostate weight, testis weight and epididymal weight after a single dose of ethane-1,2-dimethane sulphonate 75 mg/Kg I.P. Untreated rat closed circles, E.D.S. treated rat open circles. Three-week adrenalectomised castrated rat open square. Mean ± S.E.M. $n = 5$.

not show any significant weight changes until 1 week after EDS ($P < 0.05$). However, the maximal weight loss was also seen at 5 weeks similar to the weight loss in the ventral prostate and epididymis. The epididymis however had not regained the weight loss at 7 weeks ($P < 0.01$).

DISCUSSION

The results presented here clearly demonstrates that EDS impairs the ability of the testis to secrete androgen into the blood. The action of a single dose of EDS is immediate and similar to castration in that plasma androgen is almost totally absent (when compared to the adrenalectomised castrated rat serum) after 24 h [8]. However, unlike castration the effects are reversible as also reflected by the decline in organ weights and their recovery at 7 weeks. The failure of the epididymal weight to recovery is related to the period of infertility when sperm production has been arrested [3] and probably reflects the degree to which sperm contribute to the weight of this organ.

The results do not completely reflect the suppression of metabolism *in vitro* of pregnenolone to testosterone by testicular incubates. This impairment was only 75% of control values at day 1 and reached a maximum at days 7–18. However, by 7 weeks recovery is almost complete as the *in vivo* results would suggest [4]. The *in vivo* effects of EDS more closely reflect the fall in the testicular cyclic AMP response to HCG which declines almost immediately [5]. These observations are consistent with the histological observations of Leydig cell degeneration in which the membrane is disrupted [9]. As the LH receptor on the Leydig cell is associated with the membrane fraction [10] the ability of LH to drive steroidogenesis will be impaired, the preformed enzymes of steroid transformation taking longer to decay. In conclusion the results demonstrate the reversible suppression of testicular androgen secretion by EDS and also indicates that the *in vitro* steroidogenic studies do not accurately reflect testicular activity *in vivo*.

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