Ethacrynic Acid Hyperglycaemia in Mice

The hyperglycaemic and potential diabetogenic properties of benzothiadiazine derivatives have received much attention in recent years and several clinical and laboratory reports appear in the literature[1-4]. Ethacrynic acid, a diuretic chemically unrelated to the benzothiadiazines has been shown to produce hyperglycaemia in rats[5,6] but preliminary clinical investigation in a limited number of patients has not revealed any impairment of carbohydrate metabolism[7,8].

In this paper a comparison of the diuretic and hyperglycaemic properties of ethacrynic acid in mice is presented together with preliminary results which suggest a role for the adrenal medulla in the production of the hyperglycaemic response.

Material and methods. Female albino mice (Tucks No. 1) weighing 25–35 g for diuresis experiments and 30–40 g for experiments on hyperglycaemia, were used throughout.

(a) Diuretic effects. Mice were deprived of food, but given water ad libitum for 16 h before the experiment. An oral dose of 2.5 ml/100 g normal saline was administered to all mice simultaneously with the i.p. injection of the drug or saline (control). Urine was collected using metabolic cages (Mouse Metabowls, Jencons Ltd.). Urinary levels of sodium and potassium were determined using an Eel flame photometer.

(b) Blood glucose effects. The mice were allowed food and water ad libitum until the experiment. An initial blood sample of 0.1 ml was obtained from the femoral vein of each mouse under light ether anaesthesia, the skin incision being closed with a single suture clip. Ethacrynic acid was injected i.p. or adrenaline i.m., a second blood sample being obtained 2 h or 30 min after injection, respectively. Parallel saline or solvent controls were used throughout.

Blood glucose was estimated colorimetrically by the method of HASELWOOD and STROOKMAN[9]. Liver glycogen was determined colorimetrically by the phenolsulphuric acid method[10] using 1 g samples of liver removed 2 h (ethacrynic acid) or 17 min (adrenaline) after treatment. Adrenalectomy or adrenal demedullation was carried out under ether anaesthesia through a single dorsal skin incision, the animals being used 1 and 3 weeks respectively after surgery. Thoroughness of demedullation was checked histologically.

Results. Figures 1 and 2 reveal that of the doses studied, 5 mg/kg produced the largest and most rapid water,

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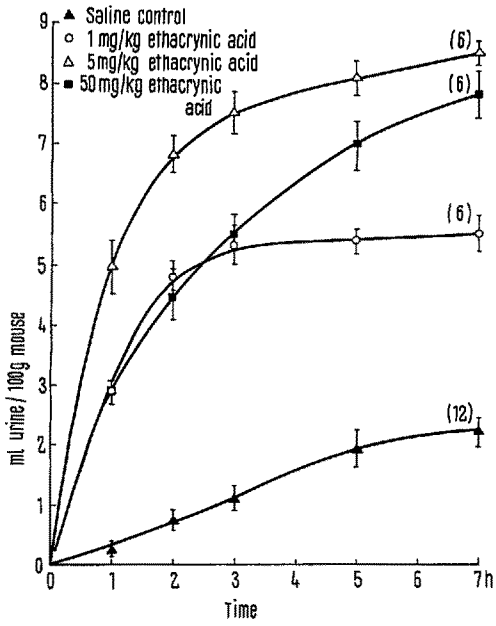

Fig. 1. Urine output in mice following saline load and either i.p. saline in the case of the controls or the dose of ethacrynic acid indicated. Figures in parentheses indicate the number of observations made, each from the combined output of 2 animals in 1 cage. Vertical bars represent the standard error of the mean.

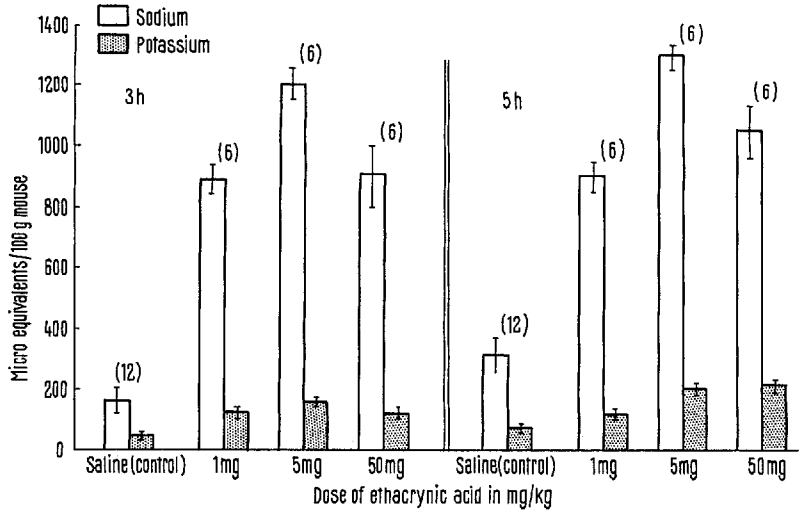

Fig. 2. Cumulative sodium and potassium output (in microequivalents/100 g mouse) at 3 and 5 h following saline load and either i.p. saline in the case of the controls or the dose of ethacrynic acid indicated. Figures in parentheses indicate the number of observations made, each from the combined output of 2 animals in 1 cage. Vertical bars represent the standard error of the mean.

sodium and potassium output. The dose used for the hyperglycaemic studies (50 mg/kg) actually produced a smaller diuretic effect than 5 mg/kg.

Figure 3 indicates the dose-effect relationship of ethacrynic acid in elevating the blood sugar.

Figure 4 shows the attenuation of ethacrynic acid induced hyperglycaemia by adrenalectomy and adrenal demedullation. The sham operative procedures did not affect the response. Daily treatment with cortisone acetate failed to restore the response in the adrenalectomized animal. Adrenaline hyperglycaemia was unaffected by adrenalectomy.

Liver glycogen in ethacrynic acid treated animals was 34.3 ± 4.0 mg/g compared with 50.4 ± 2.9 mg/g in saline control animals (sig. at $p < 0.001$). Adrenaline treated animals showed a liver glycogen of 40.6 ± 6.6 mg/g compared with 66.3 ± 5.9 in the control group.

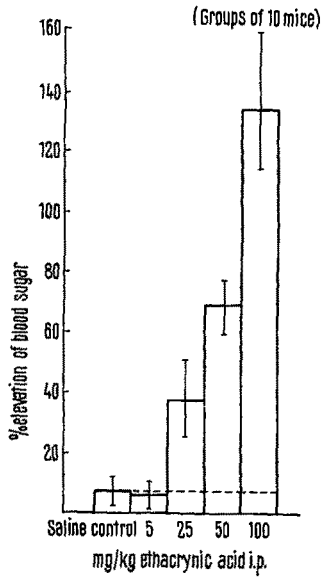

Fig. 3. The percentage elevation of blood sugar in mice 2 h after the i.p. injection of either 0.9% sodium chloride solution (control) or the dose of ethacrynic acid indicated. The elevation induced by saline is projected through the drug responses as a broken line and standard errors are indicated by vertical bars.

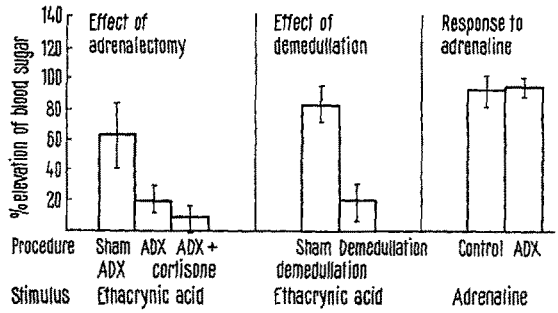

Fig. 4. The percentage elevation of blood sugar in mice 2 h after the i.p. injection of either 50 mg/kg ethacrynic acid, or 30 min after the i.m. injection of 0.05 mg/kg adrenaline. Groups of 10 mice were treated as indicated; vertical bars represent the standard error of the mean.

Discussion. From the results it is clear that ethacrynic acid is capable of producing hyperglycaemia and a reduction of liver glycogen in the mouse. However, there appears to be a separation, on a dose basis, of the hyperglycaemic and diuretic properties of this drug. A dose of 5 mg/kg, whilst producing a water and electrolyte output greater than a 50 mg/kg dose, did not produce any elevation of blood sugar 2 h after injection. The smaller diuretic effect of the 50 mg/kg dose may be similar to the phenomenon observed with mercurial diuretics[11] and probably indicates the threshold of toxic effects. Further work is necessary to show whether or not the pronounced sodium, potassium and water depleting effect makes any contribution to the production of the hyperglycaemia. However, ethycrynic acid can produce hyperglycaemia in the rat[5,6], in which species the drug has no diuretic properties[12].

The hyperglycaemic response is markedly attenuated by adrenalectomy, as has been previously demonstrated in the rat[6]. Adrenaline hyperglycaemia is unmodified by adrenalectomy, indicating that the carbohydrate reserves are adequate despite the absence of the adrenal glands. The failure of daily cortisone treatment to restore the response in the adrenalectomized animal together with the attenuation of the response by adrenal demedullation suggests that the adrenal medulla may play an important role in the production of the response. This is in contrast to diazoxide, the hyperglycaemic effect of which is reported to be fully restored in the cortisone treated, adrenalectomized animal[13] and is not attenuated by demedullation alone[14]. This suggests some dissimilarity between benzothiadiazine and ethacrynic acid induced hyperglycaemia.

Futher work is in progress on the role of the adrenal medulla in the production of the hyperglycaemic response and to establish whether or not lower doses of ethacrynic acid impair carbohydrate metabolism[15].

Résumé. L'administration de grandes doses de l'acide Ethacrynic par injection i.p. aux souris produit l'hyperglycémie. Les investigations préliminaires indiquent un rôle important pour la médulle surrénale dans la production de la réponse hyperglycémique.

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