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The effect of chlorthalidone on blood sugar and glucose tolerance in the rat

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Chlorthalidone, in high doses, failed significantly to influence blood sugar or intravenous glucose tolerance in the rat, following the intraperitoneal injection of single doses or 28 day oral treatment with the drug (100 mg/kg daily). The significant effect of the drug in stimulating glucose transfer in everted sacs prepared from rat intestine may explain the trend towards altered oral glucose tolerance observed. Chlorthalidone failed significantly to influence the blood sugar of alloxan diabetic rats and did not modify glucose uptake by rat-hemidiaphragm muscle or epididymal adipose tissue incubated *in vitro* in the presence or absence of insulin.

Chlorthalidone, like the benzothiadiazine diuretics, has been reported to produce an impairment of glucose tolerance in some patients (Reutter & Labhardt, 1961, Carliner, Schelling & others, 1965; Dobrzanski, 1969). In animal experiments, large doses of chlorthalidone when injected intraperitoneally have been shown to elevate the blood sugar (Tabachnick, Gulbenkian & Yannell, 1966; Foy, 1967; Wales, Grant & Wolff, 1968). However, the mechanism of this acute hyperglycaemic response has not been investigated and the relation between the effect in rats and the clinical findings has not been established. The experiments now described have been made to evaluate the significance of this action of chlorthalidone in producing hyperglycaemia in the rat. Some of the results presented were communicated to the British Pharmaceutical Conference (Furman, 1971).

METHODS

All experiments were made on male Wistar rats, 200–250 g. Glucose in blood or incubation-media was determined on 0·05 ml samples by the microcolorimetric copper-reduction technique of Haslewood & Strookman (1939).

Acute experiments

Blood glucose. Blood glucose was determined on blood samples removed from the femoral vein under light ether anaesthesia at 1 or 2 h intervals after the injection of chlorthalidone or control solutions.

Oral glucose tolerance. Rats were starved for 18–24 h and injected with chlorthalidone or control solution. Two h later, oral glucose tolerance was determined under pentobarbitone sodium anaesthesia (60 mg/kg, i.p.). The femoral artery and vein of one leg were cannulated and the animal heparinized intravenously (100 units/rat). A fasting blood sample was removed from the femoral artery and glucose (1g/kg) was administered via a stomach tube. Blood samples were removed at 15 min intervals up to 1 h after glucose administration.

Intravenous glucose tolerance. The procedure was essentially as described for "oral glucose tolerance" but, after removing a fasting blood sample, glucose (1g/kg:

2·0 ml/kg of a 50% solution) was injected into the femoral vein over 30 s. A blood sample was removed 3 min later and thereafter at 10 min intervals. The glucose excess above the fasting level (on a logarithmic scale) was plotted against time, the line being fitted freehand to the points, since there was usually a small scatter of the points about the line. The $t_{\frac{1}{2}}$ value (time for glucose excess to be reduced to 50%) was determined and the intravenous glucose tolerance constant, k, was determined (Amatuzio, Stutzman & others, 1953) using the formula

$$k = \frac{0{\cdot}693}{t_{\frac{1}{2}}} \text{ min}^{-1} \text{ or } k = \frac{0{\cdot}693}{t_{\frac{1}{2}}} \times 100\% \text{ min}^{-1}$$

Alloxan-diabetes. Alloxan diabetes was produced by the injection of alloxan (60 mg/kg) into a femoral vein under ether anaesthesia 48 h before using the rats.

In vitro *studies*

Hemidiaphragms. Hemidiaphragms from starved rats were incubated at 37° for 60 min in a shaking incubator bath according to the method of Vallance-Owen and Hurlock (1954) but using Krebs-bicarbonate buffer gassed with 5% carbon dioxide in oxygen. The glucose disappearance from the medium was determined and expressed as glucose uptake in mg/g wet weight muscle. Chlorthalidone was dissolved in sodium carbonate solution and diluted in buffer. An equivalent amount of sodium carbonate solution was diluted with Krebs for the control experiments. In some experiments, insulin (10^{-3} units/ml) was present in the Krebs solution.

Adipose tissue. Glucose uptake by pieces of epididymal adipose tissue was determined in a similar manner to that described above but using an incubation period of 2 h. In some experiments the effect of chlorthalidone was examined in the presence of insulin (10^{-3} units/ml).

Intestinal glucose transfer. Rats fed freely were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.) Everted intestinal sacs were prepared and incubated according to the method of Barry, Matthews & Smyth (1961) but using 25 ml incubating medium containing 5 mg/ml glucose in both mucosal and serosal fluids. The sacs employed were "2" and "3" (the jejunum and ileum being divided in five equal parts and numbered from the jejunal end), as these were the most active with respect to glucose transfer.

Twenty eight day oral treatment. Fasting blood glucose and intravenous glucose tolerance were determined in chlorthalidone treated (100 mg/kg daily by mouth for 28 days) and control solution-treated rats. Chlorthalidone was dissolved in the minimum quantity of N sodium carbonate solution and administered in the total daily ration of drinking water. Control animals received drinking water containing an equivalent concentration of sodium carbonate solution to that of the drug-treated rats. Each drug treated rat was weight-matched with a control animal and a pair feeding technique was employed to minimize the possibility that any effect of drug treatment was due to altered carbohydrate intake. Additionally, water intake was monitored to ensure that the rats received their daily dose of drug.

Drugs used

Chlorthalidone (Geigy) was prepared for injection by dissolving it using the minimum of N sodium hydroxide solution which for the highest dose injected (200 mg/kg–2 ml/kg of a 100 mg/ml solution) required a solution of pH 12·0. Control solutions

of the same pH were used throughout. Because of the possibility of degradation of chlorthalidone in these very alkaline solutions, solutions of the drug were examined using ultraviolet spectroscopy (Pulver, Wirz & Stenger, 1959). None of the alkaline degradation product was detectable.

We had found that the largest dose of chlorthalidone used (200 mg/kg) produced a significant diuresis and natriuresis in saline-loaded rats, thus demonstrating that, under the conditions employed, the drug was capable of exerting its pharmacological actions. The doses of chlorthalidone used (5–200 mg/kg) were maximal or supra-maximal in relation to the production of a natriuretic effect, according to published figures (Timmerman, Springham & Thoms, 1964).

Other drugs used were alloxan monohydrate (B.D.H.), bovine insulin (Calbiochem), pentobarbitone sodium (Nembutal, Abbott).

Statistical significance was assessed by the use of Student's *t*-test or, where appropriate, analysis of covariance, significance being accepted where $P < 0{\cdot}05$.

RESULTS

Blood sugar studies

The administration of chlorthalidone (200 mg/kg, i.p.) to rats fed freely produced a slight and statistically insignificant reduction in blood sugar when determined at 1 h or 2 h after injection. Lower doses (5 and 100 mg/kg) produced no effect (Fig. 1).

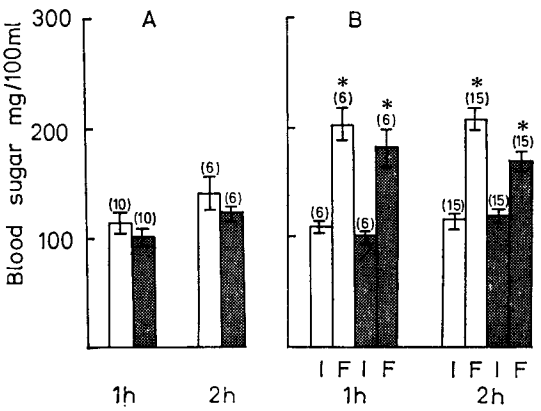

FIG. 1. Effect of chlorthalidone (200 mg/kg, i.p.) on the blood sugar of rats, fed freely, at 1 or 2 h after injection. In experiments A, blood was removed from the rats at 1 or 2 h after injection. In experiments B, a pre-treatment blood sample was removed under ether anaesthesia and a further blood sample removed at 1 h or 2 h after injection. Open columns refer to blood sugar in control rats (± s.e.) Shaded columns refer to blood sugar in chlorthalidone treated rats (± s.e.) I refers to pre-treatment blood sugar values. F refers to post-treatment blood sugar values. The number in brackets indicates the number of observations.

* Represents a statistically significant difference between pre- and post-treatment values.

Chlorthalidone (200 mg/kg) produced a qualitatively variable effect on fasting blood sugar, ranging from a statistically significant decrease in one series of experiments (Fig. 3) to a small and insignificant increase. In animals fed freely from which a pre-treatment blood sample had been removed under ether anaesthesia, blood sugar in both chlorthalidone (200 mg/kg, i.p.) and control-solution treated rats, was significantly higher than the pre-treatment value at 1 and 2 h. In an experiment in which the

effect on blood sugar of the injection of the alkaline saline solution was compared with that of 0·9% sodium chloride solution, the alkaline saline itself produced a significant rise in blood sugar compared with the control saline solution (control values pre and post treatment were 98 ± 10 mg/100 ml; and in alkaline saline-treated rats 185 ± 20 mg/100 ml; n = 10). This suggests that the rise in blood sugar produced by chlorthalidone dissolved in alkaline saline is due to the solvent, rather than to the drug itself. This effect is likely to be related to the irritant effect of the alkaline solution since a brief but marked writhing response was observed following the administration of drug or alkaline saline control solution. Examination of the peritoneal cavity showed inflammation of the serosal surface of the lower part of the gastrointestinal tract together with blood stained exudate.

Blood sugar in alloxan diabetic rats measured 2 h after the injection of chlorthalidone (200 mg/kg, i.p.) was 554 ± 58 mg/100 ml (n = 6) compared with a value of 592 ± 27 mg/100 ml (n = 6) in alloxan diabetic rats receiving alkaline saline. The difference was statistically insignificant.

Oral glucose tolerance

Fig. 2 shows that 15 min after glucose administration (1 g/kg, orally) the increase in blood sugar above the fasting level in chlorthalidone-treated rats (200 mg/kg, i.p. 2 h

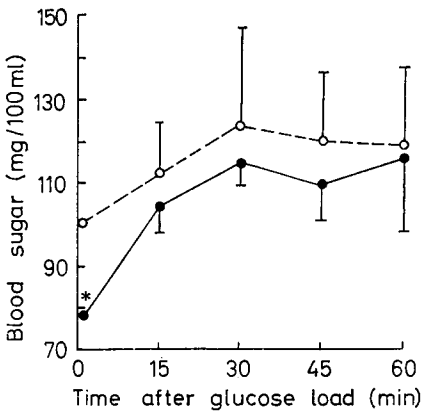

FIG. 2. Effect of chlorthalidone (200 mg/kg, i.p. 2 h beforehand) upon oral glucose tolerance. Each point represents the mean (± s.e.) of six observations. – – ○ – – Control. –●– Chlorthalidone-treated. * Indicates a statistically significant difference between drug and control values.

beforehand) was almost twice that seen in control solution-treated animals although analysis of covariance showed the difference to be insignificant. However, in this experiment fasting blood sugar in chlorthalidone-treated rats was significantly lower than in animals receiving the control solution ($P < 0{\cdot}05$).

Intravenous glucose tolerance

Chlorthalidone (5 or 200 mg/kg) produced no significant effect upon intravenous glucose tolerance determined 2 h after drug administration, although there was a trend towards a decrease in the k value in animals receiving the high dose of the drug (Fig. 3). It is of interest to note that the k values in animals receiving the high dose of chlorthalidone, or appropriate control solution, are considerably lower than the k

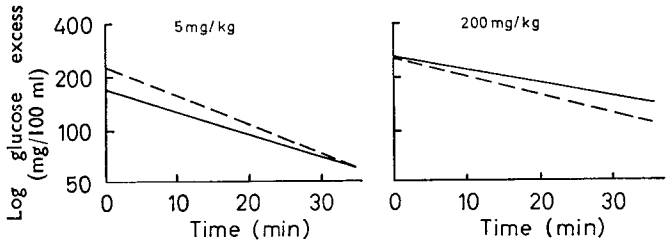

FIG. 3. Effect of chlorthalidone (5 or 200 mg/kg, i.p. 2 h beforehand) upon intravenous glucose tolerance. – – – Control; —chlorthalidone treated, K values for the control and 5 mg/kg dose were 3·7 ± 0·4 and 0·3 ± 0·3 (n = 5); K values for control and 200 mg/kg dose were 2·5 ± 0·1 and 2·1 ± 0·4 (n = 9).

values determined in animals receiving the low dose of chlorthalidone or appropriate control solution.

In vitro studies

Glucose uptake by muscle or adipose tissue. Chlorthalidone ($1{\cdot}5 \times 10^{-5}$M, 5 μg/ml to $0{\cdot}6 \times 10^{-3}$M, 200 μg/ml) produced no significant effect upon glucose uptake by rat hemi-diaphragm muscle or epididymal adipose tissue. The highest concentration used also failed to modify the insulin-stimulated glucose-uptake of either tissue.

Intestinal transfer of glucose. Chlorthalidone (1×10^{-3}M) produced a significant increase in the mucosal to serosal glucose transfer in sac 2 but not in sac 3: glucose transfer, mg/100 ml of fluid transferred per g empty sac was for control sac 2: 463 ± 25 and sac 3:669 ± 147 (n = 6). With chlorthalidone, 10^{-3}M, it was respectively 701 ± 92* and 635 ± 69 (n = 6) (*$P = <0{\cdot}05$).

Twenty eight day oral treatment. Chlorthalidone in a daily oral dose of 100 mg/kg produced no detectable effect on fasting blood sugar or intravenous glucose tolerance when compared with pair-fed control-treated animals. (k [control] = 4·6 ± 0·4; k [chlorthalidone-treated] = 4·7 ± 0·8; n = 5 in each group).

DISCUSSION

These experiments demonstrate that chlorthalidone has no significant effect upon blood sugar (except in one experiment in which a decrease was observed) when administered acutely by intraperitoneal injection.

As both solutions of chlorthalidone, of sufficient concentration to administer the high doses employed in some experiments, and the appropriate control solutions were very irritant, the irritant effect must be attributed to the large amount of alkali required to dissolve the chlorthalidone. The observation that the alkaline saline control solution produced a marked hyperglycaemic response when compared with a normal saline control solution confirmed that the irritant action of the solutions was responsible for the marked hyperglycaemia observed in both drug and control-treated animals.

The discrepancy between these results and those of previous workers, who have reported a hyperglycaemic reponse to chlorthalidone in the rat, is difficult to explain.

Although chlorthalidone did not exert any significant effect upon oral glucose tolerance, it is attractive to suggest a relation between the trend towards larger post-glucose-administration increases in blood sugar in chlorthalidone treated rats, with

the observed effect of the drug in stimulating glucose transfer in the everted intestinal sacs. The concentration of chlorthalidone used in these experiments could possibly be achieved *in vivo* following the intraperitoneal injection of large doses. The interpretation of the effects of chlorthalidone upon oral glucose tolerance is rendered difficult by the possible influence of the drug upon the gastrointestinal absorption of glucose. However, the failure of the drug significantly to modify intravenous glucose tolerance further suggests that the drug is without effect on carbohydrate metabolism. The possibility that a compensatory increase in insulin secretion was masking some effect of the drug on blood sugar or glucose tolerance is unlikely, in view of the failure of the drug to significantly influence blood sugar in alloxan-diabetic rats or to modify the *in vitro* glucose uptake by hemi-diaphragm muscle or epididymal adipose tissue.

The failure of chlorthalidone to influence blood sugar or glucose tolerance in the rat, following the acute or 28 day administration of large doses, does not necessarily indicate that chlorthalidone is not a "diabetogenic" diuretic. It may simply serve to demonstrate that the administration of diuretic drugs to the normal rat does not provide a useful model for human diuretic-induced or precipitated diabetes mellitus. Moreover, these results emphasize the care required in the interpretation of elevations in blood sugar seen after the acute intraperitoneal injection of diuretic drugs or indeed, any other substance.

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