RESEARCH PAPERS

THE EFFECT OF HORMONES AND THEIR ANALOGUES UPON THE UPTAKE OF GLUCOSE BY MOUSE SKIN *IN VITRO*

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An experimental system is described for measuring the uptake of glucose by mouse skin *in vitro*. Insulin is found to stimulate and hydrocortisone to depress the glucose uptake of this system when added to the medium in concentrations comparable to those which may occur *in vivo*. Oestrogens, androgens and progestational agents, in contrast, have no effect at approximately physiological levels, but cause some depression of glucose uptake at high concentrations.

OUR object was the development of an *in vitro* technique for studying the influence of steroid hormones upon an aspect of skin metabolism. A major contribution to this problem had previously been made by Bullough,[1] who developed a method for studying the effect of hormones upon the rate of mitosis in a preparation of the skin of the mouse. Employing this technique Bullough was able to demonstrate a stimulatory effect of insulin and of oestrogens, and an inhibitory effect of cortisone, upon the rate of mitosis in mouse ear epidermis. He ascribed the stimulatory effects to direct intervention in the glucokinase system. A method based upon determination of mitotic activity, however, is not readily adaptable for routine screening of a large number of compounds. We therefore turned our attention to the measurement of glucose uptake of mouse skin, hoping thereby to develop an assay based upon a more convenient parameter.

Mouse ear shows a very slow rate of glucose uptake, and difficulty was experienced in the development of a method of preparation of the skin which would allow comparable samples of adequate size to be taken for test and control experiments. This was accomplished by mechanical chopping of the skin, by thoroughly mixing the strips of skin from a large number of animals, and by taking large replicate samples. This procedure took longer than would be acceptable with more delicate tissues, but no evidence was obtained that it led to a serious deterioration in response of the skin. A number of experiments were carried out using single pairs of flasks containing samples rapidly prepared in an oxygen atmosphere, but no alteration in sugar uptake or response to oestrone was observed.

EXPERIMENTAL

Mice

Male albinos of the Schofield strain aged 9 to 12 weeks were used. All had been employed five times previously for routine insulin assays. They were starved for 24 hours before use.

Procedure

(i) The mice were killed by breaking the neck, and the ears were removed and cut into strips 1 mm. wide with a mechanical tissue chopper (Hospital and Laboratory Supplies, Ltd.). Strips from 40 pairs of ears were bulked in ice-cold Krebs-Ringer phosphate buffer pH 7·4, and thoroughly mixed. Samples weighing 250 to 350 mg. were then removed, blotted on filter paper, weighed on a torsion balance, and transferred to eight 50-ml. conical flasks each containing 8 ml. of a solution of glucose (0·4 mg./ml.) in Krebs-Ringer phosphate buffer. 0·04 ml. of a solution of the material under test was added to four flasks, and 0·04 ml. of the solvent was added to the remaining four. Propanediol was used for steroids and 0·01N hydrochloric acid for insulin. The flasks were then incubated for four hours at 37° with lateral shaking in an atmosphere of air. Three 2-ml. samples of fluid were taken from each flask, pipetted into 15-ml. centrifuge tubes, and 1 ml. of 0·3N barium hydroxide solution and 1 ml. of 5 per cent w/v zinc sulphate heptahydrate solution were added. After centrifugation, 2 ml. of the supernatant solution was taken for glucose determination by the method of Somogyi[2], using Nelson's colour reagent[3].

(ii) A number of experiments were made in which the conditions used by Bullough and Johnson[4] were followed as closely as possible. Six mice were killed, the ears were chopped rapidly, and the strips were mixed in oxygenated saline. Two 100-mg. samples were rapidly weighed, and transferred to Warburg flasks containing 4 ml. of a medium identical with that used by Bullough and Johnson, except that it contained a lower glucose concentration of 0·4 mg./ml. The flasks were attached to manometers, gassed with oxygen, and incubated at 38° for 4 hours. Three 1-ml. samples were then removed from each flask for glucose assay.

Results

Glucose uptake under the conditions used was found to be approximately linear over 6 hours. The rate of uptake varied from experiment to experiment within the range 0·5 to 1·5 mg. glucose/g. wet tissue/hour. The method of calculation was as follows. For each experiment the ratio of the glucose uptake in each flask containing test material to that of each control flask was calculated; an experiment comprising four control and four test flasks would thus yield sixteen ratios. The ratios of similar experiments were then combined to give a single mean. Results are expressed in Table I in terms of the percentage alteration of glucose uptake at each concentration of test substance in the medium. The limits given are the standard deviations of the means. N is the number of ratios used in the calculation of the results.

No effect of insulin was demonstrable at a concentration of 0·1 μg./ml., but from concentrations of 1 μg./ml. upwards an increasing stimulatory effect on glucose uptake was observed.

Hydrocortisone and prednisolone both markedly inhibited sugar uptake, the effect being demonstrable at concentrations down to 0·01 μg./ml.

TABLE I

THE EFFECT OF ADDED SUBSTANCES ON THE GLUCOSE UPTAKE OF MOUSE EAR *in vitro* USING EXPERIMENTAL PROCEDURE (i)

Substance	Concentration μg/ml.	N*	Per cent change in glucose uptake
Insulin	100	16	+29·2 ± 5·4
	10	20	+19·8 ± 4·7
	1	16	+ 5·4 ± 3·9
	0·1	16	0
Hydrocortisone	10	32	−53·6 ± 6·6
	1	52	−47·3 ± 13·4
	0·1	24	−41·1 ± 16·9
	0·01	32	−11·2 ± 5·0
	0·001	16	0
Prednisolone	1	34	−42·2 ± 10·6
	0·1	16	−39·6 ± 2·7
	0·05	16	−27·2 ± 1·6
	0·01	37	− 5·2 ± 7·5
Cortisone	1	27	−26·5 ± 5·3
	0·1	4	−33·1 ± 3·3
Cortisone acetate	1	9	−30·4 ± 14·0
Desoxycorticosterone acetate	10	16	−29·0 ± 5·1
	1	32	− 7·2 ± 10·6
	0·1	28	0
4-Methylcortisone acetate	1	16	− 6·7 ± 5·5
2-Chloroprednisone acetate	1	4	−21·4 ± 5·6
	0·1	4	0
Oestradiol	10	16	−42·0 ± 18·6
	5	16	−10·7 ± 5·4
	1	96	0
Oestrone	10	20	−16·5 ± 7·9
	1	32	− 4 ± 5·8
	0·1	16	0
Testosterone	10	16	−19·9 ± 12·9
	1	16	0
Pregnenolone	10	32	− 2·8 ± 6·2
	1	16	0
Progesterone	10	32	− 6·2 ± 7·3
	1	16	0
Cholesterol	10	12	0
Glycyrrhetinic acid	10	16	− 6·6 ± 5·8

* N = Number of ratios used in calculating the results.

Cortisone and its acetate were also active. 2-Chloroprednisone acetate, 4-methylcortisone acetate, and desoxycorticosterone acetate showed inhibitory activity only at relatively high concentrations.

Oestrone, oestradiol and testosterone all inhibited glucose uptake at a concentration of 10 μg./ml., but showed little or no effect at lower concentrations. In addition, a further series of experiments showed that no effect of oestrone at a concentration of 1 μg./ml. was demonstrable when experimental procedure (ii) was used.

Progesterone and pregnenolone had a slight inhibitory effect at a concentration of 10 μg./ml. Glycyrrhetinic acid also had a slight inhibitory effect at this concentration, but cholesterol had no effect at a concentration of 10 μg./ml.

The following experimental compounds, not listed in the Table, showed no activity at concentrations of 1 or 10 μg./ml. 3β-Naphthol-Δ^2-*cyclo*penten-1-one-2-acetic acid: 21-acetoxy-16α-methylprogesterone: 16α-methoxydesoxycorticosterone: 1,2-dimethyloestradiol diacetate: 2-chloro-1-methyloestradiol dipropionate: 4-chloro-1-methyloestradiol dipropionate: 4-chlorotestosterone: 11β,20β-dihydroxypregn-4-en-3-one: 3β-hydroxy-6-methylpregn-5-en-20-one.

The last three compounds, at a concentration of 1 μg./ml., did not inhibit the action of cortisone at a concentration of 0·1 μg./ml.

Discussion

The glucose uptake of mouse skin *in vitro* under the conditions used is influenced both by insulin and by the glucocorticoids at concentrations similar to those found *in vivo*. Thus the normal concentration of hydrocortisone in peripheral blood lies within the range 0·02 to 0·23 μg./ml.[5], while mouse skin responds to concentrations below this range. The minimum concentration of insulin at which a response of skin is detectable is just above the upper limit of normal serum concentrations, which are believed to lie within the range 0·005 to 0·5 μg./ml.[6], but is not so high as to be of doubtful physiological significance. Thus these two hormones show effects *in vitro* in accord with their actions *in vivo*, and in agreement with Bullough's observations of their effect upon mitosis in skin *in vitro*. It is therefore of interest that similar agreement is not obtained with Bullough's observations of the effect of oestrogens. Whereas Bullough found that stimulation of mitosis was caused by oestrogen, we find that the only demonstrable effect on glucose uptake is an inhibitory one at high oestrogen concentrations. Our findings are more in accord with those of Shelley and Hurley[7], who found that implanted oestrogens had no demonstrable effect upon human skin.

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