MECHANISM OF ACTION OF ACTH AND HYDROCORTISONE ON TISSUE HEXOKINASE ACTIVITY

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Hydrocortisone and, to a greater degree, ACTH prevent the decrease in hexokinase activity in the muscles, heart, and liver of fasting rabbits. The liver glycogen content is unchanged after administration of hydrocortisone but falls after ACTH is given. It is postulated that the stimulant action of hydrocortisone on hexokinase activity is connected with activation of the pancreatic α-cells, while that of ACTH is connected with activation of the β-cells.

The complex character of the effect of ACTH and hydrocortisone on the tissue hexokinase activity has been described [6, 7], but the mechanisms of this effect are not clear. The writer has previously shown that in experimental pancreatic diabetes of varied etiology ("hunger diabetes," dithizone diabetes of different degrees of severity) the metabolic effect of ACTH differs from that of hydrocortisone. During starvation and moderately severe dithizone diabetes ACTH inhibits, while hydrocortisone stimulates, the development of hyperglycemia and hypercholesteremia. In severe dithizone diabetes, on the other hand, ACTH has the same action as hydrocortisone [5].

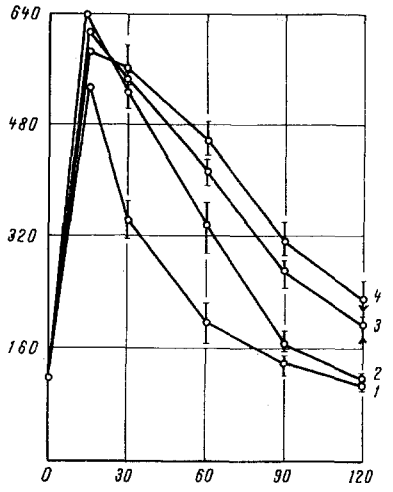

Fig. 1. Effect of ACTH and hydrocortisone on the character of the blood sugar curves of rabbits during starvation: 1) normal; 2) starvation + ACTH; 3) starvation + hydrocortisone; 4) starvation. Ordinate: blood sugar (in mg %); abscissa: time (in min).

The object of the present investigation was to examine the character of action of these hormones on tissue hexokinase activity and other metabolic indices, allowing for their possible effects on the incretory system of the pancreas.

EXPERIMENTAL METHOD

Hexokinase activity (of muscle and heart) and total phosphorylating power (of liver) were determined from the glucose absorption during incubation for 20 min, and expressed in μg sugar/mg protein [1]. To each test sample, 500 μg glucose was added. Sugar was determined in the protein-free filtrate by the Hagedron–Jensen method. Glycogen was determined by Seifter's anthrone reagent [12].

To test the pancreatic islet function of rabbits, blood sugar curves were plotted after intravenous injection of glucose (3 g/kg) under different conditions: normal, after starvation for 5 days, and after starvation for 3 or 5 days accompanied by administration of ACTH (8 units/kg/day) or hydrocortisone (8 mg/kg/day).

The state of the islet system was also studied under the microscope, after specific staining for α- and β-cells by the Gomori and Heidenhain methods, and staining for RNA by Brachet's method and for DNA by Feulgen's method.

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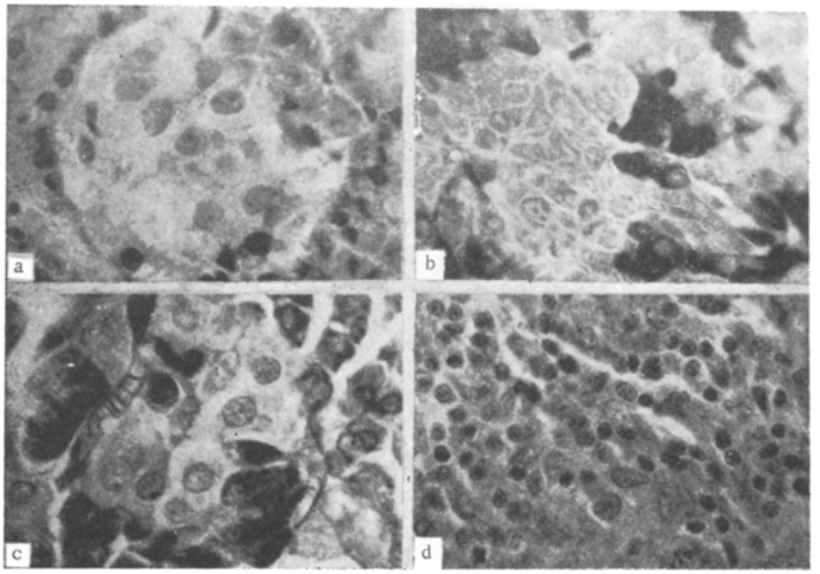

Fig. 2. Microscopic changes in pancreas after administration of ACTH (in units/day) and hydrocortisone (8 mg/day) for 12 days to rabbits with dithizone diabetes: a) islet of Langerhans after administration of hydrocortisone: nuclei of α-cells hypertrophied, β-cells destroyed (hematoxylin–eosin, 600×); b) pancreas, endocrine portion after administration of hydrocortisone. Reversion to simpler structure of acini, individual cells contain crimson granules, joined into a continuous mass (Heidenhain's method, 600×); c) islet of Langerhans after administration of ACTH and hydrocortisone: α-cells contain crimson granules (Heidenhain, 600×); d) pancreas after administration of ACTH: proliferation of cells with light and dark nuclei (hematoxylin–eosin, 600×).

TABLE 1. Effect of Hydrocortisone on Tissue Hexokinase Activity during Starvation (M±m, 6 experiments)

Experimental conditions	Muscles	Heart	Liver	Liver glycogen (in %)
Normal	26,8±2,03	35,7±4,2	27,1±6,9	5,3±1,49
Starvation for 3 days + hydrocortisone, 8 mg/kg/day	24,1±6,5	51,5±9,1	12,5±3,7	4,5±0,08
Starvation for 5 days + hydrocortisone, 8 mg/kg/day	30,3±2,3	32,8±5,6	8,0±1,7	5,3±0,42
Starvation for 5 days	11,6±5,7	11,5±4,6	4,0±2,3	0,7±0,08

EXPERIMENTAL RESULTS

During starvation of the rabbits for 5 days, a marked decrease in hexokinase activity was observed in the muscles, heart, and liver (Table 1).

This confirms the results of earlier work by other investigators [2-4]. This decrease evidently took place because of an increase in production of glucocorticoids and a decrease in insulin production during starvation [8-10]. As an energy-yielding material, glycogen was utilized rapidly until it disappeared completely from the liver. It could be postulated that the administration of hydrocortisone to fasting rabbits would inhibit the hexokinase reaction still further, or at most would leave it unaffected. However, the experimental results showed that hydrocortisone prevented the decrease in hexokinase activity in the muscles and heart, while the activity of the enzyme in the liver also remained high. The liver glycogen content was normal.

TABLE 2. Effect of ACTH on Hexokinase Activity in Tissues during Starvation (M±m; 6 experiments)

Experimental conditions	Muscles	Heart	Liver	Liver glycogen (in %)
Normal	26,8±2,03	35,7±4,2	27,2±6,9	5,3±1,49
Starvation for 3 days + ACTH, 8 units/kg/day	26,7±4,3	45,9±2,7	21,4±3,0	0,54±0,08
Starvation for 5 days + ACTH, 8 units/kg/day	30,0±3,4	42,6±3,6	9,6±3,3	1,01±0,12
Starvation for 5 days	11,6±5,7	11,5±4,6	4,0±2,3	0,7±0,08

These results can be understood if it is recalled that glucocorticoids stimulate glyconeogenesis in the liver. This accounts for the higher blood glucose level and its utilization by the tissues with bypassing of the hexokinase reaction. An increase in the blood glucose concentration itself is known to promote insulin secretion [11].

Determination of the action of ACTH on tissue hexokinase activity under precisely the same conditions as in the preceding case showed that ACTH has a stronger stimulant action than hydrocortisone, especially on hexokinase activity in the liver on the third day of starvation (Table 2).

It is interesting to note that administration of ACTH in the same dose to rabbits with severe dithizone diabetes, and kept on a normal diet, lowered the liver hexokinase activity on the 5th day to 7.9 μg/mg protein.

Differences between the action of ACTH and hydrocortisone on the insular apparatus were revealed particularly clearly by comparing the sugar curves obtained against the background of "hunger diabetes" (Fig. 1). These results suggest that the β-cells of the islets of Langerhans were stimulated to a higher degree by ACTH than by hydrocortisone. Both hormones evidently can stimulate insulin production, but the mechanism of their action differs. Hydrocortisone probably acts through stimulation of glyconeogenesis, while the action of ACTH is connected with a direct effect on the β-cells. Further evidence in support of this view was provided by microscopic analysis. The action of ACTH and hydrocortisone, separately and in combination, was studied in animals with dithizone (60 mg/kg) diabetes, in order to determine the potential function of the remaining β-cells. These experiments showed that hydrocortisone stimulates the α-cells, but not the β-cells, of the islets of Langerhans. As a result, the nuclei of the α-cells were hypertrophied (Fig. 2a), and the cytoplasm of the α-cells and of some of the excretory glandular cells was packed with crimson granules, characteristic of glucagon (Fig. 2b). Some acini showed reversion to a simpler type of structure. The combined action of hydrocortisone and ACTH led to a similar picture: accumulation of the characteristic secretion in the α-cells (Fig. 2c, d). This distinctive feature of hydrocortisone action, together with the stimulation of glyconeogenesis in the liver, evidently led to some degree of stimulation of the β-cells of the islets of Langerhans, and this would apparently explain the paradoxical character of action of the hormone on hexokinase activity in the muscles and heart. After administration of ACTH alone, proliferation of β-cells was observed, and they contained the appropriate secretion. In sections stained by Brachet's method intensive accumulation of RNA was observed in the cytoplasm of both β-cells and acinar cells.

It was thus concluded from analysis of the morphological data and their comparison with the biochemical changes that ACTH and hydrocortisone act differently on the α- and β-cells of the islets of Langerhans, and that this factor must be taken into consideration when the metabolic effects of both hormones are assessed.

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