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CHANGES IN SOMATOTROPHIC AND LACTOTROPHIC FUNCTIONS OF THE ADENOHYPOPHYSIS IN RATS WITH ACUTE ALLOXAN DIABETES

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By electrophoresis followed by colorimetric study of the stained gels an increased content of growth hormone and prolactin was found in the pituitary of rats during the development of alloxan diabetes. The STH and prolactin levels 4-5 days after injection of alloxan were higher by 45-58 and 38-43% respectively than in intact animals. Experiments on primary cell cultures using [^{14}C]-L-leucine as labeled precursor revealed increased secretory activity of the somatotrophs and lactotrophs of rats with alloxan diabetes.

KEY WORDS: alloxan diabetes; culture of adenohypophysis; secretion of growth hormone and prolactin.

Despite considerable progress in recent years in the study of the pathogenesis of diabetes many aspects of this problem are still far from understood. During the development of diabetes changes take place in the functions of several endocrine glands. These changes may be compensatory in character, but at certain stages of the disease they aggravate its course. The problem of whether the insular function of the pancreas can be controlled by the pituitary gland has not yet been solved. On the other hand, there is evidence that the pituitary plays a definite role in the pathogenesis of diabetes mellitus. Removal of the pituitary from dogs and other animals [12] has been shown to alleviate the course of experimental diabetes included by subtotal pancreatectomy. Meanwhile pituitary hormones such as growth hormone (STH) and prolactin, under certain conditions, can have a diabetogenic action [8].

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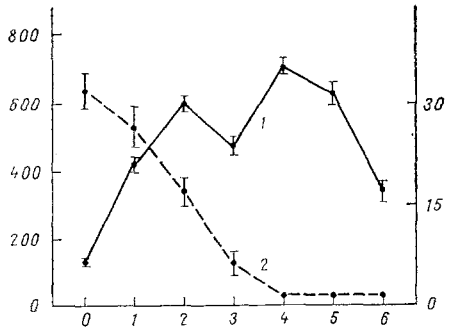

Fig. 1. Changes in glucose (1) and insulin (2) concentration in blood of rats with alloxan diabetes. Abscissa: time after injection of alloxan (in days); ordinate: left) glucose concentration, mg %, right) insulin concentration (in microunits/ml).

As regards the secretion of these hormones it is increased in diabetes mellitus complicated by ketoacidosis [10, 14]. A raised plasma STH level has also been found in patients with juvenile diabetes [13]. The plasma STH level has been shown to be increased in rats even in the early stages of alloxan diabetes [4]. Similar results were obtained in experiments on totally pancreatectomized dogs [9]. However, R. and B. Hazelwood [11] found no change in growth-stimulating activity in the pituitary at these stages of experimental diabetes. The character of changes in the lactotropic function of the pituitary in experimental diabetes remains virtually unstudied.

Changes in the STH and prolactin levels in the pituitary gland during development of alloxan diabetes in rats were investigated. Some differences in the secretion of these hormones in vitro in cultures of adenohypophyseal cells obtained from rats with alloxan diabetes also were studied.

METHODS

Male Wistar rats weighing 160-180 g were used. Diabetes was induced by intraperitoneal injection of alloxan in a dose of 180 mg/kg after starvation for 24 h. The blood glucose concentration was determined by the glucose oxidase method [1] and the plasma insulin concentration by a radioimmunological method using the kit from IRE-Sorin (France). Rats with glycemia exceeding 300 mg % were used. The STH and prolactin concentrations in the adenohypophyses were determined by the method of Kurts et al. [3]. The method of preparing the primary monolayer culture of adenohypophyseal cells was described previously [2]. The cell suspension ($8 \cdot 10^5$ cells/ml medium) was seeded into glass test tubes. The culture was grown in medium 199 containing 20% embryonic calf serum. After 3 days the medium was changed and at the same time [^{14}C]-L-leucine(Czechoslovakia) was added to the tubes for 24 h in a concentration of 5 μCi/ml. After washing to remove the isotope the cells were incubated for 3 h in nonradioactive medium. Labeled STH and prolactin were isolated from the incubation medium and cell homogenates by electrophoresis in polyacrylamide gel and the radioactivity of the samples was measured on a liquid scintillation counter (Intertechnique, France). Full details of the method were described previously [6].

RESULTS

Hyperglycemia was observed 24 h after injection of alloxan into the rats, and after 48 h the insulin level began to fall progressively (Fig. 1). On the 4th day after injection of alloxan the insulin concentration was below the threshold of sensitivity of the method used. The hyperglycemia remained steady at a high level. Typical features of diabetes (polydipsia, polyuria) were observed in the animals. The body weight and absolute weight of the adenohypophysis were lower in rats on the 4th and 5th days of alloxan diabetes than in intact animals, but the relative weight of the adenohypophysis (in mg/100 g body weight) was unchanged (Table 1).

Comparative data for the STH and prolactin concentrations in adenohypophyses taken from rats with alloxan diabetes and from intact animals are given in Table 2. On the 4th day after injection of alloxan the STH and prolactin concentrations in the adenohypophyses of the experimental rats were higher than in the intact animals by 45 and 38% respectively. On the 5th day this increase was 58 and 43% respectively. These results are in agreement with those obtained by Sadovnikova et al. [4], who found an increase in the concentration of immunoreactive STH in the blood plasma of rats on the 4th day of development of alloxan diabetes.

TABLE 1. Body Weight and Weight of Adenohypophysis of Intact Rats and Rats with Alloxan Diabetes (M ± m)

Group of animals	Body weight, g	Weight of adenohypophysis	
		absolute, mg	relative, mg/100 g body weight
1) Intact	186±6 (8)	10,4±0,6 (6)	5,56±0,30 (6)
2) Diabetes for 4 days	145±5 (10)	7,4±0,4 (6)	5,08±0,30 (6)
P_{1-2}	<0,001	<0,01	>0,05
3) Diabetes for 5 days	146±6 (10)	6,8±0,4 (6)	4,67±0,29 (6)
P_{1-3}	<0,001	<0,001	>0,05

Legend. Here and in Tables 2 and 3, number of animals in parentheses.

TABLE 2. STH and Prolactin Concentrations (in μg/mg wet weight of tissue) in Adenohypophyses of Intact Rats and of Rats with Alloxan Diabetes (M ± m)

Group of animals	STH	Prolactin
1) Intact	39,29±3,77 (6)	33,64±1,51 (6)
2) Diabetes for 4 days	56,95±5,24 (5)	46,59±4,48 (5)
	P_{1-2}<0,05	P_{1-2}<0,05
3) Diabetes for 5 days	61,76±4,34 (6)	48,21±3,80 (6)
	P_{1-3}<0,01	P_{1-3}<0,01

Similar results were obtained in experiments on dogs 4, 8, and 14 days after total pancreatectomy [9].

The increase in the STH and prolactin concentrations in the pituitary gland of the animals evidently cannot be explained by retention of the hormones in the cells, possibly connected with the toxic action of alloxan on the first days after its administration. This is confirmed by experiments *in vitro* on cultures of adenohypophyseal cells. An increase in the STH and prolactin concentration both in the medium and in the cells was found in cultures of adenohypophyseal cells taken from rats with diabetes for 3 days, even after culture *in vitro* for 4 days, suggesting a higher level of pituitary hormone production (Table 3). Similar changes also were found in cultures taken on the 10th day of development of diabetes. Preservation of functional changes acquired *in vivo* during transfer of the cells to *in vitro* culture is evidence of the stability of the intracellular changes in the synthesis and secretion of STH and prolactin during the development of diabetes.

The results are evidence of increased functional activity of the eosinophils of the rat adenohypophysis in the early stages of development of alloxan diabetes. This phenomenon is probably part of the compensatory changes taking place in the body of cell nutrition from glucose to products of lipid metabolism [7]. The increase in the secretory activity of the lactotrophs in the early period of development of alloxan diabetes is at present difficult to explain. The research must evidently be continued in order to elucidate the mechanism of this phenomenon. The results of determination of the STH concentration differ from those obtained by the Hazelwoods [11], who found no changes in the STH concentration, determined by the tibia test, in the adenohypophysis of rats on the 4th and 7th days of development of alloxan diabetes, but found a fall in the STH level on the 14th and 28th days. This disagreement may be connected with the inadequate sensitivity of the tibia test and also with its inadequate specificity for determination of the STH concentration in the pituitary or in material incubated from it, for certain other adenohypophyseal hormones also possess growth-stimulating activity [5].

The results of the present experiments demonstrate yet again the involvement of the adenohypophysis in the development of the symptom-complex of diabetes mellitus. An increase

TABLE 3. Content of Labeled STH and Prolactin (in cpm/mg protein; M ± m) in Incubation Medium and in Cells of 4-day Monolayer Cultures of Adenohypophyses Taken From Intact Rats and Rats with Alloxan Diabetes

Group of animals	STH		Prolactin	
	medium	cells	medium	cells
Experiment 1:				
intact	11 610±1 050 (5)	28 180±4 930 (5)	13 140±770 (5)	26 960±1 800 (5)
diabetes for 3 days	23 280±4 280 (5)	133 180±25 910 (4)	22 360±1 820 (5)	138 570±30 350 (4)
P	<0,05	<0,01	<0,01	<0,01
Experiment 2:				
intact	19 522±1 906 (6)	72 321±4 144 (5)	30 514±3 408 (5)	27 174±1 974 (5)
diabetes for 10 days	20 174±1 746 (6)	84 273±2 482 (6)	52 080±2 478 (6)	35 231±2 247 (4)
P	>0,05	<0,05	<0,001	<0,05

in the secretion of growth hormone as early as on the 4th or 5th day was detected in the experimental model of this disease. Increased secretion of prolactin also was observed at the same time.

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