INSULIN SENSITIVITY OF MUSCLE TISSUE ISOLATED FROM RATS WITH MANIFEST ALLOXAN DIABETES OF VARIED DURATION

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Sensitivity of muscle tissue to insulin was studied by measuring the intensity of its action on incorporation of labeled glucose into glycogen by the isolated diaphragm of 20 control rats and 27 rats with alloxan diabetes. If the duration of diabetes was 5-8 days the sensitivity of the muscle tissue to insulin was unchanged. After alloxan diabetes lasting 22-24 days, a significant decrease was found in the sensitivity of the muscle tissue to insulin. With an increase in the duration of decompensated alloxan diabetes to 5-8 months, a further decrease in incorporation of labeled glucose into glycogen by the diaphragm was observed. The results show that the sensitivity of muscle tissue to insulin is reduced by metabolic disturbances characteristic of diabetes mellitus.

KEY WORDS: alloxan diabetes; muscle tissue; insulin.

In the early stages of spontaneous diabetes mellitus the cause of the metabolic disturbances is now considered to be not an absolute, but a relative insulin deficiency [1, 9, 12], probably resulting from a decrease in the sensitivity of the tissues, especially adipose and muscular, to the action of insulin [7, 10, 11, 14].

However, the sensitivity of the diabetic organism to insulin can vary in the course of development of the disease. A decrease in sensitivity to insulin during prolonged, uncontrolled diabetes is known to take place, whereas sensitivity increases during compensation of diabetes [4, 5]. In rats with diabetes caused by total pancreatectomy, a marked increase in sensitivity to insulin was observed after establishment of normoglycemia [5]. Very probably the tissue sensitivity to insulin participates in this change of sensitivity to insulin. Accordingly, to assess the role of tissue sensitivity to insulin in the pathogenesis of spontaneous diabetes mellitus, it is important to study changes in tissue sensitivity during the course of this disease.

Previous investigations showed that with an increase in the duration of decompensated manifest alloxan diabetes the sensitivity of adipose tissue to insulin falls [6]. The need thus arose for a study of the dynamics of sensitivity of muscle tissue to insulin during the course of alloxan diabetes. The information thus obtained could answer the question of whether the decrease in the sensitivity of adipose tissue to insulin is specific for diabetes mellitus or whether it reflects a general decrease in tissue sensitivity to insulin in this disease.

As a first step, the sensitivity of muscle tissue to insulin was shown to be reduced in animals with alloxan diabetes [3], although in the investigation, cited sensitivity to insulin was tested only once, and no attempt was made to study its dynamics. Moreover, in that investigation the duration of the diabetes when the sensitivity of the muscle tissue to insulin was studied is not mentioned.

EXPERIMENTAL METHOD

Sensitivity of muscle tissue to insulin was studied in 20 control and 27 experimental male Wistar rats of reproductive age (3-7 months) with alloxan diabetes. To produce this diabetes alloxan was injected as a freshly prepared 5% aqueous solution, in a dose of 180 mg/kg body weight. The blood sugar level was determined by the method of Somogyi and Nelson.

The rats were investigated when the duration of manifest alloxan diabetes was 5-8 days (10 rats), 22-24 days (nine rats), and 5-8 months (eight rats). Hyperglycemia and glucosuria were found in all rats with dia-

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TABLE 1

Group of animals	Duration of diabetes	Number of rats	Age of rats, months	Radioactivity of glycogen in rat diaphragm after incubation, cpm/mg		P
				in buffer	with insulin	
1- (Control)	Normal	10	3—4	3750±250	4830±300	<0,02
2-	5—8 days	10	3—4	3605±220	4500±260	<0,02
3-	22—24 days	9	3—4	2550±150	2675±170	>0,02
4- (Control)	Normal	10	8—11	3220±210	4120±240	<0,02
5-	5—8 months	8	8—11	1870±130	1910±145	>0,02
				$P_{1,3}<0,001$ $P_{2,3}<0,002$ $P_{4,5}<0,001$ $P_{3,5}<0,01$	$P_{1,3}<0,001$ $P_{2,3}<0,001$ $P_{4,5}<0,001$ $P_{3,5}<0,01$	

betes. The severity of the diabetes was the same in the different groups of rats regardless of the duration of the diabetes and the fasting blood sugar varied from 270 to 317 mg%. The control for the rats with manifest alloxan diabetes consisted of 20 intact rats, 10 with a mean body weight of 181 g and aged 3-4 months serving as the control for rats with diabetes for 5-8 and 22-24 days, whereas 10 rats with a mean body weight of 260 g, aged 8-11 months, were the controls for rats with alloxan diabetes for 5-8 months.

Sensitivity of the muscle tissue to insulin was judged from the intensity of incorporation of labeled glucose into glycogen of the isolated diaphragm. The diaphragm was removed immediately after decapitation of the rats and divided into two halves, one of which was placed in a tube containing bicarbonate buffer and the other into a tube with insulin solution in a concentration of 100 microunits/ml. Stable glucose (200 mg%), bovine albumin (2%) and uniformly labeled ^{14}C-glucose (0.2 μCi) were added to the buffer and insulin solutions. The diaphragm was incubated for 2 h at 37°C and on a shaker at 120 rpm. After incubation, the diaphragm was removed from the incubation medium and glycogen was extracted from it by boiling in an alkaline medium, followed by precipitation and washing with ethanol [11]. The washed and dried glycogen residue was placed in a 4P gas-flow counter and the radioactivity counted, allowing for the coefficient of self-absorption, expressed as the number of counts per minute per milligram residue of dry glycogen.

EXPERIMENTAL RESULTS AND DISCUSSION

Between 5 and 8 days after the development of alloxan diabetes the level of radioactivity of glycogen in the diaphragm of the rats after incubation either in buffer solution or in insulin solution was indistinguishable from the corresponding level in the control rats (Table 1). Under the influence of insulin a statistically significant increase was observed in the incorporation of labeled glucose into glycogen in the diaphragm compared with incubation in buffer solution, both in rats of the two control groups and in rats with alloxan diabetes lasting 5-8 days.

In rats with manifest alloxan diabetes lasting 22-24 days and 5-8 months, the radioactivity of glycogen in the diaphragm was significantly lower than in the control rats or in rats with diabetes lasting 5-8 days, whether the diaphragm was incubated with buffer or insulin. Insulin completely failed to stimulate the incorporation of labeled glucose into glycogen in the diaphragm. In rats with alloxan diabetes lasting 5-8 months, the radioactivity of glycogen in the diaphragm was significantly lower than in rats with diabetes lasting 22-24 days, whether incubated in buffer solution or with insulin.

These results show that during the first days after the development of alloxan diabetes the sensitivity of the muscle tissue to insulin was unchanged. With an increase in the duration of diabetes the sensitivity of the muscle tissue to insulin fell or it even ceased to react to insulin.

The results show that under the influence of metabolic disturbances characteristics of diabetes mellitus the sensitivity of the muscle tissue to insulin falls. In manifest alloxan diabetes in rats, sensitivity of both adipose tissue and muscle tissue to insulin is reduced, suggesting a general decrease in the tissue sensitivity to insulin in diabetes mellitus. This lowering of tissue sensitivity to insulin under the influence of metabolic disturbances evidently plays a definite role in the general fall in sensitivity of the body to insulin and the increased insulin demand observed in patients with chronic decompensated diabetes mellitus.

The cause of the lowering of tissue sensitivity to insulin in chronic decompensated diabetes may be, first, a decrease in the activity of enzymes responsible for glycolysis and glycogen synthesis [2] and, second, a raised blood level of insulin antagonists, notably growth hormone, preventing the action of insulin at the tissue

level. It has been shown [3] that the sensitivity of muscle tissue of hypophysectomized rats with alloxan diabetes to insulin is not reduced.

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