EFFECT OF ADAPTATION TO COLD ON SENSITIVITY OF ANIMALS TO THE DIABETOGENIC ACTION OF ALLOXAN

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The increase in specific resistance of an organism on adaptation to a certain factor regularly changes its sensitivity to the action of other external environmental conditions. For instance, adaptation to hypoxia prevents stress injuries of the heart [4]. Physical training of rats leads to a marked increase of working capacity, but is accompanied by lowered resistance of the animals to the toxic action of barbiturates and inhibition of structural and functional repair processes in organs [8]. If animals are kept constantly under low temperature conditions, their heat producing capacity is increased but their resistance to other forms of stress, hypoxia for example, is reduced [13]. The response of an organism to a long-acting unfavorable external environmental factor is accompanied by changes in activity of the antioxidant defensive system [7]. However, the general rules and the role of these changes in the development of crossed resistance have not yet been explained. In the modern view, the mechanism of the harmful action of alloxan on pancreatic β-cells is connected with induction of free-radical oxidation [14]. The selective action of alloxan is due to the rapid accumulation of this compound in pancreatic β-cells and the low activity of the enzymic component of inactivation of free radicals in these cells [12].

The object of this investigation was to study the sensitivity of control animals and animals adapted to cold to alloxan.

EXPERIMENTAL METHOD

Experiments were carried out on 130 male Wistar rats obtained from the "Stolbovaya" Nursery in January to April. The rats were put into single cages at the age of 2.5-3 months, and some animals were placed in a low-temperature chamber at 4 ± 1°C (adaptation to cold). Control animals were kept at a temperature of 22 ± 1°C. Feeding schedules and alternation of light and darkness were identical in both groups, and water was allowed ad lib. Some animals from the control and experimental groups 50 days after the beginning of adaptation to cold were given an intraperitoneal injection of alloxan hydrate (from Chemapol, Czechoslovakia) in a dose of 17 mg/kg body weight in 0.4 ml of 0.03 M acetate buffer (pH 4.0), 18 h after the last meal. During the first 5 days after injection of the compound the percentage of animals which died was determined. The animals were decapitated 14-20 days after injection of alloxan. The glucose concentration in the blood was determined by the orthotoluidine method [6] and the immunoreactive insulin (IRI) level in the blood plasma was determined with kits for radioimmunoassay of insulin (Hungary). Morphological and stereometric investigations of the pancreas were carried out on 12 animals (three from each of the four experimental groups). Paraffin sections stained with hematoxylin and eosin, by Van Gieson's method, and with Schiff's reagent and also semithin sections stained with azure II were examined under the light microscope. A stereologic analysis was undertaken on sections 3-4 μ thick under a magnification of 50; a multipurpose test system of short sections (n = 36, P = 72) was used. The relative volume of the endocrine and exocrine parts of the parenchyma and also of the stromal component of pancreatic tissue was determined. The whole area of the section was measured. The significance of differences between mean values was estimated by Student's test.

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TABLE 1. Blood Glucose Concentration and Plasma IRI Level in Animals Subjected to Various Experimental Procedures (M ± m)

Experimental conditions	IRI, μU/ml	Glucose, mg/100 ml
1. Control (n = 31)	38,3±2,9	85,3±2.5
2. Adaptation to cold (n = 24)	26,1±2,3	79,5±3.0
3. Injection of alloxan into control animals (n = 21)	16,4±1,9	210,1±18,2
4. Injection of alloxan into animals adapted to cold (n = 10)	22,1±4,1	83,5±7.7
	$P_{1-2}<0,01$	$P_{1-3}<0,001$
	$P_{1-3}<0,001$	

Legend. n) Number of animals in group.

EXPERIMENTAL RESULTS

During adaptation of the animals to cold their plasma IRI level fell significantly, but the blood glucose concentration was unchanged compared with that of the control rats (Table 1).

Insulin secretion is known to be under the control of the nervous system and, in particular, the sympathetic nervous system has an inhibitory action on pancreatic β-cells [1]. During adaptation of animals to cold their catecholamine production rises and the sensitivity of the tissues to noradrenalin increases [13]. The fall in the plasma IRI level of rats adapted to cold observed in these experiments agrees with data in the literature [9, 10] and may be associated with increased sympathetic activity in these animals. The fall in the blood insulin concentration in the phase of resistance is regarded as one indication of transition of the animal to a more economic level of regulation of energy metabolism [5].

During the first 5 days after injection of alloxan 58% of the animals in the control group died, and in the surviving rats persistent hyperglycemia was observed on the 14th-20th days, with a marked fall in the plasma IRI level (Table 1).

In the group of animals adapted to cold, 28% of the rats died during the 5 days after injection of alloxan, whereas in the surviving animals normoglycemia was observed after 14-20 days, and the plasma IRI level was unchanged.

In rats exposed to cold no significant destructive changes were observed in the parenchyma of the pancreas. The relative volume of islet tissue was somewhat reduced (11.3 ± 0.2 mm^3/cm^3 compared with 13.1 ± 2.7 mm^3/cm^3 in the control; $P > 0.05$), due mainly to a decrease in the number of islets, with no change in their size or shape. The stromal component of the tissue exhibited high reactivity in response to this treatment. Edema was severe in the interlobular connective tissue. The periductular and perivascular tissue was moderately infiltrated with cells of the mononuclear series.

Considerable changes in structure of the pancreas were observed in the control animals after injection of alloxan. Although the general lobular structure of the organ was preserved, there was some increase in the relative volume occupied by the terminal zones (596 ± 42.8 mm^3/cm^3). The relative density of the endocirne part was sharply reduced by 64% compared with the control ($P < 0.05$), due to a decrease in both the number and size of the islets of Langerhans. Morphological analysis revealed that initially the dense packing of the cells disappeared and the center of the islet became pale. The cells here were large and pale and their cytoplasm vacuolated in some places. Gradually at the periphery of the islet a border of densely packed cells with darkly stained long or irregular nuclei was formed. At the same time, the normal round shape of the islets themselves remained. Later they became irregular in outline. Among typical cells of the endocrine part of the gland, cells with a structure similar to that of the acinar cells appeared.

The results of morphological and morphometric analysis suggest that dying islet cells are replaced by the exocrine parenchyma. In all animals of this group there was hyperemia, with small areas of extravasation.

Data obtained on animals in which the development of alloxan diabetes took place in animals exposed to general cooling are of the greatest interest. By contrast with the previous group of rats, changes in the endocrine part were less marked and took the form of dystrophy, necrobiosis, and necrosis of cells of the islets of Langerhans, more especially the insulocytes. However, the intensity and extent of these lesions were less, and this was reflected in the quantitative data. The relative volume of the islets was 7.6 ± 1.6 mm^3/cm^3, almost twice that in the control animals after injection of alloxan, but 42% less ($P < 0.01$) than in the control.

The greatest destructive changes thus affected the islet apparatus of the pancreas in this model of experimental diabetes. During exposure to cold, the diabetes followed a milder course in the animals. It can be postulated that general hypothermia to some degree neutralizes the harmful action of alloxan on the endocrine part of the pancreas.

A study of alloxan diabetes in rats under high mountain conditions showed that the course of the disease depends on the degree of adaptation of the rats in the mountains: mortality among the animals and biochemical changes after injection of alloxan were greater in unadapted animals [2]. The probable common factor, with a bearing on increased resistance of the animals to alloxan, is an increase in power of the antioxidant systems of the body both during adaptation to hypoxia [4] and during adaptation to cold [3]. It is also known that administration of the natural antioxidant vitamin E reduces the sensitivity of rats to the toxic effect of alloxan [11].

It can be tentatively suggested that the leading role in the increase of resistance of animals to the diabetogenic action of alloxan during adaptation to cold is played by increased reactivity of the antioxidant defensive systems of the body.

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