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EFFECT OF PHENTOLAMINE AND OBSIDAN ON MORPHOMETRIC INDICES OF THE ISLETS OF LANGERHANS IN RATS RECEIVING ALLOXAN

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Chronic administration of phentolamine and obsidan to rats previously treated with alloxan alleviated the destructive action of alloxan on the B cells and promoted new islet formation. It is suggested that phentolamine and obsidan block one of the possible mechanisms of the destructive action of alloxan on the islet tissue, connected with the intensification of adrenalin secretion.
KEY WORDS: islets of Langerhans; alloxan; adrenoreceptors; phentolamine; obsidan.

Both the parasympathetic and the sympathetic nervous systems participate in the regulation of the endocrine function of the pancrease [6]. An inhibitory effect of sympathetic nerves on B cell function has been found in experiments with sympathetic denervation of the pancreas [4, 7] and administration of α- and β-adrenoreceptor-blocking drugs. In the last case hyperemia of the islet-cell apparatus, an increase in the Zn concentration in the cytoplasm of the B cells, and the formation of new islets of Langerhans are observed, evidence of an increase in insulin-forming function during adrenoreceptor blockade [5].

The question naturally arises whether this stimulating action of chemical desympathization is manifested after injection of alloxan and whether administration of blocking agents would alleviate to some extent the action of alloxan on the islets of Langerhans.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats with an initial mean weight of 250 g. Diabetes was produced by subcutaneous injection of alloxan in a dose of 15 mg/100 g body weight. In a high proportion of the animals the first injection of alloxan did not cause the development of diabetes, as reflected in the level of the diuresis and the appearance of sugar in the urine. Alloxan was injected again into these animals in the same dose on the 12th day of the experiment. The total duration of the experiment was 24 days.

Phentolamine and obsidan were injected intramuscularly throughout the period of the experiment in doses of 2 mg/100 g body weight and 2-3 mg per rat, respectively, daily. On the days when alloxan was injected, phentolamine and obsidan were injected intramuscularly 2 h before the alloxan.

Sections through the pancreas 5 μ thick were stained with aldehyde–fuchsin by Gomori's method in Dyban's modification. The following morphometric indices were used: the relative percentage of islet tissue, the number of islets per 10 mm^2 area of section, the mean area of an islet, the number of B and A cells per islet, the ratio between the numbers of B and A cells, and the area of the nuclei of the B and A cells.

The pancreases of intact rats served as the control.

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TABLE 1. Effect of Phentolamine and Obsidan on Morphometric Indices of Islets of Langerhans in Rats Previously Receiving Alloxan (M ± m)

Group of animals	Number of animals	Islet tissue, %	Number of islets per 10 mm² parenchyma	Area of islet mm²	Number of B cells per islet	Number of A cells per islet	B/A ratio	Area of nucleus of B cell, μ²	Area of nucleus of A cell, μ²
1. Intact	6	0,55±0,06	10,6±1,7	0,0056±0,0004	31±4,07	6±0,9	5,17	21,22±0,4	15,89±0,33
2. Receiving alloxan	8	0,19±0,05	5,2±1,2	0,0045±0,0007	14±2,3	29±3,6	0,48	42,25±0,6	20,41±0,36
3. Receiving alloxan and phentolamine (mild diabetes)	12	0,28±0,02	12,5±0,9	0,0024±0,0002	22±2,0	15±2,2	1,47	25,63±0,46	16,48±0,36
4. Receiving alloxan and phentolamine (severe diabetes)	2	0,15±0,1	3,6±1,3	0,0035±0,001	11±2,1	15±2,1	0,73	30,3±2,3	16,5±1,4
5. Receiving alloxan and obsidan (mild diabetes)	13	0,35±0,07	12,3±1,5	0,0031±0,0004	24±2,5	13±2,0	1,69	22,76±0,42	15,89±0,35
6. Receiving alloxan and obsidan (severe diabetes)	2	0,20±0,01	5,3±0,4	0,0039±0,0005	15±4,6	22±5,1	0,68	34,5±3,6	18,6±1,3
P_{1-2}		<0,01	<0,05	>0,05	<0,01	<0,001		<0,001	<0,001
P_{2-3}		>0,05	<0,001	<0,001	<0,05	<0,01		<0,001	<0,001
P_{2-4}		>0,05	>0,05	>0,05	>0,05	<0,05		<0,001	>0,05
P_{2-5}		>0,05	<0,001	>0,05	<0,01	<0,001		<0,001	<0,001
P_{2-6}		>0,05	>0,05	>0,05	>0,05	>0,05		>0,05	>0,05

EXPERIMENTAL RESULTS

Alloxan caused severe degenerative changes in the islets of Langerhans, as manifested by a sharp decrease in the mass of islet tissue, the number of islets per 10 mm^2 of parenchyma, and the number of B cells per islet (Table 1). The remaining B cells were deficient in specific blue-violet granules and had hypertrophied nuclei, evidence of the increased function of these cells. Alloxan also caused a marked increase in the number of A cells and an increase in the area of their nuclei, pointing to increased functional activity of the A cells and in agreement with clinical observations showing an increase in the glucagon concentration in the blood of diabetics [2]. Modern views on the relations between B and A cells [2, 13] indicates that activation of the A cells in alloxan diabetes is a compensatory reaction leading to an increase in the insulin-forming function of the remaining B cells. Activation of the A cells in alloxan diabetes may also be the result of an increase in the concentration of catecholamines [3] and glucocorticoids [10, 11] in response to shock, and also to loss of sensitivity of the A cells to the inhibitory action of hyperglycemia [9].

Chronic administration of phentolamine and obsidan against the background of alloxan injections considerably alleviated the harmful action of alloxan on the islets in most animals (in 12 of 14 and in 13 of 15, respectively).

In the rats which received phentolamine as well as alloxan (group 3) the number of A cells per islet was reduced, the number of B cells was increased, and the B/A ratio was higher than in the control group of animals with alloxan diabetes (group 2). The cytoplasm of the B cells of these rats contained brightly stained blue-violet granules, indicating preservation of the usual insulin-forming function of the B cells. This was also confirmed by normalization of the area of the nuclei of these cells compared with the control group of animals with diabetes. The B cells of this group thus differed sharply both quantitatively and qualitatively from the B cells of the diabetic animals not receiving the blocker. These differences suggest a protective action of phentolamine against the destructive action of alloxan on the B cells. It is interesting to note that under these conditions the mass of islet tissue was not much greater than in the animals with diabetes. Undoubtedly this was because of the sharp increase in the number of small, newly formed islets, compared not only with the diabetic, but also with the intact animals.

Comparison of these last indices suggested that following administration of phentolamine the process of new islet formation took place intensively in the animals with diabetes. This evidently compensated to some extent the effect of the destructive action of alloxan on the B cells. Only in two animals was such compensation not observed (group 4). The basic morphometric indices in this group were similar to those in the control diabetic animals.

After injection of obsidan into the animals receiving alloxan the same general pattern was observed as after injection of phentolamine. In most animals (group 5) obsidan alleviated the destructive action of alloxan on the B cells and promoted new islet formation. Only in two animals was this protective action of obsidan not observed (group 6). The protective action of the adrenoblockers described above explains the considerable alleviation of alloxan diabetes observed in the writers' laboratory even after a triple blockade of the α-adrenoreceptors by phentolamine (a sharp decrease in the mortality among the animals, a smaller decrease in body weight, and a higher percentage of animals without glycosuria and polyuria) [1].

The mechanism of the protective action of the adrenoblockers after administration of alloxan can be represented as follows. Alloxan evidently acts on the B cells not only directly, but also indirectly, by intensifying adrenalin secretion. Adrenalin, which acts on α- and β-adrenoreceptors through cyclic AMP, inhibits the secretion of insulin, stimulates the secretion of glucagon [12], and reduces the ability of the pancreas to form new islets. Naturally blockade of the α- and β-adrenoreceptors abolishes all these influences of the catecholamines on the islet-cell apparatus after alloxan administration. Adrenoblockers thus stimulate the insulin-forming function not only in animals with an intact pancreas, but also, as was shown previously [5], after injection of alloxan, i.e., in the pathologically changed pancreas. This protective action of the adrenoblockers may be interesting in connection with the search for substances alleviating the severity of diabetes [8].

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