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EFFECT OF DIABETOGENIC AGENTS ON ZINC AND CALCIUM CONCENTRATIONS IN RABBIT PANCREATIC ISLET CELLS

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KEY WORDS: zinc, calcium, diabetes, pancreatic islets

Considering the important role of zinc and calcium in maintenance of the integral structure and function of cell membranes [4, 7], cytochemical studies of these metals in the pancreatic islets of animals receiving diabetogenic agents are interesting from the point of view of an explanation of the mechanism of the damaging action of these substances on insulin-producing cells. The present investigation was carried out by highly sensitive methods of determination of zinc and calcium in cells, developed by the writers [3].

EXPERIMENTAL METHOD

Experiments were carried out on 185 rabbits (intact and receiving single intravenous injections of the substances). Substances injected into the animals are widely used for the production of experimental diabetes [1, 2, 5]: dithisone, 8-(p-toluenesulfonylamino)-quinoline (8-TSQ), 8-(benzenesulfonylamino)-quinoline (8-BSQ) in doses of 40-50 mg/kg, and alloxan, in a dose of 100-200 mg/kg. In a separate group of investigations, rabbits received an injection of oxin in a dose of 50 mg/kg. This is an analog of 8-TSQ and 8-BSQ and, according to some workers [8, 10], it also exhibits diabetogenic properties.

The blood sugar level of the rabbits was determined by the Hagedorn Jensen method before and after injection of the substances. The animals were killed 5 days after the injection. Pieces of pancreas were used for fixation in Bouin's fluid, cold acetone, and 70 degrees alcohol, saturated with H₂S, and also to prepare frozen sections.

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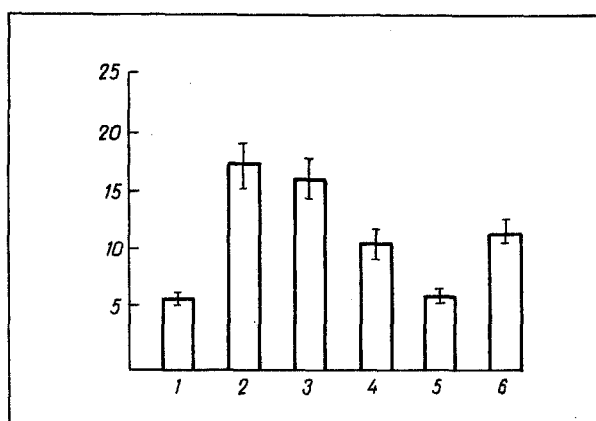


Fig. 1. Blood sugar of normal rabbits (1) and rabbits after injection of dithisone (2), 8-(p-toluenesulfonylamino)-quinoline (3) 8-(benzenesulfonylamino)-quinoline (4), oxin (5), and alloxan (6).

TABLE 1. Cellular Composition of Pancreatic Islets and Intensity of Cytochemical Reaction of 8-(p-Toluenesulfonylamino)-Quinoline (for zinc) and Chlortetracycline (for calcium) in Insulin-Producing Cells in Rabbits Receiving Diabetogenic Agents

Agent	Number of B-cells in islet ($\bar{X} \pm m$)	Functional index	Intensity of reaction ($\bar{X} \pm m$)	
			zinc	calcium
Control	31,5±0,40	4,7	1,9±0,07	0,5±0,03
Dithisone	8,5±0,31	1,0	0,3±0,11	0,1±0,03
8-TSQ	10,5±0,53	1,3	0,5±0,16	0,1±0,04
8-BSQ	11,8±0,97	1,6	1,1±0,25	0,2±0,06
Alloxan	11,2±0,84	1,6	0,9±0,28	0,2±0,07

Legend. Differences from control significant in all cases.

Paraffin sections 5-10 μ thick, prepared from pieces of the pancreas, were fixed in Bouin's fluid and stained with aldehyde-fuchsine and hematoxylinphloxine by Gomori's method. The first stain was used to detect specific granules of B-cells of the pancreatic islets, the second to study the structure of the islets and to count the number of cells in them, and to calculate the functional index (the ratio of the number of B-cells to the number of A-cells in the islets).

For the cytochemical detection of calcium in the islets, frozen sections of the pancreas were fluorochromed with chlortetracycline (CTC). Paraffin sections of the pancreas, fixed in acetone and H₂S-alcohol, were stained with dithisone and 8-TSQ to reveal zinc in the cells by methods developed in [3].

EXPERIMENTAL RESULTS

The results of investigations of the blood sugar level in the rabbits are given in Fig. 1. They show that dithisone had the strongest diabetogenic activity, that of 8-TSQ was somewhat weaker, and that of 8-BSQ and alloxan was weaker still. According to our data, oxin has no diabetogenic action.

In preparations stained with aldehyde-fuchsine, bluish violet granules could be seen in the cytoplasm of the islet B-cells; after staining with hematoxylin-phloxine the A-cells were red and the B-cells grayish blue. Injection of the diabetogenic agents caused degranulation of the insulin-producing cells, with a reduction of their number, as a result of which the functional index fell (Table 1), but the number of A-cells did not change so considerably. The greatest changes were induced by dithisone. After

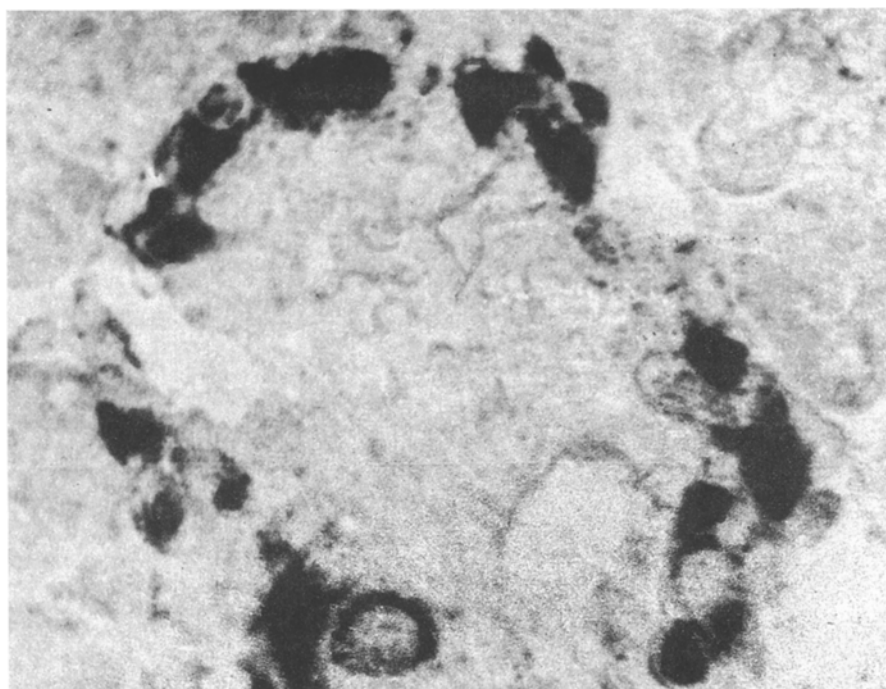


Fig. 2. Cytochemical dithisone reaction in pancreatic islet of rabbit with dithisone-induced diabetes. Marked reaction observed in A-cells arranged as a rim around the periphery; reaction virtually negative in central B-cells. 900 \times .

injection of oxin, damage to the cells was minimal and the cellular composition of the islets did not differ significantly from normal.

In intact rabbits, in sections stained with dithisone, the pancreatic islets stood out sharply against a pale yellow background of the surrounding endocrine parenchyma, because of their deep reddish purple color, due to the presence of a large quantity of dithisone granules in the cytoplasm of the islet A- and B-cells. In preparations treated with 8-TSQ, granules giving yellowish green luminescence were found in the same parts of the cells. In a separate series of investigations proof was obtained of the specificity of the reaction of dithisone and 8-TSQ with zinc in the islet cells [3]. In sections fluorochromed with CTC the islet A-cells, located in intact rabbits mainly around the periphery of the islets, gave bright green luminescence, whereas luminescence of the islet B-cells was much weaker. These data indicate a higher calcium concentration in the islet A-cells than in the B-cells.

In rabbits receiving the diabetogenic agents the zinc and calcium concentrations in the A-cells showed no significant change (Fig. 2), evidently due to the slight degree of damage to these cells. In B-cells, to which the damaging action of the diabetogenic substances mainly spreads, a marked decrease in zinc and calcium concentrations was observed (Table 1). In severe cases the cytochemical reactions in them were virtually negative (Fig. 2). After injection of oxin, a decrease in the zinc and calcium concentrations also was observed in the B-cells, but these changes were much weaker than after injection of the diabetogenic agents.

Dependence of the lowering of the zinc concentration in insulin-producing cells on the diabetogenic activity of the substances, observed in these experiments, confirms the existing view that zinc ions are involved in the internal secretory function of the pancreas [3]. If the action of dithisone, 8-TSQ, 8-BSQ, and oxin on islet B-cells is compared, correlation will be found between the reduction in the zinc concentration in these cells and the degree of affinity of the chelating agent for this metal, as shown by the value of the stability constant of the complex of the agent with zinc. For instance, the value of the constant for dithisone is four orders of magnitude higher than for oxin [9]. In view of evidence in the literature of the protective role of calcium ions against the action of pathogenic agents on the cell [6], the weak degree of damage caused to islet A-cells by the substances which we tested can be partly explained by the high calcium concentration in these cells.

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SEIZURE ACTIVITY OF ALBINO RATS AFTER IMMUNIZATION WITH A CONJUGATE OF SYDNOPHEN AND SERUM ALBUMIN

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In the clinical management of epilepsy psychotropic drugs with sedative and tranquilizing properties are widely used as anticonvulsive agents. Antidepressants with powerful stimulating properties, chiefly MAO inhibitors, in most cases have a provocative effect and potentiate the action of convulsive agents. The method of so-called inverse regulation of physiological and biochemical processes, being studied at the present time, involves active immunization against bioregulators, injected in the form of conjugates with carrier antigens. It leads to prolonged and profound effects, the direction of which it is mainly opposite to the effects of the same bioregulator as such. The bioregulator used may be identical with or similar in structure to the endogenous regulator. The regulators bind with antibodies formed during immunization, thus determining their ultimate prolonged physiological effect [1]. We showed previously that immunization of rats with a conjugate of the antidepressant and psychostimulant sydnophen (a partial catecholamine analog) with bovine serum albumin (BSA) can produce a long-term change in the state of rats, as reflected in physiological and biochemical parameters [2, 3, 6]. Changes observed in the behavior of the rats, similar in some respects to the effects of neuroleptics, led us to assess the influence of immunization on seizure activity.

The aim of this investigation was to study the effect of immunizing rats with a conjugate of sydnophen and serum albumin on seizure activity following injections of metrazol.

EXPERIMENTAL METHOD

Experiments were carried out on 115 noninbred male albino rats weighing 180-200 g. Conjugation of sydnophen with BSA was carried out by the glutaraldehyde method, as described previously [2, 3]. Rats of the experimental groups were

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