discovered may reflect compensatory changes in the GABA-ergic systems of the rat striatum during poisoning by GABA antagonists.

The possibility of membranotoxic effects of picrotoxin and bicuculline likewise cannot be ruled out.

LITERATURE CITED

1. M. M. Bordyukov, G. N. Kryzhanovskii, E. V. Nikushkin, et al., Byull. Éksp. Biol. Med., No. 12, 686 (1985).
2. A. K. Tonkikh, V. I. Kuznetsov, M. V. Karanova, and A. A. Sadykov, Neirokhimiya, **4**, No. 3, 260 (1985).
3. G. N. Shilov and A. I. Balakleevskii, Zdravookhr. Belorussii, No. 9, 21 (1989).
4. E. Chung, F. Yocca, and M. Woert, Life Sci., **36**, No. 11, 1051 (1985).
5. J. R. Hammond and I. L. Martin, Eur. J. Pharmacol., **137**, No. 1, 49 (1987).
6. M. Lazarova and R. Roussinov, Acta Physiol. Pharmacol. Bulq., **8**, No. 1-2, 78 (1982).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, No. 1, 265 (1951).
8. E. Lewin, J. Peris, V. Bleck, et al., Pharmacol. Biochem. Behav., **33**, No. 2, 465 (1989).
9. A. K. Mehta and M. K. Ticku, Pharmacol. Biochem. Behav., **30**, No. 4, 995 (1988).
10. H. Ninomiya, T. Nanihouchi, M. Kameyama, and F. Motohatsu, J. Neurochem., **51**, No. 4, 1111 (1988).
11. M. R. L. Sandoval and J. Palermo-Neto, Eur. J. Pharmacol., **167**, No. 1, 117 (1989).

EFFECT OF THE DIABETOGENIC AGENT DITHISONE ON ZINC CONCENTRATION IN PANCREATIC ISLETS OF ANIMALS OF DIFFERENT SPECIES

E. D. Gol'dberg, V. A. Eshchenko, and V. D. Bovt

UDC 616.329-008.64

KEY WORDS: glycemia; diabetes; pancreatic islets: zinc

The high selectivity of action of dithisone on the insulin-producing cells is one of its advantages over other substances (alloxan etc.) used as a model of diabetes mellitus [1, 2, 4, 6, 8-11]. In view of the high affinity of dithisone for zinc ions, the study of changes in the content of this metal in the pancreatic islets of animals of different species receiving dithisone must be interesting from the point of view of the study of the role of disturbances of zinc metabolism in the endocrine part of the pancreas in the mechanism of development of diabetes. Highly sensitive and selective methods of cytochemical and biochemical determination of zinc in the insular tissue of the pancreas have been developed in order to carry out such investigations.

In this paper the zinc concentration in the pancreatic islets in animals of different species was studied after injection of dithisone.

EXPERIMENTAL METHOD

The experiments were carried out on 42 cats, 293 rabbits, 49 golden hamsters, 68 mice, and 25 pigeons. Dithisone was injected intravenously in doses of 50-100 mg/kg. The blood sugar level was determined by the Hagedorn—Jensen

Research Institute of Pharmacology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 53-55, January, 1992. Original article submitted June 7. 1991.

0007-4888/92/0001-0075$12.50

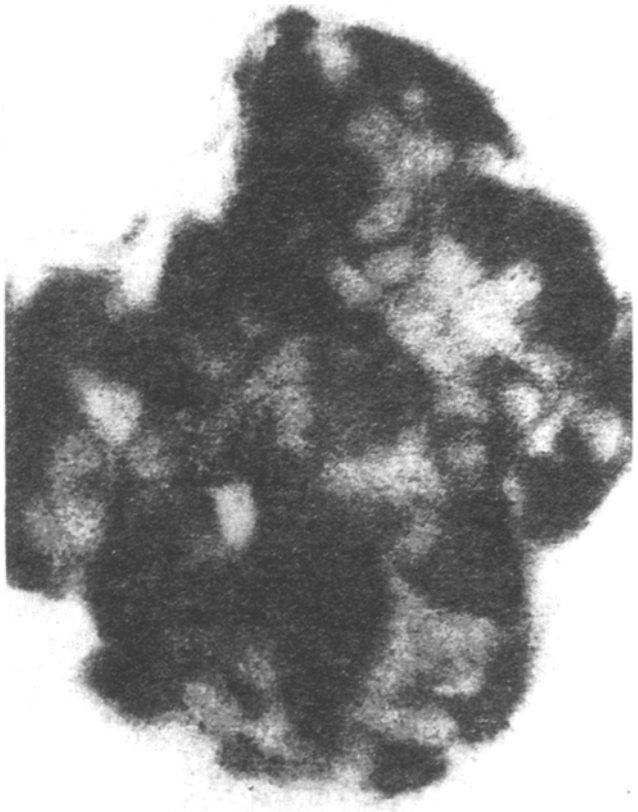

Fig. 1. Dithisone reaction in pancreas of control rabbit. Positive reaction discovered in islet cells: reaction negative in cells of exocrine tissue surrounding islet. 900.

method before and 5-15 days after injection of dithisone. The animals were killed at these times and pieces of the pancreas were used for biochemical determination of zinc, and for fixation in cold acetone and 70% alcohol, saturated with hydrogen sulfide (for the cytochemical study of zinc), and in Bouin's fluid (for staining sections of the gland by the modified hematoxylin-phloxine and aldehyde-fuchsine methods of Gomori) [3, 4]. Considering the ability of dithisone to bind zinc selectively in the pancreatic islets (Fig. 1) with the formation of a complex readily soluble in acetone, a method of extracting this metal from homogenized pancreatic tissue by shaking it in a solution of dithisone in acetone has been developed [5]. Zinc was determined in the mineralized extracts by a fluorometric method, using 8-(p-toluenesulfonylamino)-quinoline (8-TSQ) as the reagent (sensitivity of the method 10^{-7} mmole zinc in 1 ml of solution). For cytochemical detection of zinc paraffin sections 5-10 μm thick were stained with dithisone and 8-TSQ by the method described in [3]. A- and B-cells in the islets were differentiated by the hematoxylin-phloxine method. The specificity of the granules in the B cells (an indicator of their insulin content) was revealed by staining the sections with aldehyde-fuchsine. A combination of Gomori's method and reactions for zinc on the same or on adjacent sections enabled the distribution of insular zinc between cells of the two types to be studied.

EXPERIMENTAL RESULTS

Of all the species of mammals studied, the pancreatic islet-cell tissue of rabbits contained the most zinc, whereas that of cats contained least (Table 1). Zinc was detected cytochemically in A- and B-islet cells and was not found in the exocrine cells of the gland (Figs. 1 and 2).

Administration of dithisone to the animals led to a reduction of the amount of zinc in the pancreatic islet tissue (Table 1). The index of the decrease in the content of this metal in the islets (its relative amount in insular tissue in control animals to that found in experimental animals) was highest in the case of rabbits and lowest in the case of cats

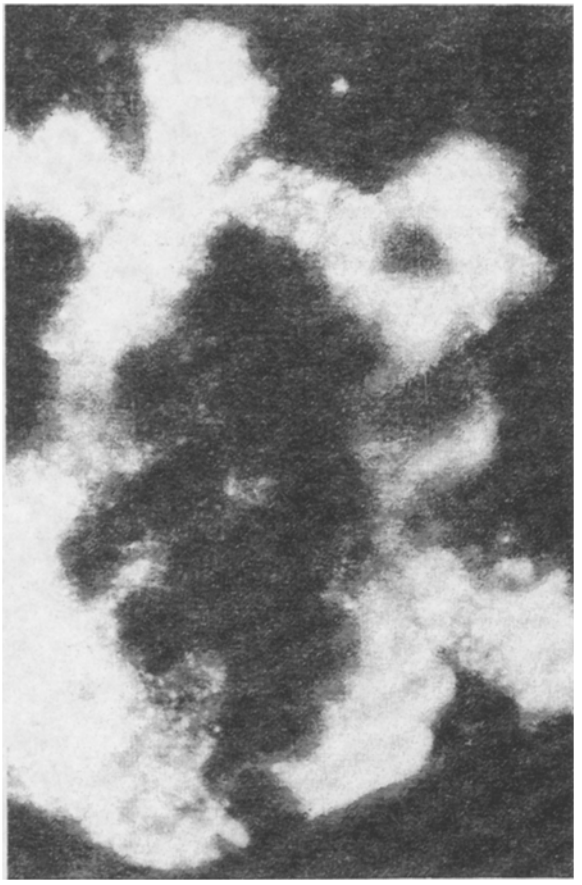

Fig. 2. Highly selective luminescence reaction of 8-TSQ with zinc in pancreatic islet of a rabbit receiving dithisone. Restoration of zinc concentration in A cells arranged around periphery of islet. Insulin-producing cells with sharply reduced quantity of zinc seen in middle part of islet. 900×.

TABLE 1. Zinc Content in Pancreatic Islet Tissue of Normal Animals and Animals Receiving Dithisone in Doses of 50-100 mg/kg

Species of animal	Zinc concentration in μg/g wet weight of gland (X ± m)		Index of reduction of zinc concentration in islets
	control	control	
Rabbits	13,8±0,35	4,3±0,27	3,2
Golden hamsters	9,0±0,51	4,5±0,28	2,0
Mice	7,4±0,38	5,2±0,44	1,4
Cats	4,0±0,22	2,9±0,30	1,3

Legend. Here and in Table 2 difference from control significant in all cases.

(Table 1). The values of this index largely agreed with another parameter, namely the glycemic index (the ratio of the blood sugar of the experimental animals to that of the controls) (Table 2).

It will be clear from Tables 1 and 2 that the diabetogenic action of dithisone in mammals depends on the initial zinc content in the pancreatic islets. This conclusion is confirmed by the results of experiments on starving animals and animals receiving glucose. For instance, in rabbits after 48 h of starvation the zinc content in the insular tissue was 1.7 times greater than in control (satiated) animals, whereas after intravenous injection of glucose in a dose of 10 g/kg it was

TABLE 2. Blood Sugar Concentration in Normal Animals and Animals Receiving Dithisone in Doses of 50-100 mg/kg

Species of animal	Blood sugar, mmoles/liter (X ± m)		Glycemic index
	control	exptl.	
Rabbits	5,7±0,03	22,1±1,05	3,9
Golden hamsters	7,0±0,11	10,0±1,05	1,4
Mice	6,8±0,26	10,0±1,09	1,5
Cats	5,1±0,10	5,9±0,56	1,2

1.7 times less. Correspondingly, the diabetogenic action of dithisone in starving rabbits was stronger, whereas in animals receiving glucose, it was weaker than in the control rabbits.

Despite the high zinc content in the islets of pigeons, it is impossible to obtain dithisone diabetes in them. The reason is that in pigeons most of the insular tissue is accounted for by glucagon-producing cells, by contrast with the mammals which were studied, in which most zinc is contained in the insulin-producing cells. On injection of dithisone into animals it binds in the pancreatic islets with zinc in both A- and B-cells [2], although irreversible disturbances of the metabolism of zinc arise chiefly in the B cells (Fig. 2). The reversibility of disturbances of this kind in the A-insulocytes may be the result of the mild damaging action of dithisone on them. One of the causes of this is the high calcium concentration in the A-cells, which lowers their sensitivity to the pathogenic agent [7].

Thus the essential role of disturbances of zinc metabolism in the insulin-producing cells in the mechanism of development of diabetes was demonstrated.

LITERATURE CITED

1. E. D. Gol'dberg and V. A. Eshchenko, Patol. Fiziol., No. 3, 81 (1989).
2. E. D. Gol'dberg and V. A. Eshchenko, Probl. Éndokrinol., No. 3, 78 (1989).
3. V. A. Eshchenko, Tsitologiya, No. 8, 927 (1978).
4. I. V. Toroptsev and V. A. Eshchenko, Experimental Dithisone Diabetes [in Russian], Tomsk (1975).
5. V. G. Troshkin and V. A. Eshchenko, Trace Elements in the Biosphere and Their Use in Agriculture and Medicine in Siberia and the Far East [in Russian], Ulan-Udé (1973), pp. 468-469.
6. V. G. Baranov, I. M. Sokoloverova, É. G. Gasparyan, et al., Experimental Diabetes Mellitus. Role in Clinical Diabetology [in Russian], Leningrad (1983).
7. K. V. Atchuta, N. Ramajah, and A. Mookerjee, Indian J. Exp. Biol., **16**, 857 (1978).
8. J. Kadota, J. Lab. Clin. Med., **35**, 568 (1950).
9. H. Kawanishi, Endocrinol. Jpn., **13**, 384 (1966).
10. K. Okamoto, Acta Scholae Med. Univ., **27**, 43 (1949).
11. K. Okamoto, Tohoku J. Exp. Med., **61**, Suppl. III, 1 (1955).