DISTRIBUTION OF ZINC IN THE ISLETS OF LANGERHANS OF HEALTHY AND DIABETIC ANIMALS RECEIVING TOLBUTAMIDE

I. V. Toroptsev* and V. A. Eshchenko

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Oral administration of tolbutamide to healthy and diabetic rabbits and rats led to the accumulation of zinc in the B cells of the islets of Langerhans, to a decrease in their insulin content and a decrease in their enzyme activity. The complex of the sulfonamide with zinc formed in the cytoplasm of the B cells evidently depresses activity of the enzymes preventing the secretion of insulin into the blood stream.

Of the sulfonamides given for the oral treatment of diabetes mellitus, tolbutamide is most extensively used. The mechanism of its action is explained by stimulation of the secretion of insulin by the B cells of the islets of Langerhans [1, 2, 9]. The presence of a sulfonamide group in the molecule is responsible both for its hypoglycemic [9] and its chelating [20] properties.

The object of the present investigation was to examine the role of zinc in the hypoglycemic action of tolbutamide, which can be postulated on the basis of existing data concerning its participation in insulin secretion [8, 17].

EXPERIMENTAL METHOD

Experiments were carried out on 73 rabbits and 32 rats. Diabetes was produced in the rabbits by intravenous injection of 20-40 mg/kg dithizone in 0.25% ammonia solution. Tolbutamide was given by mouth in a dose of 500 mg/kg body weight to healthy animals and also to diabetic animals in different stages of the development of the disease. The blood sugar was determined by the Hagedorn-Jensen method before and 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after administration of the tolbutamide. The animals were sacrificed at the same times, the pancreas was fixed in Bouin's fluid and by Timm's method, and frozen sections of the pancreas were cut. The frozen sections, 10-20 μ in thickness, were cut in a cryostat, and paraffin sections 5-10 μ in thickness also were prepared.

A histochemical reaction with zinc was obtained by means of 8-(p-tosylamino)-quinoline (8-TQ) and dithizone [3-5]. Acid phosphatase activity was determined at pH 5.0 by the azo-coupling method [7], carbonic and hydrase activity by a modified Kurata's method [14], glucose-6-phosphatase activity by Chiquoine's method [10, 11], and adenosinetriphosphatase activity by the method of Padykula and Herman [18, 19]. To detect acid phosphatase, frozen sections were fixed in 0.01% acetone solution of 8-TQ at 4°C for 30 min, so that the reactions for zinc and the enzyme could be observed in the same section. The activity of the

*Academician of the Academy of Medical Sciences of the USSR.

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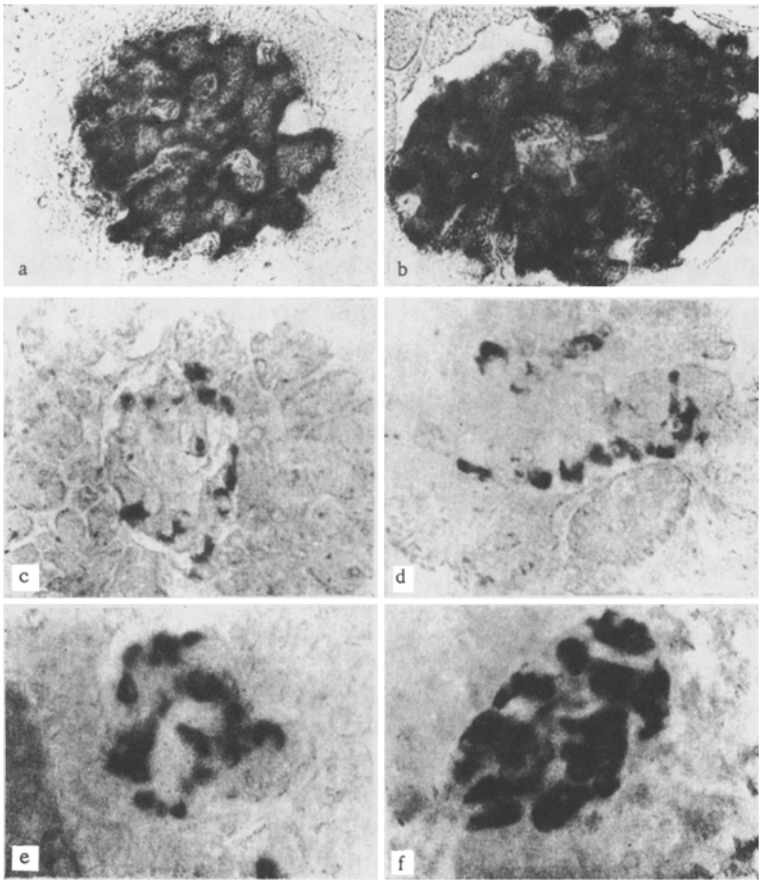

Fig. 1. Histochemical dithizone reaction for zinc in islets of Langerhans: a) healthy intact rabbit; b) healthy rabbit 6 days after administration of tolbutamide; c) intact rabbit with severe diabetes; d) rabbit with severe diabetes 6 h after administration of tolbutamide; e) intact rabbit with mild diabetes; f) rabbit with mild diabetes 6 h after administration of tolbutamide. Fixation by Timm's method, 400×.

remaining enzymes was determined in unfixed frozen sections. By staining with aldehydefuchsin [4] the "depot" form of insulin was detected [6]. A specific reaction for insulin was obtained by fluorochroming sections of the gland, fixed in Bouin's fluid, with pseudoisocyanin [12]. Differentiation between A and B cells of the islets of Langerhans was carried out on frozen sections examined in the dark field of the microscope, and on paraffin sections stained with hematoxylin-phloxine by Gomori's method [4].

EXPERIMENTAL RESULTS

In sections of the gland treated with 8-TQ, a yellowish-green luminescence appeared in areas containing zinc, and in sections stained with dithizone, reddish-purple granules were found. With the aldehyde-fuchsin method, bluish-violet granules were found in the B cells, and in the sections fluorochromed with pseudoisocyanin the cytoplasm of the cells showed a yellow luminescence.

Large quantities of zinc were found in the A cells of both rabbits and rats, and it was uniformly distributed among the cytoplasm. The B cells in rabbits had a high zinc content (Fig. 1a), but in rats the zinc content was low. Granules of the metal were concentrated mainly in the apical zones of the B cells, especially on the side facing the sinusoidal capillaries. In the healthy animals, the localization of zinc, reactions with aldehyde-fuchsin and pseudoisocyanin, and activity of the enzymes in the B cells were similar. Paranephric procaine block intensified these reactions.

In severe diabetes the B cells had very low enzyme activity and were almost free from zinc (Fig. 1c), and gave a negative reaction for insulin. In moderately severe diabetes, traces of zinc and low enzyme

activity were detected in the B cells, and a negative reaction was obtained for insulin; in mild diabetes the zinc content (Fig. 1e) and enzyme activity were higher, but no insulin could still be detected.

Tolbutamide had no significant effect on the A cells of the islets, but it produced marked changes in the B cells. The intensity of the histochemical reaction for zinc in the B cells increased in proportion to the decrease in the blood sugar. Zinc granules not only were concentrated in the apical zones of the B cells, but they frequently filled the remainder of the cytoplasm. However, the content of aldehyde-fuchsin granules, the intensity of the pseudoisocyanin reaction, and the activity of the enzymes (especially carbonic anhydrase) were actually slightly reduced, and the insulin-like activity of the blood serum was increased.

The most severe histochemical changes after administration of tolbutamide occurred in the healthy animals (Fig. 1b), especially in animals receiving a paranephric block. In mild diabetes the changes were less severe (Fig. 1f), while in severe diabetes they were absent (Fig. 1d). The changes were more marked in rabbits than in rats. The impression was thus obtained that the strength and duration of the action of tolbutamide were dependent on the initial zinc concentration in the B cells of the islets of Langerhans. For example, paranephric procaine block, leading to the accumulation of zinc in the B cells, increased the sensitivity of the animals to the hypoglycemic action of tolbutamide, whereas rabbits with a severe form of diabetes, in which hardly any zinc could be detected in the B cells, were resistant to the compound. However, further investigations are necessary before this finding can be finally confirmed.

It is doubtful whether the increase in the zinc content in the B cells of the islets after administration of tolbutamide could be due entirely to hypoglycemia. For instance, injection of glucose into animals treated with tolbutamide does not prevent the accumulation of zinc in the islets of Langerhans. On the basis of the writers' own observations and data published in the literature [13, 15, 16, 20], the role of zinc in the mechanism of the hypoglycemic action of tolbutamide cannot yet be exactly identified. In the present experiments, the preliminary (a few hours before) administration of the powerful chelating agent sodium diethyldithiocarbamate largely prevented the decrease in the blood sugar concentration and the changes in the histochemical reactions for insulin and enzymes in the islets of Langerhans after administration of tolbutamide.

It can be postulated that after administration of tolbutamide it forms a complex with zinc and accumulates in the cytoplasm of the B cells, which inhibits activity of the enzymes (carbonic anhydrase, acid phosphatase, etc.) which prevent the secretion of insulin into the blood stream.

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