POSSIBLE ROLE OF ZINC BLOCKING IN THE DEVELOPMENT OF DITHIZONE DIABETES

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Experiments on 76 rabbits showed that sodium diethyldithiocarbamate exerts its preventive action on dithizone diabetes directly in the islets of Langerhans. It appears very rapidly and continues, gradually becoming weaker, for 2 days. Zinc blocking takes place not only in the pancreatic islets, but also in other tissues. Sodium diethyldithiocarbamate, if injected preliminarily, binds Zn firmly and prevents the formation of the colored Zn dithizonate, the agent apparently associated with the selective damage of the islets of Langerhans by dithizone.

During the study of the pathogenesis of dithizone diabetes, discovered by Okamoto [10] and investigated in the writers' laboratory [1, 2, 4, 7, 8, 9], attention has been directed to the combination of the diabetogenic properties of dithizone and its ability to form complexes (chelates) with Zn. However, by no means are all chelating agents diabetogenic. Sodium diethyldithiocarbamate (DEDTC), for instance, which forms water-soluble chelate salts with Zn, does not induce diabetes even when injected intravenously in massive doses. Preliminary injection of DEDTC 30-60 min before injection of dithizone completely prevents the development of diabetes [6]. It can be postulated that the preliminary injection of the nondiabetogenic chelating agent DEDTC, which forms a complex with Zn in the islets of Langerhans in the pancreas, obstructs the subsequent binding of Zn by dithizone and thereby counteracts the diabetogenic effect of dithizone. The writers subsequently showed that DEDTC has a prophylactic action not only against dithizone diabetes, but also against diabetes induced by 5-(N,N-diethylaminophenylazo)-8-hydroxyquinoline, 5-(phenylazo)-8-hydroxyquinoline, and 8-(p-toluenesulfonylamino)-quinoline [3-5].

On the basis of these arguments, an attempt was made in the investigation described below to determine the point of application of the antidiabetogenic action of DEDTC, its duration, its stability, and the rapidity of its appearance. Attempts were also made to obtain further evidence of the possibility of Zn blocking not only in the pancreas, but in other tissues.

EXPERIMENTAL METHOD

Experiments were carried out on 76 noninbred rabbits, deprived of food for 2 days before the experiment. To determine the point of application of the antidiabetogenic action of DEDTC, the experimental animals received injections of 500-1000 mg/kg DEDTC followed 30 min later by 30-40 mg/kg of an ammoniacal solution of dithizone. Rabbits receiving dithizone only acted as the controls. The animals were sacrificed by air embolism. Sections of the pancreas, 10 μ in thickness, were cut on a freezing microtome and examined and photographed in a dark field. To determine Zn in the islets of Langerhans, pieces of pancreas were fixed by the method [11, 12] in alcohol saturated with hydrogen sulfide. After the sections had been dewaxed in the usual way, Zn was detected by the writers' own method [3].

To determine the duration and stability of the antidiabetogenic action of DEDTC, after the initial blood sugar had been estimated, the rabbits were given an intravenous injection of 250-1000 mg/kg DEDTC

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TABLE 1. Preventive Action of DEDTC on Development of Dithizone Diabetes

Series of experiments	Time of injection of DEDTC (1000 mg/kg) relative to injection of dithizone (30-54 mg/kg)	Expt. number of animals	Expt. blood sugar (in mg%) initial	Expt. blood sugar (in mg%) after injection of dithizone	Control number of animals	Control blood sugar (in mg%) initial	Control blood sugar (in mg%) after injection of dithizone	
I	6 h before	7	112 ±7.7	121 ±4.0	5	103 ±12.4	482 ±51.3	< 0.001
II	24 h before	8	126 ±6.6	124 ±3.4	6	111 ±5.8	399 ±25.5	< 0.001
III	48 h before	8	112 ±5.0	337 ±32.4	10	106 ±2.9	428 ±23.2	< 0.02
IV[1]	5 min after	7	112 ±5.4	127 ±2.4	10	111 ±9.4	418 ±45.5	< 0.001
	5 min after	3	127 ±3.3	206 ±26.4				< 0.001

[1]Dose of DEDTC 250 mg/kg.

followed, after different time intervals, by 30-54 mg/kg dithizone in 0.25% ammonia solution. Animals receiving dithizone only acted as controls. The presence or absence of diabetes was judged from the results of repeated blood sugar determinations by the Hagedorn-Jensen method.

EXPERIMENTAL RESULTS

The point of application of DEDTC in preventing the development of diabetes was found to be in the islets of Langerhans. After administration of dithizone to the rabbits the islets of Langerhans became packed with bright ruby-red granules of Zn dithizonate. Luminescence of the granules in the cells prevented detection of the structure of the islet and made it impossible to recognize the A and B cells in it (Fig. 1A). When a preceding injection of DEDTC was given, after the subequent injection of dithizone the cells had the usual structure and contained no dithizonate granules. One such islet is shown in Fig. 1B; B cells with dark nuclei and brightly luminescent silver-colored A cells at the periphery are clearly visible in it. Preliminary blocking of Zn in the islet cells by DEDTC evidently hinders the formation of the brightly luminescent Zn dithizonate. In these cases diabetes never developed [5]. Confirmation that DEDTC blocks Zn is given by Fig. 1C and D, showing the histochemical reaction for Zn with the specific reagent 8-(p-toluensulfonylamino)-quinoline [3]. When a preliminary injection of DEDTC was given, binding the metal (Fig. 1C), the reaction for Zn was negative, whereas it was strongly positive in sections from the pancreas of an intact rabbit (Fig. 1D).

To determine how DEDTC exerts its preventive action and its duration and stability, dithizone was injected after preliminary injection of DEDTC and the time between the injections of the two compounds was progressively increased. In the first group of experiments this interval was 6 h. It is clear from Table 1 (series 1) that diabetes did not develop in any of the 7 experimental rabbits, whereas all the 5 control animals, receiving the same doses dithizone, developed severe diabetes.

When the interval between injections of DEDTC and dithizone was lengthened to 24 h (Table 1, series II), the development of diabetes likewise was completely prevented in all 8 experimental rabbits. All 6 control rabbits receiving the same dose of dithizone developed severe diabetes.

Nine rabbits received DEDTC 48 h before the injection of dithizone. Eight of these animals developed diabetes, but it was significantly less severe than the diabetes in the control group (Table 1, series III). The diabetes in 3 of the 8 experimental rabbits of this series cured itself spontaneously.

The results of these experiments indicate that the preventive action of DEDTC on the development of dithizone diabetes continues for a long time. However, they do not tell how rapidly this action develops. An attempt was made previously [5] to answer this question by injecting dithizone and DEDTC simultaneously into different blood vessels, but these experiments had to be discontinued because of the high mortality among the animals. Diabetes was prevented by this means in only 2 surviving rabbits. On this occasion, DEDTC was injected, not simultaneously with dithizone, but 5 min

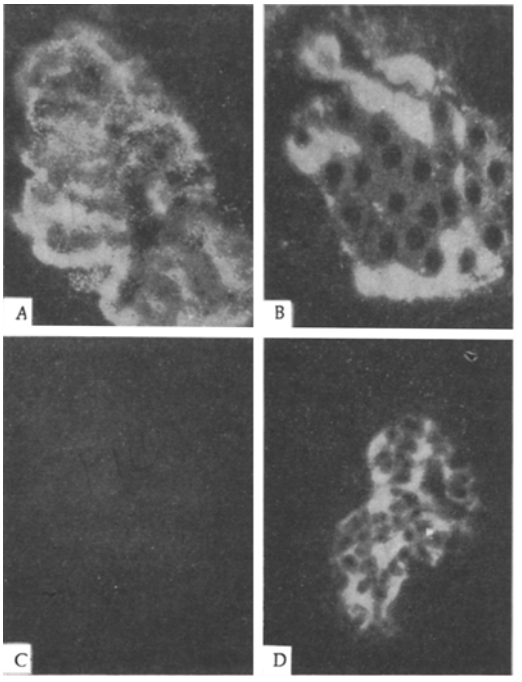

Fig. 1. Sections of rabbit's pancreas: A) after injection of dithizone: entire surface of islet is studied with brightly luminescent dithizonate granules; B) the same after preliminary injection of sodium diethyldithiocarbamate: dithizonate granules absent, normal structure of islet visible, B cell in center, silver A cells at periphery (dark field; 180 ×); C) absence of luminescent reaction for zinc in pancreas of rabbit receiving diethyldithiocarbamate; D) positive reaction for zinc in pancreas of intact rabbit (180 ×).

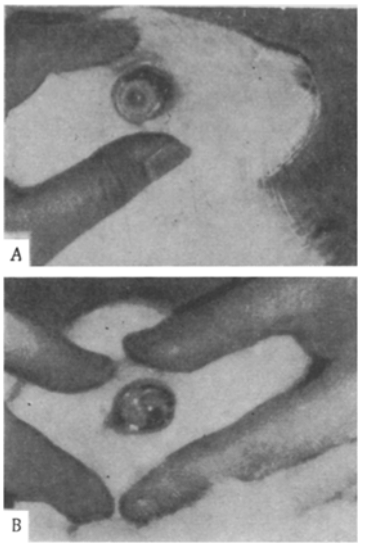

Fig. 2. Positive reaction for zinc in nictitating membrane of intact rabbit (A) and absence of reaction in a rabbit receiving preliminary injection of diethyldithiocarbamate (B).

later. In these experiments, 7 of the 10 animals did not develop diabetes, while in the remaining 3 animals the mild diabetes which developed cured itself spontaneously (Table 1, series IV). All 10 rabbits in the control group developed severe diabetes. The results of these experiments indicate that the strongest factor in the competitive relationships developing between DEDTC and dithizone is the chelating power of DEDTC, even if it is injected 5 min after dithizone.

In the last series of experiments, DEDTC was found to prevent the formation of Zn dithizonate not only in the pancreas, but also in other organs and tissues. In model experiments, the rabbits of one group received an injection of 0.9% solution of Zn sulfate into the skin of the ear and abdomen and into the nictitating membrane, after which 40 mg/kg dithizone was injected intravenously. A reddish-violet color (Zn dithizonate) appeared after 10-15 min at all sites of injection, and reached a maximum after 1 h. The islets of Langerhans were stained the same color. Staining of the nictitating membrane with Zn dithizonate can be see in Fig. 2A. The other group of rabbits received an injection of zinc sulfate, followed by an injection of 500-1000 mg/kg DEDTC in the same place, and after an interval of 1 h, an injection of 40 mg/kg dithizone. The pancreatic islets of these animals remained unstained. The nictitating membrane also remained unstained (Fig. 2B). The skin of the abdomen and ear became pale pink in color at the site of injection of the zinc sulfate, in sharp contrast to the color observed in the first case.

Summarizing the results of these investigations, they showed that the preventive action of DEDTC on dithizone diabetes is effected directly in the islets of Langerhans. It appears very rapidly and persists, gradually becoming weaker, for 2 days. Zinc is blocked, as the mother experiments showed, not only in the pancreatic islets, but also in other tissues. A preliminary injection of DEDTC bound the zinc firmly, thus explaining the selective injury to the islets of Langerhans by dithizone and the development of diabetes.

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