PREVENTION OF DITHIZONE DIABETES BY COMPOUNDS CONTAINING SULFHYDRYL GROUPS

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Preliminary administration of cysteine, glutathione, BAL, and unithiol prevents the development of diabetes produced in rabbits by intravenous injection of dithizone.

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The study of experimental diabetes produced by dithizone and 8-hydroxyquinoline derivatives [1-3, 7, 8] has directed attention to the combination of diabetogenic properties of these compounds with ability to form complexes (chelates) with metals. The developing diabetes is produced essentially by blocking of metals (most probably zinc) in the β-cells, leading to death of the cells and to the development of primary insulin deficiency. This hypothesis was confirmed by our experiments in which experimental diabetes was prevented by preliminary administration of sodium diethyldithiocarbamate [1, 3]. This compound, with marked chelating properties, forms complexes with many metals, including those present in the pancreas. However, it has no diabetogenic action. If the mechanism of action of diabetogenic compounds were simply that of blocking metals, preliminary administration of nondiabetogenic sodium diethyldithiocarbamate might prevent damage to them [1].

According to one report [10], compounds containing sulfhydryl groups react with zinc. Zinc has a marked affinity for groups containing sulfur, with which it forms stable complexes. Zinc combines with many proteins by reacting with their free SH-groups. Metals are bound even more strongly by amino acids, such as cysteine, containing thiol groups. Its stability constant is 18.2 [9].

In this investigation an attempt was made to prevent experimental diabetes with the aid of compounds containing SH-groups.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rabbits kept on a normal diet (oats, hay, bread, vegetables) with water ad lib. Before the experiment the animals were kept without food for 1-2 days. The presence or absence of diabetes was judged from the results of repeated estimations of glucose in the blood by Roe's anthrone method.

Diabetes was produced by intravenous injection of dithizone (40-50 mg/kg) in 0.25% aqueous ammonia. After the initial blood sugar concentrations had been established, the test compounds were injected into the rabbits, followed by dithizone after various time intervals. Cysteine, glutathione, and unithiol were given as a single intravenous injection, and BAL by repeated intramuscular injections. Animals injected with dithizone only acted as controls.

EXPERIMENTAL RESULTS

In the experiments of series I (Table 1) the rabbits were injected with 1000 mg/kg of neutralized cysteine hydrochloride 5 min before receiving an injection of a diabetogenic dose of dithizone. This completely prevented the development of diabetes in all the experimental animals. Of the 7 control animals, 6 developed marked diabetes. Injection of reduced glutathione 5 min before dithizone also prevented the development of diabetes in all the experimental rabbits although it developed in all the controls. To make sure that the preventive action of glutathione was due to the presence of SH-groups in its structure, in the

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TABLE 1. Prevention of Diabetogenic Action of Dithizone by Cysteine, Glutathione, BAL, and Unithiol

Compound injected	No. of animals	Blood sugar (in mg) (M±m)		
		before injection of dithizone	after injection of dithizone	P
Cysteine (1000 mg/kg) followed after 5 min by dithizone (50 mg/kg)	5	117±9.9	104±5.3	> 0.5
Dithizone (50 mg/kg)	6	99±5.6	357±20.6	< 0.001
Reduced glutathione (1000 mg/kg) followed after 5 min by dithizone (50 mg/kg)	3	104±9.7	127±11.4	> 0.5
Oxidized glutathione (1000 mg/kg) followed after 5 min by dithizone (50 mg/kg)	3	98±12.8	381±17.7	< 0.001
Dithizone (50 mg/kg)	4	95±4.1	451±14	< 0.001
BAL (80-252 mg/kg) followed by dithizone (50 mg/kg) 30-50 min after last injection	5	101±6.1	111±14.6	> 0.5
Dithizone (50 mg/kg)	8	109±8.5	331±35.3	< 0.001
Unithiol (110-200 mg/kg) followed after 3 min by dithizone (50 mg/kg)	8	119±6.3	132±5.4	> 0.1
	3	127±21.2	297±35.3	< 0.02
Dithizone (50 mg/kg)	8	114±5.7	402±14.0	< 0.001
	1	–	136	

experiments of series IV rabbits under the same conditions were given oxidized glutathione, which has no sulfhydryl group. This compound did not prevent the development of diabetes.

Five experimental animals received BAL intramuscularly. One rabbit was injected with 0.8-1 ml daily for 4 days and twice with 1 ml at an interval of 3 h on the day of the experiment. The second rabbit received 1 ml daily for 3 days and 1 ml twice at an interval of 3 h on the day of the experiment. The third and fourth rabbits received 1 ml BAL daily and 1 ml twice at an interval of 3 h on the day of the experiment. Finally, the fifth rabbit received BAL only on the day of the experiment, as 2 injections at an interval of 3h. Dithizone was injected when the rabbit gave off a pungent odor of BAL (usually 30-50 min after the final injection). In all the animals preliminary intramuscular injection of BAL in a dose of 80-252 mg/kg prevented the development of diabetes. In the last series of experiments, 3 min before injection of dithizone, 11 rabbits received an injection of 110-200 mg/kg unithiol. Eight control animals received dithizone only. The preventive action of unithiol was very clearly demonstrated (in 8 of the 11 experimental rabbits it completely prevented the diabetes), although it was somewhat weaker than the action of cysteine, glutathione, and BAL, which prevented the diabetes in all the animals without exception.

These experiments provided convincing proof of the marked preventive action of a series of chemical compounds containing a sulfhydryl group. This action was strongest in the case of cysteine, glutathione, and BAL, in agreement with the few observations reported in the literature [4-6].

It may be supposed that the preventive action of sulfhydryl compounds is dependent on their ability to form stable compounds with the metal contained in the active centers of enzymes, which do not prevent the activity of enzymes concerned in the synthesis of insulin [3]. The subsequent injection of the diabetogenic chelating compound dithizone does not displace the sulfhydryl compound from their bond with the metal. Consequently, the introduction of cysteine, glutathione, BAL, or unithiol into the β-cells of the islets places an obstacle in the way of the formation of the diabetogenic complex which causes the destruction of these cells.

The results of our investigations [2] and those reported by other workers [9, 10] suggest that this metal is most probably zinc.

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