EXPERIMENTAL DIABETES CAUSED BY 5-(N,N-DIETHYLPHENYLAZO)-8-HYDROXYQUINOLINE

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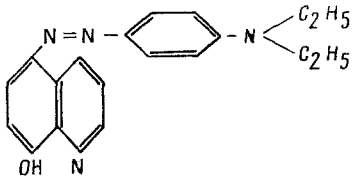
UDC 616.379-008.64-092.9-02:615.756.2

It has been shown [3, 8, 10] that administration of dithizon to rabbits is accompanied by the development of diabetes. Attention has been drawn to the combination of the diabetogenic properties of dithizon and its ability to form complexes (chelates) with zinc found in the pancreas [11]. This explains the interest in the study of the diabetogenic action of other chelating agents, of which 8-hydroxyquinoline and, in particular, its derivatives merit particular attention. The study of 15 derivatives of quinoline and quinaldine [5, 6], showed that three of them produced a temporary increase in the blood sugar level. Okamoto [11] described 15 derivatives of quinoline and quinaldine capable of causing diabetes in rabbits.

The object of the present investigation was to obtain new compounds of hydroxyquinoline possessing a powerful diabetogenic action, and to study the dynamics of the developing diabetes and the mechanism of its origin.

EXPERIMENTAL METHOD

Having proved conclusively that 8-hydroxyquinoline itself has no diabetogenic action [4], the authors synthesized and studied its derivatives 5-(N,N-diethylphenylazo)-8-hydroxyquinoline (5EP8Q):



The synthesis was based on the principles published in the papers by Matheus [9] and N. V. Vorozhtsov and I. M. Kogan [1].

5EP8Q is insoluble in water, readily soluble in organic solvents, and much less soluble in aqueous solutions of acids. It was injected into 20 rabbits previously deprived of food for 48 h. A weighed sample of 5EP8Q was dissolved in 4-5 ml of 0.2 N hydrochloric acid and the volume made up with water to 20-25 ml. The resulting cherry red solution was slowly injected into the auricular vein.

The fluctuations in the blood sugar level very characteristic of diabetogenic substances were studied first. After establishment of the initial blood sugar concentration, 5EP8Q was injected in a dose of 30 mg/kg. The blood sugar was determined 2, 4, 8, 12, 16, 20, and 24 h later and on the following day (by the Hagedorn — Jensen method).

EXPERIMENTAL METHOD

Two hours after injection of 5EP8Q a clear hyperglycemia occurred, and was followed by hypoglycemia, reaching its minimum after 8-12 h. The third phase was a secondary hyperglycemia, followed by permanent diabetes. The pattern of the phasic changes in the blood sugar described above is typical of chemical diabetogenic substances selectively damaging the β-cells of the islets of Langerhans [2, 3].

The remaining experiments were devoted to the study of the dynamics of the diabetes until the moment of death or sacrifice of the animals. After death the pancreas was excised, fixed in Bouin's solution,

Department of Pathological Physiology, Karaganda Medical Institute (Presented by Academician V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 7, pp. 45-48, July, 1967. Original article submitted November 10, 1965.

TABLE 1. Diabetes in Rabbits Produced by Injection of 5EP8Q

Period of observation (in days)	No. of rabbits	Weight of rabbits (in g)	Dose of 5EP8Q (in mg/kg)	Blood sugar (in mg%, M±m and limits of variations)	
				before experiment	maximal level
8—14	3	1630—2945	30,0	114±12,97 (99—137)	617±53,27 (550—706)
21—56[1]	4	2240—3100	20,0—31,2	99±12,12 (76—126)	554±55,03 (392—620)
143—170	3	1400—3240	30,0—32,1	112±2,09 (109—115)	485±32,75 (446—542)

*One of the rabbits of this group, after the blood sugar level had reached its maximum of 392 mg% of the 5th day, recovered on the 10th day of observation.

and sections were stained with aldehyde-fuchsin by Gomori's method. A distinct parallel is observed between the content of granules in the β-cells of the islets and the insulin content in the pancreas [7]. In most cases the diabetes was studied for a considerable length of time (Table 1). In the two rabbits which died quickly, weakness, anorexia, and a rapid decrease of body weight were observed. Acetone and sugar were found in the urine. The blood sugar level reached 450-700 mg%. The picture resembled that of diabetic coma.

In the histological sections stained by Gomori's method no β-cells could be found in the islets. The islets were filled with a crumbling necrotic mass. No aldehyde-fuchsin granules could be found. This picture may be described as total necrosis causing complete cessation of insulin section. Only one animal recovered. The remainder survived for a fairly long period. The animals were in a satisfactory state, maintained their body weight, ate twice or three times as much food as healthy animals, drank large quantities of water, and excreted up to 600-1000 ml of urine daily. Sugar was constantly found in the urine. Microscopic examination of the pancreas revealed a decrease in the size and number of the islets. The β-cells became much less numerous than in healthy animals, while the number of α-cells increased both relatively and absolutely. No aldehyde-fuchsin granules were found in the β-cells. When recovery took place, more β-cells were present than in chronic diabetes, but fewer than in intact animals. Aldehyde-fuchsin granules were present in them, but in smaller numbers than in healthy rabbits. The presence of granules demonstrated the incretory activity of the cells. Newly formed transitional β-cells were found in the exocrine tissue and in the walls of the small efferent ducts.

It was thus found that 5EP8Q possesses a powerful diabetogenic action, no less marked than the action of alloxan and dithizon. According to Okamoto [11], as a result of the entry of dithizon into the pancreatic islets, chelates with zinc [dithizonates) are formed in them, producing destructive changes in the β-cells of the pancreas, as a result of which diabetes develops. Critical examination of Okamoto's arguments cannot adequately justify the conclusion that zinc is the only metal in the pancreas forming chelates with dithizon. The specificity of the methods used to determine zinc histochemically was low [12]. A color reaction similar to that of zinc dithizonate may also be obtained with other metals found in the islets.

These objections to the exclusive role of zinc in the pathogenesis of diabetes do not discredit the fundamental idea that the chelates of metal found in the pancreas may have a diabetogenic action. If this view is correct it may be assumed that the preliminary administration of a powerful complexing agent blocking all the metals in the islets but not possessing a diabetogenic action must prevent the development of diabetes following the subsequent injection of the diabetogenic chelating agent 5EP8Q. To investigate this possibility experiments were carried out on 10 rabbits weighing 1650-3240 g; five rabbits were experimental (receiving injections of sodium diethyldithiocarbamate in a dose of 400 mg/kg, followed 15-30 min later by 5EP8Q in a dose of 30 mg/kg), and the other five animals were controls (receiving only the diabetogenic complexing agent 5EP8Q). The results of these experiments are given in Table 2, which shows that diabetes did not develop in the experimental animals.

The results of these experiments suggest that the diabetogenic action of 5EP8G is most probably attributable to the formation of chelates by this substance with the metal in the islet tissue. The main role among these metals probably belongs to zinc, but further proof of this is still required. If the cause of

TABLE 2. Effect of Sodium Diethyldithiocarbamate on the Diabetogenic Action of 5EP8Q

Rabbit	Blood sugar (in mg%) before experiments and during first 7 days after injection of 5EP8O (M ± m)							
	before expt.	1st day	2d day	3d day	4th day	5th day	6th day	7th day
Control	111± ±7,29	177± ±57,20	457± ±44,30	517± ±43,10	511± ±88,30	446± ±27,20	—	526± ±53,40
Experimental	106± ±4,05	117± ±6,15	113± ±5,94	113± ±6,53	114± ±8,19	114± ±3,26	109± ±5,34	113± ±7,17
P			<0,00 –	<0,001	<0,01	<0,001		<0,001

*The values of P are given when the differences between the control and experimental results are significant.

development of diabetes is blocking by 5EP8Q of the metals located in the active centers taking part in insulin synthesis, the mechanism of action of diethyldithiocarbamate can be understood. The metalloenzymes may react with some chelating agents without depression of their activity [13]. Clearly, therefore, administration of diethyldithiocarbamate, which does not cause the development of diabetes [4] but which forms complexes with metals, may obstruct the subsequent binding of metals by 5EP8Q and thus prevent its diabetogenic action. It may be concluded from the experiments with the preliminary administration of diethyldithiocarbamate that the diabetogenic action of 5EP8Q may be explained by the formation of chelates of metals in the islets injuring the β-cells, as a result of which a primary insulin deficiency develops.

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