Investigation of Diabetogenic Action of Xanthurenic Acid

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Repeated administration of xanthurenic acid to experimental animals completely eliminates insulin stores in pancreatic B cells and attenuates normalization of blood glucose level in the glucose tolerance test. Xanthurenic acid inhibits insulin secretion by isolated pancreatic islets and decreases zinc-specific luminescent reaction in B cells.

Key Words: *xanthurenic acid; B cells; insulin*

Xanthurenic acid (XA, 4,8-dihydroxyquinoline-2-carboxylic acid), a product of altered tryptophan metabolism, induces experimental diabetes mellitus in rats maintained on tryptophan-enriched diet [11]. It has been noted that tryptophan-enriched diet without pyridoxine leads to accumulation of XA in experimental animals, which can be prevented by vitamin B_6 treatment [9]. Further studies demonstrated the presence of XA in the urea of rabbits, dogs, and patients with diabetes mellitus. It has been shown that XA loses its diabetogenic properties after elimination of the hydroxyl group in the eighth position. Study of the mechanisms of diabetogenic action of other 8-hydroxyquinoline derivatives showed that blockage of active groups in the eighth position leads to complete loss of the diabetogenic properties and prevents the formation of their toxic complexes (chelates) with zinc ions in B cells. These processes are of crucial importance for the pathogenesis of experimental diabetes caused by these complex-forming substances [3,4,10].

The aim of the present study was to explore the effect of XA on insulin production in B cells and to elucidate the mechanisms of its potential diabetogenic action.

MATERIALS AND METHODS

Experiments were carried out on 15 albino mice, 44 rats, and 5 outbred rabbits. In chronic experiments, the effect of a diet promoting the generation of XA in combination with repeated injections of XA (200 mg/kg body weight) on tissue structure and insulin stores in B cells of pancreatic islets was examined. To this end, the animals maintained for 1 month on tryptophan-enriched diet [9,11] were weekly intraperitoneally injected with XA. One week after the last injection the animals were sacrificed; pancreas sections fixed in Bouin's fluid were stained with diethyl pseudoisocyanine [7] and aldehyde fuchsin [8] and examined under luminescent and light microscope. The intensity of diethyl pseudoisocyanine staining indicating the presence of insulin stores was measured fluorometrically [6]. The effect of XA on the dynamics of blood glucose level was also studied using the hexokinase-peroxidase method. In all animals the glucose tolerance test was carried out before and after the experiment.

For evaluation of the direct effect of XA on insulin secretion by B cells, pancreatic islets isolated with collagenase were exposed to XA, and the level

TABLE 1. Effect of XA on Functional State of B Cells Assessed by Glucose Tolerance Test (mmol/liter)

Experimental conditions	Control	XA, 200 mg/kg weekly
Initial	5.02±0.2	4.42±0.25
After glucose load:		
30 min	7.97±0.67	8.78±0.64
2 h	4.73±0.16	6.20±0.5*

Note. *$p<0.05$ compared with the control.

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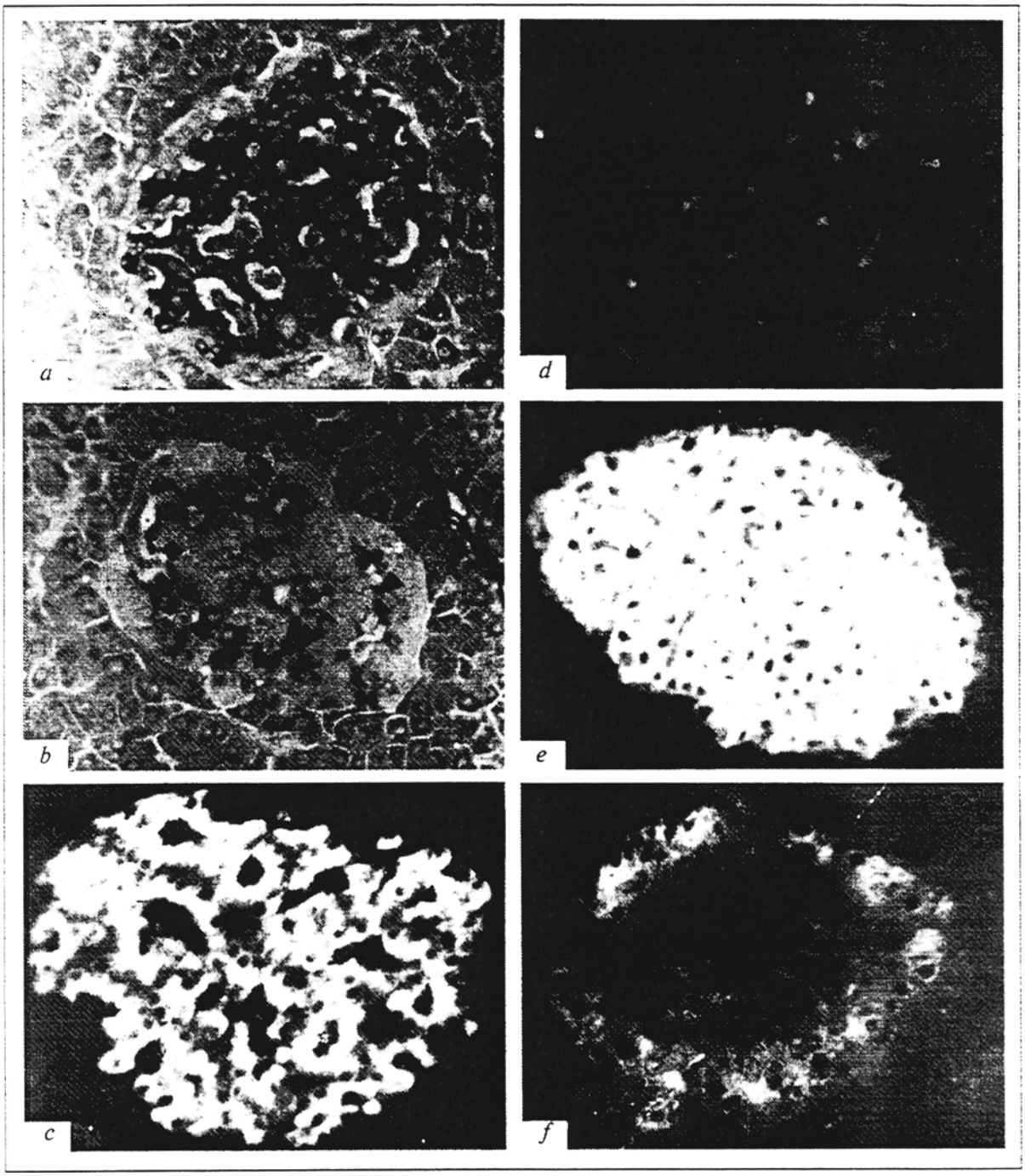

Fig. 1. Pancreatic islet of intact (*a*, *c*, *e*) and experimental (*b*, *d*, *f*) rats. *a* and *b*) aldehyde fuchsin staining, ×280; *b*) degranulation and necrosis of B cells; *c* and *d*) diethyl pseudoisocyanine, ×120; insulin-positive (*c*) and insulin negative (*f*) reaction in B cells; *e* and *f*) ×120; Zn-positive (*e*) and Zn-negative (*f*) luminescent reaction in B cells.

of immunoreactive insulin in the incubation medium was measured.

The interaction between XA and zinc ions in B cell cytoplasm was assessed using Zn-specific histochemical luminescent reaction with 8-p(toluene sulfonylamine)quinoline (8TSAQ) [2]. To this end the sections were treated with 2% XA buffer solution and than 8TSAQ acetone solution. The sections were examined under a luminescent microscope; the reaction was measured fluorometrically.

RESULTS

Periodic glycemia (to 12.9-14.9 mmol/liter) was observed in the majority of experimental animals. By the end of the experiment, the blood glucose level

TABLE 2. Effect of XA on Insulin Secretion by B cells of Isolated Pancreatic Islets (ng/islet/3 h)

Experimental conditions	Without XA	XA, μg/ml/30 min			
		75	150	300	600
1.5 mmol glucose	1.14	21.07	7.78	2.89	1.79
5.0 mmol glucose	1.13	18.80	10.59	2.97	1.42
10.0 mmol glucose	4.84	9.95	10.95	1.49	1.31
10.0 mmol glucose+0.1 mmol IBMX	20.83	27.82	9.28	4.62	3.59
15.0 mmol glucose	14.76	16.93	9.14	13.14	5.31
ng/islet	129.51	103.42	67.34	65.35	26.83

TABLE 3. Effect of XA on the Intensity of Luminescence of B Cell Cytoplasm (rel. units)

Staining	Control	XA
Diethyl pseudoisocyanine (insulin)	1.00±0.04	0.08±0.01
8TSAQ (zinc)	1.00±0.06	0.1±0.01

was within 9.8-12.3 mmol/liter *vs.* 7.9-9.9 mmol/liter in the control.

The glucose tolerance test revealed considerable differences in blood glucose level between the control and experimental group 2 h after administration (Table 1); in experimental animals, the blood glucose did not return to normal.

Table 2 demonstrates that XA has pronounced and dose-dependent inhibitory effect on insulin secretion in pancreatic B cells.

Histochemical analysis showed that insulin almost completely disappears from the cytoplasm of B cells (Fig. 1, *a-d*, Table 3).

In the reaction with 8TSAQ practically no zinc-positive staining was observed in B cells treated with XA (Fig. 1, *e, f*), which under our experimental conditions may be due to the formation of XA—Zn complexes.

Thus, our findings suggest that both *in vivo* and *in vitro* XA treatment is accompanied by a marked decrease in the content of insulin in B cells, inhibition of insulin secretion and disappearance of Zn-positive reaction in B cells. The last phenomenon is of great importance, since XA is a 8-hydroxyquinoline derivative, and the ability of such compounds to form toxic complexes with insular zinc (chelates), which induce irreversible changes in B cells as soon as 15-20 min postinjection resulting in the development of type I diabetes, plays a chief role in the pathogenesis of experimental diabetes mellitus induced by these agents [4,5]. This problem becomes more interesting when the data on diabetogenic effects of 8-hydroxyquinoline derivatives are taken into consideration:

- 8-hydroxyquinoline derivatives (including XA) form a most toxic 1:1 chelate with zinc ions [1];
- preliminary elimination of zinc ions from B cells or temporary binding of these ions in more stable complexes with nondiabetogenic compounds prevents the development of diabetes mellitus in experimental animals [4,5];
- elimination of 8-hydroxyl group from XA molecule leads to the loss of both complex-forming and diabetogenic properties [10].

Our findings and recent data [12] suggest that zinc blockage probably plays a role in the pathogenesis of XA-induced disturbances.

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