EFFECT OF CYCLOSPORIN A ON THE INSULAR APPARATUS OF MICE

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KEY WORDS: cyclosporin; blood sugar; pancreatic islets; zinc

Cyclosporin A (CSA), which affects the T-cell component of immunity, is being increasingly used in immunosuppressive treatment [6-8]. Accordingly, research into the side effects of the preparation is indicated. The contradictory nature of information on its effect on the insular apparatus [5] served as the basis for the present investigation. Techniques developed by ourselves, based on the obtaining of a highly selective cytochemical reaction of 8-(p-toluenesulfonylamino)-quinoline (8-TSQ) for zinc were used. We showed previously, that this reaction, obtained in sections of the pancreas, is a sensitive indicator of the functional state of insulin-producing cells [3]. In response to injection of 8-TSQ into animals selective damage to these cells takes place, so that different forms of pancreatic diabetes can be obtained depending on the dose of the agent [1].

EXPERIMENTAL METHOD

Experiments were carried out on 88 mice. CSA was injected intramuscularly: as a single dose of 145 mg/kg or as a course of daily injections of 7 mg/kg for 3 weeks [2]. Injections of 8-TSQ were given intravenously in a single dose of 50 mg/kg [1]. In a separate series of investigations a single injection of CSA was given 24 h before injection of 8-TSQ. The blood sugar level of the control and experimental animals was determined (in the latter case, 5 days after the last injection). Blood samples were taken, after which the mice were sacrificed and pieces of the pancreas were fixed in cold (4°C) acetone for 12 h. Dewaxed sections 10 μ thick were treated for 0.5-1 min with a 0.01% acetone solution of 8-TSQ, washed with distilled water, and examined in luminescent light (FS-1 and ZhS-18 filters), The intensity of the cytochemical reaction was assessed on a 3-point scale: weak (1 point), moderate (2 points), and intensive reaction (3 points).

EXPERIMENTAL RESULTS

The blood sugar was within normal limits in all groups of mice tested except animals receiving combined injections of CSA and 8-TSQ, which in most cases developed diabetes (Table 1). In sections fluorochromed with 8-TSQ, yellowish green luminescence of the islets was observed against the dark background of the exocrine parenchyma of the gland (Fig. 1). Under high power of the microscope, small granules giving the same color of fluorescence, were found in the cytoplasm of the pancreatic B cells.

In all the experimental animals the intensity of the cytochemical reaction of 8-TSQ in insulin-producing cells was significantly lower than in intact mice (control; Table 1). The most marked changes were observed after combined injection of CSA and 8-TSQ.

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TABLE 1. Blood Sugar and Intensity of Cytochemical Reaction of 8-(p-Toluenesulfonylamino)-quinoline (8-TSQ) in Insulin-Producing Cells of Mice after Injection of Cyclosporin A (CSA) and 8-TSQ

Agent	Number of animals	Blood sugar, mmoles/liter		Intensity of reaction with 8-TSQ	
		$(\bar{X}\pm m)$	p	$(\bar{X}\pm m)$	p
Control	40	6,8±0,45		2,1±0,18	
CSA	12	7,4±0,59	>0,5	1,5±0,23	<0,05
8-TSQ	25	8,1±0,73	>0,05	1,4±0,19	<0,02
CSA + 8-TSQ	11	11,5±0,62	<0,001	1,1±0,20	<0,001

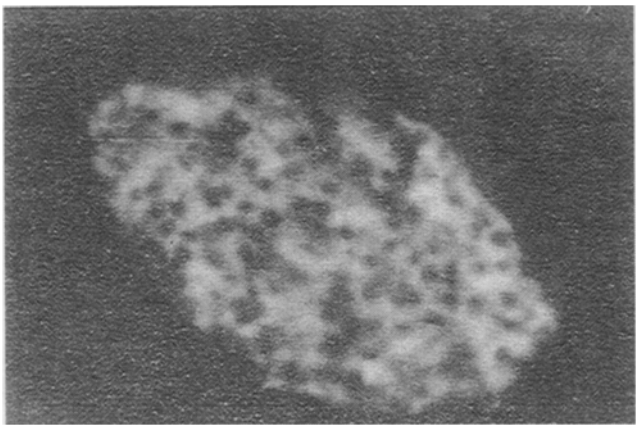

Fig. 1. Cytochemical luminescence reaction of 8-(p-toluenesulfonylamino)-quinoline in a mouse pancreatic islet. 280×.

Previous investigations [3] showed that since bound with the hormone insulin, can be detected by the cytochemical reaction of 8-TSQ in pancreatic B cells. It can be tentatively suggested that in our experiments the decrease in the zinc concentration in these cells was an indicator of insulin deficiency in them. In response to the combined injection of CSA and 8-TSQ these changes were accompanied by hyperglycemia, evidence of the development of a manifest form of diabetes, but in cases when only one of these substances was injected, the decrease in the zinc concentration in the insulin-producing B cells was accompanied by normoglycemia. Evidently in the last cases there was a latent deficiency of the insular apparatus. These results may be confirmation of the view expressed by a number of workers [5], that administration of CSA to persons with signs of diabetes in a manifest or latent form is contraindicated.

After injection of CSA into mice we observed zinc deficiency also in the cells of other organs besides the pancreas. These disturbances evidently can also be classed as complications caused by this agent. In this case it might be supposed that measures aimed at correcting disturbances of cellular metabolism of zinc ought to lead to weakening or abolition of some of the side effects of CSA and, in particular, disturbances of the internal secretory function of the pancreas.

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EFFECT OF MPTP ON NEURONAL UPTAKE OF MONOAMINES

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The substance 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) causes the development of clinical symptoms characteristic of parkinsonism in man and is used to create a model of experimental parkinsonism in monkeys and certain other laboratory animals [1, 6, 8, 9, 11]. The mechanism of the toxic effect of MPTP, leading to degeneration of dopamine-synthesizing neurons of the substantia nigra of the brain, is considered to be the formation of 1-methyl-4-phenylpyridine (MPP) from MPTP in the astroglia under the influence of monoamine oxidase. MPP, it is considered, is taken up by the neuronal dopamine uptake system and interacts with components of the cell to form toxic substances, which cause death of the neurons and, as a result, reduce production of dopamine and its metabolites in the striatum [5, 7, 10]. However, injection of L-dopa, which raises the dopamine level in the striatum, does not affect the development of symptoms of parkinsonism and manifestation of the neurotoxicity of MPTP [13]. Considering that the dopamine transport system is closely interconnected with other systems of monoamine transfer of high and low affinity [2-4], the aim of the present investigation was to study the effect of MPTP on the systems for synaptosomal reuptake of dopamine, noradrenalin, adrenalin, and serotonin.

EXPERIMENTAL METHOD

Uptake of monoamines was determined by a modified method of Snyder and Coyle [14]. The coarse synaptosomal fraction was obtained by centrifugation of a 10% brain homogenate from male albino rats weighing 180-200 g, in 0.32 M sucrose at 1000*g* for 20 min. The supernatant was recentrifuged at 11,000*g* for 15 min. The residues containing synaptosomes, mitochondria, and myelin were resuspended in 0.32 M sucrose. For the experiments 50 μl of a suspension of synaptosomes was taken (on average 0.3 mg protein) and this was added to 1 ml of an incubation medium of the following composition: 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.14 mM $MgCl_2$, 5 mM Na_2PO_4, 10 mM glucose, 10 mM sucrose, 0.125 mM pargyline, and 30 mM Tris-HCl, pH 7.4 The incubation medium also contained MPTP and the monoamine: H-D,L-noradrenalin (specific radioactivity 280 TBq/mole), ^{3}H-D,L-adrenalin (93 TBq/mole), ^{3}H-dopamine (492 TBq/mole, from Izotop Leningrad): ^{3}H-serotonin (407 TBq/mole, from Amersham International UK), Incubation was carried out at 37°C for 3 min with continuous shaking. Binding of the mediator by synaptosomes was stopped by filtration of 0.5 ml of the incubation medium through "Millipore" membrane

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