PHARMACOLOGY AND TOXICOLOGY

Effect of Phensuccinal on Pancreatic β-Cells in Rats with Neonatally Induced Streptozotocin Diabetes Mellitus

N. I. Gorbenko, V. V. Poltorak, A. I. Gladkikh, and O. V. Ivanova

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The effect of phensuccinal, a low-toxic succinic acid derivative, on the function of pancreatic β-cells in the evolution of absolute insulin insufficiency was studied in rats with neonatally induced streptozotocin diabetes mellitus. Phensuccinal (25 mg/kg body weight) prevented disorders in the secretory response of β-cells to glucose load at all stages of the study (2, 5, and 14 days after diabetes induction). This effect was realized via stimulation of the regenerative processes in the insulin-producing system of the pancreas and activation of the antioxidant system in diabetic animals.

Key Words: *phensuccinal; streptozotocin-induced diabetes; pancreatic β-cells; insulin secretion*

The principal physiological function of pancreatic β-cells is the synthesis, accumulation, and secretion of insulin. However their functional activity can be insufficient because of autoimmune destruction, which is observed in patients with type I diabetes mellitus, or due to increased resistance of peripheral tissues to insulin in type II diabetes. Secretory reaction of β-cells to increased blood glucose in type II diabetes mellitus can be reduced due to various metabolic defects: impaired synthesis of glucose transporter, glucokinase mutation, increased activity of glucose-6-phosphatase, low activity of glycerolphosphate dehydrogenase and impaired glycogen synthesis [11]. Recent studies demonstrated that these disorders in glucose oxidation, leading to energy exhaustion of β-cells, can be corrected by succinic acid esters, which can penetrate into pancreatic β-cells and enter the Krebs cycle, thus restoring ATP synthesis needed for adequate insulin secretion [9].

We previously showed that phensuccinal, a low-toxic succinic acid derivative now recommended for clinical trials, produces a pronounced antioxidant effect and prevents the development of experimental insulin resistance [1,7]. Here we studied the effect of phensuccinal on the function of pancreatic β-cells after their subtotal destruction with streptozotocin in newborn rats.

MATERIALS AND METHODS

The study was carried out on 82 newborn Wistar rats. Absolute insulin insufficiency was induced by intraperitoneal injection of streptozotocin (100 mg/kg) to one-day-old rat pups [10]. Phensuccinal (25 mg/kg, intraperitoneally) or placebo was injected 1 h after streptozotocin and then daily for 2, 5, and 14 days. These time points were chosen basing on the dynamics of the development of absolute insulin insufficiency in animals with neonatally induced diabetes. Basal glycemia, secretory reaction of isolated pancreatic islets to glucose, and oxidative status of experimental animals were evaluated. Blood glucose was measured by the glucose oxidase method on an Exan-G analyzer. Pancreatic islets were isolated as described elsewhere [8]. Five islets of the same size were incubated in

Laboratory of Pathophysiology, V. Ya. Danilevskii Institute of Endocrine Diseases, Ukrainian Academy of Medical Sciences, Kharkov. ***Address for correspondence:*** gorbenko@email.itl.net.ua. Gorbenko N. I.

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Krebs—Ringer buffer containing HEPES, serum albumin, 18 amino acids, and 5 or 15 mM glucose. Insulin concentration in the incubation medium was measured by radioimmunoassay using Belaris kits. Oxidative status of experimental animals was evaluated by the concentrations of MDA [3] and reduced glutathione [6] in liver homogenates. For histological analysis the caudal part of the pancreas was fixed in Carnoy fluid. The sections were stained with hematoxylin and eosin by the method of Meyer [2] and the number of islets and their area per cm^2 of histological preparation were evaluated [12].

The results were statistically processed using Student's *t* test.

RESULTS

Two days after streptozotocin injection to newborn rats acute insulin deficiency developed and basal glycemia sharply increased because of subtotal destruction of pancreatic β-cells, but on day 5 blood glucose concentration started to decrease due to spontaneous regeneration of β-cells and accumulation of insulin. Two weeks after modeling of diabetes mellitus blood glucose returned virtually to normal [4]. Despite normalization of basal glycemia, regeneration of β-cells was incomplete (39% of the control). This fact can explain the subsequent decrease in the functional activity of β-cells and the development of relative insulin insufficiency 6 weeks after streptozotocin injection [13]. The mechanisms of incomplete regeneration of β-cells after their subtotal destruction are unknown; a possible explanation is normalization of blood glucose 2 weeks after diabetes induction, *i.e.* liquidation of the main stimulus to regeneration [14].

Treatment with phensuccinal for 2 and 5 days reduced basal hyperglycemia in comparison with the diabetic control (Fig. 1). It should be noted that on day 14 of the experiment, blood glucose level was normal in all groups of animals, which indicates normalization of glucose homeostasis due to spontaneous regeneration of β-cells. The more rapid normalization of blood glucose can be due to intensification of regenerative processes in the pancreas in rats receiving phensuccinal compared to diabetic controls. This is confirmed by morphometric analysis of the endocrine part of the pancreas, which showed increased number and total area of Langerhans islets on day 5 after streptozotocin injection in animals treated with phensuccinal in comparison with diabetic controls (Table 1).

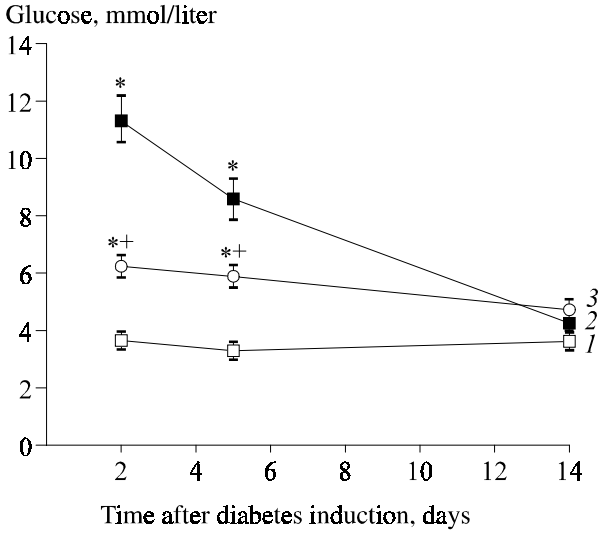

Fig. 1. Effect of phensuccinal on basal blood glucose level in rats after neonatal induction of streptozotocin diabetes. *1*) control; *2*) diabetes+placebo; *3*) diabetes+phensuccinal. Significant differences from: *control; +placebo.

Normally, pancreatic β-cells adequately respond to physiological secretogens, *e.g.* to glucose. In diabetic animals receiving placebo β-cells were tolerant to glucose stimulation: increased glucose concentration in the incubation medium did not stimulate insulin secretion (Fig. 2.) Phensuccinal therapy prevented the development of functional deficiency of β-cells at all stages of insulin insufficiency, which was confirmed by adequate secretory reaction to increased glucose level 2, 5, and 14 days after diabetes induction.

According to modern concepts of the pathogenesis of type I diabetes mellitus, free radicals act as intercellular mediators of β-cell destruction. The resistance of β-cells to damaging factors depends on activity of the antioxidant system [5]. In our experiments MDA content in liver homogenates from placebo-treated rats considerably increased on day 2 after streptozotocin injection and was virtually the same as in intact controls at later terms (Fig. 2, *c*). The inhibition

Table 1. Morphometry of Endocrine Pancreas 5 Days after Induction of Neonatal Streptozotocin Diabetes ($X \pm Sx$, $n=9$)

Parameter	Control	Diabetes	
		+placebo	+phensuccinal
Number of Langerhans islets per cm^2	5.6±0.6+	2.1±0.7*	4.0±0.7+
Mean area of islets, μ^2	72.4±4.2+	26.3±2.8*	51.2±5.1*+

Note. $p<0.05$: *compared to the control, +compared to placebo.

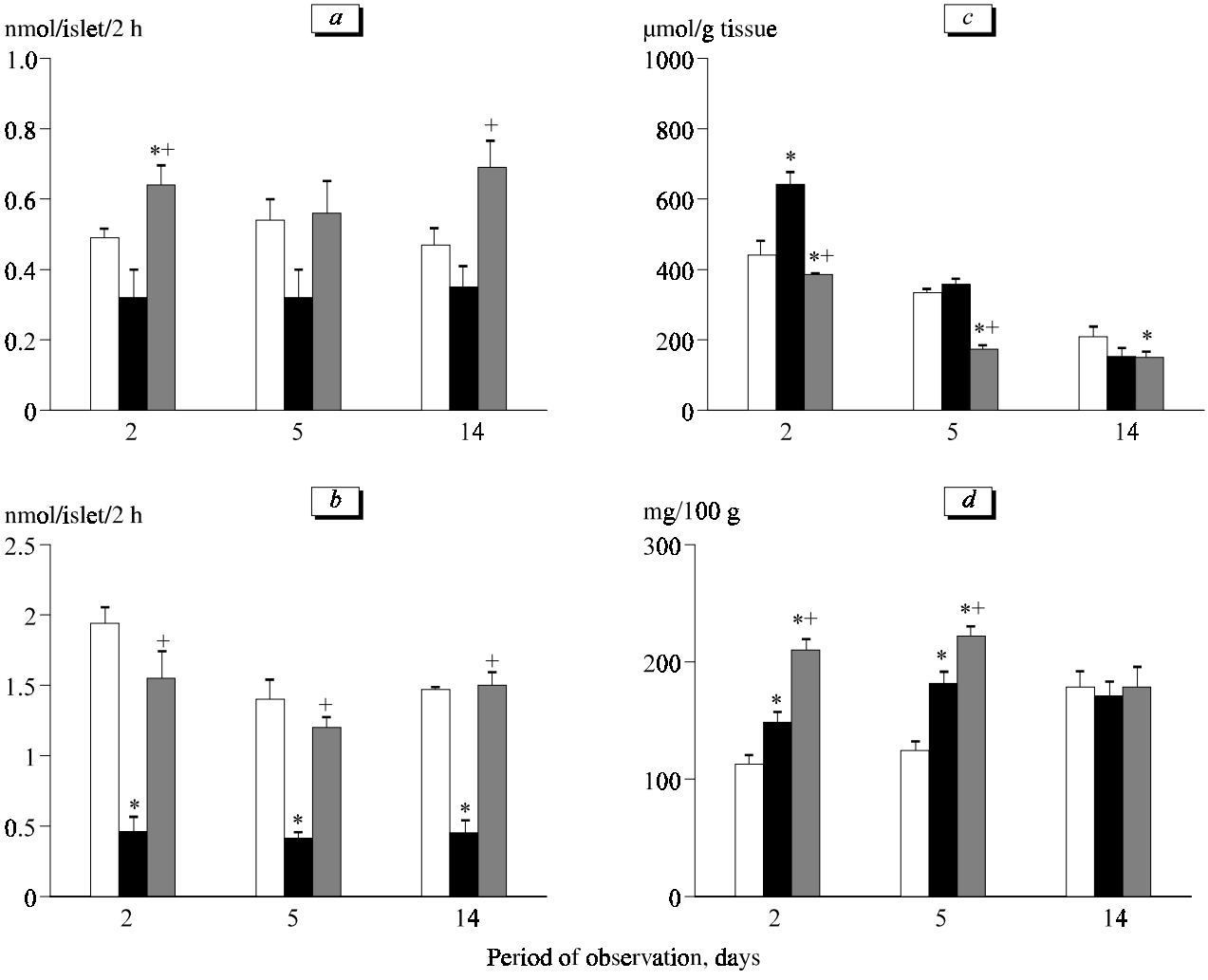

Fig. 2. Effect of phensuccinal on insulin secretion in response to 5 (*a*) and 15 mM (*b*) glucose, content of MDA (*c*) and reduced glutathione (*d*) at various terms after neonatal induction of streptozotocin diabetes. Light bars: control; dark bars: diabetes+placebo; cross-hatched bars: diabetes+phensuccinal. $p<0.05$: *compared to the control, +compared to placebo.

of LPO correlated with normalization of basal blood glucose on days 5 and 14 after induction of diabetes. It should be noted that the content of reduced glutathione in animals receiving placebo increased on days 2 and 5 days after injection of streptozotocin, which also contributes to inhibition of free radical oxidation on days 5 and 14 of the experiment. Phensuccinal prevented streptozotocin-induced increase of MDA level in the liver at the peak of hyperglycemia (2 days after diabetes induction) and reduced this parameter on day 5 of the experiment in comparison with the placebo group (Fig. 2). The antioxidant activity of phensuccinal can be explained by its effect on the content of reduced glutathione (in phensuccinal-treated rats this parameter was higher than in the placebo group 2 and 5 days after streptozotocin injection).

Thus, phensuccinal prevented functional incompetence of pancreatic β-cells in the evolution of absolute insulin insufficiency in rats with neonatally induced streptozotocin diabetes. This protective effect is realized through stimulation of regenerative processes in the insulin-producing system of the pancreas and activation of the antioxidant system in diabetic animals.

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