Mechanisms of Hyperglycemic Effect of Calcitonin

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We studied the effect of calcitonin on blood glucose level, total calcium content, and the main stages of carbohydrate metabolism: absorption in the intestine, transport from the blood to tissues, tissue sensitivity to insulin in the whole organism, glycogen content, and activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase in the liver. A hyperglycemic effect of calcitonin was demonstrated, a close negative correlation was found between glucose level and total calcium content (r=-0.834, p<0.02). Calcitonin had no effect on glucose absorption in the small intestine and its transport from the blood to tissues (glucose consumption by peripheral tissues at the organism level), but reduced glycogen content in the liver and increased activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase.

Key Words: *calcitonin; glucose; glycogenolysis, glucose-6-phosphate dehydrogenase; lactate dehydrogenase*

Calcitonin (CT) is a broad-spectrum hormone; it reduces blood concentration of calcium and affects its intracellular distribution. Wide use of CT in medical practice [5-7,9-13] necessitates detailed investigation of not only its specific, but also non-specific effects realized beyond the target organs. Of particular interest in this respect is the effect of CT on carbohydrate metabolism. Hyperglycemic effects of salmon and porcine CT [2,8] and CT-gene-related peptide [8] are well established. The level of glycemia is determined by several factors, including the proportion between the rates of different processes, glucose absorption in the intestine, their utilization by peripheral tissues, glucose release from the liver, and gluconeogenesis processes. Here we studied the effect of porcine CT, a Russian-made preparation calcitrin, on glucose adsorption in the small intestine, its transport from the blood to tissues, insulin sensitivity of tissues, and the effect of the preparation on glycogenic and glucostatic functions of the liver.

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MATERIALS AND METHODS

We performed five experimental series. In series I (110 rats) we studied the effect of single intramuscular injection of CT in a dose of 1 U/100 g body weight on blood glucose concentration at rest after fasting (basal level) and every 30 min after hormone injection over 240 min. For evaluation of the efficiency of CT, total calcium content in blood plasma was measured. Blood samples were taken under light ether narcosis from the femoral vein.

In series II (20 rats), the effect of CT on glucose absorption in the small intestine was studied by the Verzar method [3]. In brief, the rats were anesthetized with ether and after laparotomy the intestine was ligated in the proximal part of the duodenum and washed with physiological saline. The remaining solution was blown out to the large intestine and the second ligature was applied at the boundary between the small and large intestine. After that, 5% glucose solution was injected into the intestine (200 mg/100 g body weight). The precise content of glucose in the administered solution was measured spectrophotometrically. After 30 min, the intestine was removed and its content was

collected into graduated tubes. The volume of collected fluid was measured and glucose concentration was determined, after that the difference between the amounts of administered and collected glucose was calculated. Absorption was expressed in mg per 100 g body weight. CT in a dose of 1 U/100 g body weight was injected 45-60 min before the experiment.

In series III (10 rabbits), the effect of CT on glucose transport from the blood into tissues was evaluated on the basis of mathematical analysis of glycemic curves after intravenous injection of glucose by a simple exponential dependence. Each curve was transferred onto a semilogarithmic plot (where ordinate was logarithm of glucose concentration and abscissa was time). To find curve fragments approximating strait lines, the results were multiplied by 100 and the rate of glycemia decrease was expressed in percents per minute. The rate of glucose transport from the blood to tissues was calculated by the formula:

$$K = \frac{\log C_1 - \log C_2}{t_2 - t_1} \times 2.3,$$

where C_1 and C_2 are glucose concentrations in the beginning and end of the exponent, respectively, t_1 and t_2 are the corresponding time intervals after glucose injection (min), and 2.3 is a coefficient for conversion of decimal logarithms to natural ones.

To simplify calculations, the values on the axes were expressed not in logarithms of glucose concentrations, but in antilogarithms, *i.e.* the corresponding values in mmol/liter. Glucose (40% solution) was injected into the marginal ear vein (0.5 g/kg body weight). The blood for measuring glucose concentration was sampled from the marginal vein of the other ear after 1, 5, 10, 20, and 30 min. The experiments were carried out without (control) or 40 min after injection of 1 U/100 g calcitrin.

In series IV (5 rabbits), insulin sensitivity of tissues against the background of CT treatment was studied by the method proposed by Lazarus and Volk [3] implying intravenous injection of insulin together with glucose, *i.e.* insulin acts under conditions of elevated blood glucose content. In this case, anti-insulin mechanisms are not activated. The decrease of glycemic curve height during this period reflects tissue glucose absorption under the effect of insulin. The decrease in blood glucose content compared to its initial value depends on the interaction between glucose utilization in tissues and the action of compensatory factors. The experiments were performed without (control) and against the background of CT treatment. In brief, the blood was sampled from the marginal ear vein for blood glucose assay and then insulin (0.5 U/kg body weight) and 5 ml 40% glucose solution (within 20-25 sec) were injected intravenously. Blood glucose level was measured 1, 30, and 60 min after injection of insulin and glucose. CT was injected 30 min before the experiment in a dose of 1 U/kg.

In series V, CT in a dose of 1 U/100 g body weight was injected to 18 rats intramuscularly 1 h before decapitation. Homogenate of the liver (1 g) prepared on cold was centrifuged at 3000 rpm for 10 min. Glycogen content and activities of glucose-6-phosphate dehydrogenase (G-6-PD) were measured in the supernatant. Blood glucose concentration was measured by the method of Frank–Kirberger and the total content of calcium was assayed by complexonometric method with murexide indicator. Glycogen content was measured by the method of Seifter [4], G-6-PD and LDH activities were assayed using Calbiochem and Fermognost kits, respectively.

The data were processed statistically using Student–Fisher tests.

RESULTS

Injection of CT to mature rats (Table 1) induced maximum rise of blood glucose level 60 min: from 5.2±0.2 to 6.2±0.1 mmol/liter ($p<0.001$). Total calcium content decreased from 2.03±0.01 to 1.68±0.03 mmol/liter ($p<0.001$). After 240 min, glucose level approximated the initial level (5.7±0.3 mmol/liter, $p>0.1$), while calcium content was 1.83±0.05 mmol/liter, $p<0.01$). A close negative correlation was found between glucose level and total calcium content ($r=-0.834$, $p<0.02$). The maximum rise of blood glucose level after CT injection in rats was 19±2% and total calcium content decreased to 1.58±0.03 mmol/liter, which constituted 83±2% of the initial level. Analysis of these results suggests that CT produced a pronounced hyperglycemic effect in rats.

Results of experimental series II showed that glucose absorption in the small intestine of rats receiving CT did not differ from that in the control (105±3 and 101±1 mg/100 g, respectively; $p>0.2$). Hence, administration of CT to rats did not affect glucose absorption in the small intestine. In experimental series III, similar coefficients characterizing glycemia decrease in the control and against the background of CT treatment were obtained (3.4±0.4 and 3.5±0.4% per minute). Hence, CT had no effect on the rate of glucose transport from the blood to tissues, *i.e.* no effects of CT on glucose consumption in tissues were revealed in experiments on the whole organism. In series IV, blood glucose content sharply increased in both control and CT-treated animals 1 min after injection of glucose and insulin (Table 2). During the next 30 min, blood glucose content in controls progressively decreased

TABLE 1. Effect of Single Administration of CT on Glucose and Calcium Levels (*M*±*m*)

Parameter	Initial level	Time after injection, min					
		30	60	90	120	150	240
Glucose, mmol/liter	5.2±0.2 (11)	5.60±0.06* (6)	6.2±0.1**** (11)	5.9±0.2* (6)	5.70±0.06*** (11)	5.8±0.2* (11)	5.7±0.3 (11)
Calcium, mmol/liter	2.03±0.01 (6)	1.58±0.03**** (6)	1.68±0.03**** (6)	1.73±0.03**** (6)	1.80±0.02**** (6)	1.80±0.08*** (6)	1.83±0.05** (6)

Note. Number of animals is shown in parentheses. *p<0.05, **p<0.01, ***p<0.02, ****p<0.001 compared to initial values.

and approximated the initial level. During the period from minute 31 to minute 60, blood glucose content continued to decrease remaining within the hypoglycemic range. During the first 30 min of observation, the rate of glycemia decrease in rabbits receiving CT was lower than in controls (Table 2), which can be considered as reduced tissue sensitivity to insulin. Then (minutes 31-60), the rate of glycemia decrease in rabbits receiving CT was higher than in controls (p>0.1). It can be concluded that administration of CT reduced tissue sensitivity to insulin.

It is well known that the liver is an important regulator of carbohydrate metabolism. In light of this it was of primary importance to study the effect of CT on glucose depot, in particular on glycogen content and activity of liver enzymes. We found that CT reduced glycogen content in the liver from 18.71±1.30 to 11.16±1.70 μmol/g tissue (p<0.01). Activity of liver enzymes also significantly increased: G-6-PD from 4.832±0.600 to 7.254±0.100 μmol reduced NADP/min/g wet tissue (p<0.01) and LDH from 8.912±0.800 to 11.376±0.500 oxidized $NADH_2$/min/g wet tissue (p<0.05; Table 3). Hence, administration of CT under these conditions reduced glycogen content in the liver and increased activity of liver enzymes G-6-PD and LDH. In other words, CT stimulated glycogenolysis by activating liver enzymes.

Thus, our experiments showed that CT produced a hyperglycemic effect, decreased tissue sensitivity to insulin, considerably reduced glycogen content in the liver, and increased activity of G-6-PD and LDH. These findings agree with previous reports [8,14]. For instance, salmon CT stimulates glycogenolysis in skeletal muscles and inhibits glucose incorporation into glycogen [14], while CT-gene-related peptide increases plasma glucose content and stimulates endogenous production of glucose and glycogenolysis in the liver [8]. In contrast to insulin stimulating glucogenesis, CT stimulates glycogenolysis by activating the key enzymes of glycolysis in liver cells. It can be hypothesized that this effect of CT is a mechanism underlying the observed hyperglycemic effect of the hormone. It should be also taken into account that CT reduces the sensitivity of the muscular and adipose tissues to insulin [1] and inhibits insulin secretion [14]. The mechanism of hyperglycemic effects of CT preparations is poorly studied; previous findings suggest that they are related to inhibitory effects of CT on insulin secretion

TABLE 2. Effect of CT Administration on the Course of Glycemia after Simultaneous Intravenous Injection of Glucose and Insulin (*M*±*m*)

Experimental conditions	Glucose, mmol/liter				Rate of glycemia decrease, mmol/liter/min	
	initial	time after injection, min				
		1	30	60	minutes 1-30	minutes 31-60
Control	6.20±0.06	17.60±1.05	5.2±0.5	3.8±0.4	0.40±0.04	0.04±0.01
CT	6.3±0.1	16.5±1.0	7.6±0.7	5.1±0.2	0.29±0.01*	0.08±0.02

Note. Five samples were assayed in each case. *p<0.05 compared to the control.

TABLE 3. Effect of CT on Glycogen Content and Activities of G-6-PD and LDH in Rat Liver (*M*±*m*)

Experimental conditions	Glycogen, μmol/g tissue	G-6-PD, μmol reduced NADP/min/g wet tissue	LDH, μmol oxidized $NADH_2$/min/g wet tissue
Control	18.71±1.30	4.832±0.600	8.912±0.800
	(*n*=6)	(*n*=5)	(*n*=6)
CT	11.16±1.70**	7.254±0.100**	11.376±0.500*
	(*n*=12)	(*n*=10)	(*n*=7)

Note. *$p<0.05$, **$p<0.01$ compared to the control.

[14] and glucose utilization by peripheral tissues [1] and activation of glycogenolysis processes [14].

Thus, we can conclude that CT has no effect on glucose absorption in the small intestine, but modulates the main stages of its metabolism by activating glycogenolysis and increasing insulin-resistance of peripheral tissues. It can be accepted that CT participates in neuroendocrine regulation of glucose metabolism, especially under conditions of hypercalcitoninemia.

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