PHYSIOLOGY

Calcium Channel Blockers Inhibit the Hyperglycemic Effect of Calcitonin

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We studied the effect of Russian preparation of porcine calcitonin (calcitrin, 1 U/100 g) and blockers of Ca^{2+} channels nifedipine (1 mg/kg, blocker of chemosensitive Ca^{2+} channels) and isoptin (5 mg/kg, blocker of voltage-dependent Ca^{2+} channels) on glucose metabolism. Calcitonin produced a pronounced hyperglycemic effect in rats. A close negative correlation was found between glucose level and total calcium content. Injection of calcitonin reduced glucose tolerance in rats. Isoptin and nifedipine reduced plasma calcium level and did not affect significantly the blood glucose levels, did not change the pattern of alimentary hyperglycemia in response to glucose load, completely abolished the hyperglycemic effect of calcitonin, and prevented calcitonin-induced impairment of glucose tolerance.

Key Words: *calcitonin; calcium channel blockers; hyperglycemia; correlation; inhibition*

Hyperglycemic effect of calcitonin (CT) [3] and impaired glucose tolerance in children aged 10-14 years with I degree obesity against the background CT administration [2] were reported. It was also demonstrated that CT inhibits biological effect of insulin: reduces insulin-stimulated glucose uptake in adipose and muscle tissue *in vivo* and *in vitro* in rats, while isoptin, calcium channel blocker (CCB), suppresses this effect of CT [1]. There are data on metabolic neutrality of CCB [6,8,9]. At the same time, little is known on their impact on glucose metabolism and interaction with calcium-regulating hormone CT. Here we studied the effects of CT and CCB on glucose metabolism and interaction of these two classes of pharmacological agents and analyzed possible mechanisms underlying hyperglycemic effect of CT.

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MATERIALS AND METHODS

Experiments were performed on 200 male Wistar rats weighing 100-150 g. The rats were divided into 8 groups. Group 1 animals received a single intramuscular injection of porcine CT (calcitrin, Russia) in a dose of 1 U/100 g body weight. Rats of group 2 intraperitoneally received 5 mg/kg isoptin and 1 mg/kg nifedipine, respectively. Blood glucose levels and total plasma calcium content at rest on an empty stomach (initial level) and every 30 minutes after administration of these drugs over 240 min. Blood samples were taken under light ether anesthesia from dissected femoral vein. Animals of groups 4 and 5 30 min after CT (1 U/100 g body weight) received nifedipine (1 mg/kg, group 4) and isoptin (5 mg/kg, group 5); blood glucose levels were measured every 30 min over 240 min. Group 6 rats received glucose load: 30% glucose solution *per os* (1 ml/100 g body weight) and then blood samples were taken every 30 minutes (30-240 min) for measuring of glucose concentrations. Groups

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7 and 8 rats received intramuscular injections of CT (1 U/100 g body weight), and after 30 min glucose load was performed (as in the group 6) and then nifedipine (1 mg/kg, group 7) and isoptin (5 mg/kg, group 8) were injected intraperitoneally. Blood samples were taken every 30 min (30-240 min). To estimate the glycemic curves, hyperglycemic index (blood glucose ratio of maximum to the initial level) and hypoglycemic index (blood glucose ratio of minimum to the initial level) were calculated. In animals of groups 4-8, blood samples were taken from the caudal vein. A correlation coefficient between hyperglycemic effects of plasma calcium level in blood was determined. Blood glucose concentration was assessed by the method of Frank and Kirberger [11]; total calcium content in the plasma was assayed by complexonometric method [7].

The data were processed statistically using Student–Fisher test.

RESULTS

In rats in group 1, CT injection in a dose of 1 U/100 g body weight significantly increased glucose level as early as after 30 min and it surpassed the initial value at al terms of the experiment. Blood glucose level peaked 60 min postinjection (6.2±0.1 *vs.* the initial concentration of 5.2±0.2 mmol/liter, $p<0.001$). At the same time, total calcium content decreased from 2.03±0.01 to 1.68±0.03 mmol/liter ($p<0.001$). In 240 min, glucose and calcium levels practically returned to initial values: 5.7±0.4 mmol/liter ($p>0.1$) and 1.83±0.05 mmol/liter ($p<0.01$), respectively (Table 1, Fig. 1). The dynamics of hyperglycemia induced by CT injection CT-induced decrease in blood calcium concentration coincided. Close negative correlation was established between glucose levels and total calcium content ($r=-0.834$, $p<0.02$). Thus, CT administration reduced the content of total calcium in the blood plasma and in addition increased blood glucose levels with subsequent normalization of these parameters after 240 min.

In group 2, isoptin decreased the content of total calcium in the blood plasma from 2.08±0.03 mmol/liter to a minimum level (1.4±0.05 mmol/liter) on the 60th minute of the experiment; then calcium level gradually increased and returned to the initial level by the 210th minute. Glucose concentration remained virtually unchanged (Table 1, Fig. 1). Similar results were obtained with the introduction of nifedipine (group 3). Hence, isoptin and nifedipine decreased the total calcium plasma levels and had no reliable effect on blood glucose, which was consistent with previous reports [4,5].

Of particular interest was to study mutual influence of CT and CCB on glucose levels (Table 2, Fig. 2). Combined administration of CT and nifedipine (group 4) did not significantly increase glucose level. In addition, the hyperglycemic effect of CT was suppressed by nifedipine. Thus, the maximum rise of blood glucose has been achieved in 60 min like in the case of single CT, but the rise was significantly lower both compared with initial level, and with decreased glucose level after CT injection. Similar dynamics of glycemia was also observed after combined administration of CT and isoptin in experimental group 5. Therefore, CCB isoptin and nifedipine completely suppressed the hyperglycemic effect of CT.

We previously have mentioned impaired CT-induced glucose tolerance during oral glucose tolerance test [2]. We thought it appropriate to investigate the impact of combined administration of the studied preparations on glucose levels during oral glucose load.

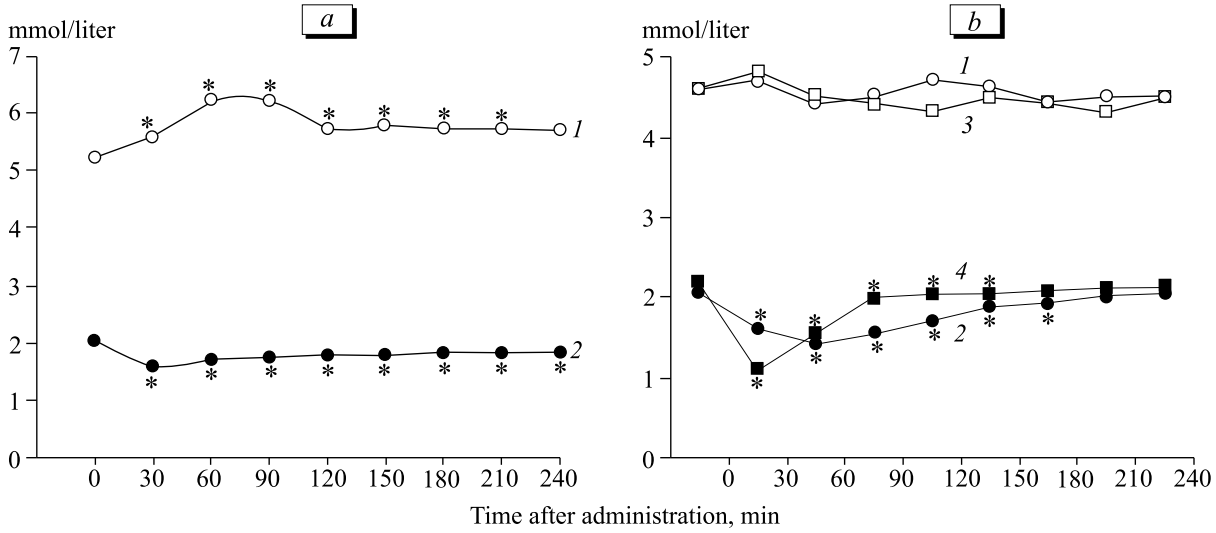

Fig. 1. Effects of single injection of CT (*a*) and CCB (*b*) on glucose and calcium levels. *a*: *1*) glucose; *2*) calcium; *b*: isoptin (*1*, glucose; *2*, calcium); nifedipine (*3*, glucose; *4*, calcium).*$p<0.05$: in comparison with initial values.

TABLE 1. Effects of CT, Isoptin, and Nifedipine on Glucose and Calcium Levels

Parameter	Initial level	Time after injection, min							
		30	60	90	120	150	180	210	240
CT									
Calcium, mmol/liter	2.03±0.01 (6)	1.58±0.03 (6)	1.68±0.03 (6)	1.73±0.03 (6)	1.8±0.02 (6)	1.8±0.08 (6)	1.83±0.05 (6)	1.83±0.01 (6)	1.83±0.05 (6)
p		<0.001	<0.001	<0.001	<0.001	<0.02	<0.02	<0.01	<0.01
Glucose, mmol/liter	5.2±0.2 (11)	5.6±0.06 (6)	6.2±0.1 (11)	6.2±0.1 (6)	5.7±0.06 (11)	5.8±0.2 (11)	5.7±0.1 (6)	5.7±0.1 (6)	5.7±0.4 (6)
p		<0.05	<0.001	<0.05	<0.02	<0.05	<0.05	<0.05	>0.1
Isoptin									
Calcium, mmol/liter	2.08±0.03 (5)	1.6±0.1 (5)	1.4±0.05 (5)	1.53±0.03 (5)	1.7±0.05 (5)	1.88±0.03 (5)	1.9±0.02 (5)	2.0±0.08 (5)	2.05±0.03 (5)
p		<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	>0.2	>0.2
Glucose, mmol/liter	4.6±0.1 (5)	4.7±0.3 (5)	4.4±0.2 (5)	4.5±0.1 (5)	4.7±0.2 (5)	4.6±0.1 (5)	4.4±0.05 (5)	4.5±0.06 (5)	4.5±0.2 (5)
p		>0.5	>0.2	>0.2	>0.5	-	>0.1	>0.2	>0.5
Nifedipine									
Calcium, mmol/liter	2.15±0.03 (5)	1.05±0.05 (5)	1.53±0.2 (5)	1.99±0.1 (5)	2.0±0.01 (5)	2.02±0.03 (5)	2.05±0.02 (5)	2.08±0.03 (5)	2.1±0.08 (5)
p		<0.001	<0.01	<0.01	<0.01	<0.02	<0.02	>0.1	>0.5
Glucose, mmol/liter	4.6±0.2 (5)	4.8±0.1 (5)	4.5±0.05 (5)	4.4±0.3 (5)	4.3±0.1 (5)	4.5±0.06 (5)	4.4±0.2 (5)	4.3±0.1 (5)	4.5±0.1 (5)
p		>0.2	>0.5	>0.5	>0.2	>0.5	>0.2	>0.2	>0.5

Note. Here and in Tables 2, 3: number of animals is shown in parentheses. *p*, significant differences compared with initial values.

TABLE 2. Effects of CT on Glucose Level against the Background of Isoptin and Nifedipine Administration

Parameter	Initial level	Time after injection, min							
		30	60	90	120	150	180	210	240
CT									
Glucose, mmol/liter	5.2±0.2 (11)	5.6±0.06 (6)	6.2±0.1 (11)	5.9±0.2 (6)	5.7±0.06 (11)	5.8±0.2 (11)	5.7±0.1 (6)	5.7±0.1 (6)	5.7±0.4 (6)
p_1		<0.05	<0.001	<0.05	<0.02	<0.05	<0.05	<0.05	>0.1
CT+nifedipine									
Glucose, mmol/liter	5.1±0.1 (6)	5.3±0.2 (6)	5.5±0.3 (6)	5.3±0.1 (6)	5.0±0.05 (6)	5.0±0.1 (6)	4.9±0.2 (6)	4.9±0.1 (6)	4.9±0.3 (6)
p_1		>0.2	>0.2	>0.1	>0.2	>0.2	>0.2	>0.2	>0.5
p_2	>0.5	>0.1	<0.05	<0.05	<0.001	<0.01	<0.01	<0.001	0.1<p<0.05
CT+isoptin									
Glucose, mmol/liter	4.9±0.1 (6)	5.1±0.2 (6)	5.4±0.3 (6)	5.3±0.3 (6)	5.0±0.3 (6)	4.9±0.3 (6)	4.8±0.2 (6)	4.8±0.1 (6)	4.8±0.3 (6)
p_1		>0.2	>0.1	>0.2	>0.5		>0.5	>0.2	>0.5
p_2	>0.1	<0.05	<0.05	>0.1	<0.05	<0.05	<0.01	<0.001	0.1<p<0.05

Note: p_1: in comparison with initial values; p_2: in comparison with CT.

The initial glucose concentration in group 6 rats corresponded to normal (Table 3, Fig. 3). After glucose load, glucose concentration peaked after 60 min increasing to 6.2±0.3 mmol/liter (p_1<0.001); in 240 min it returned to the initial level (4.8±0.2 mmol/liter). CT significantly increased the initial glucose concentration in glucose load test; pronounced hyperglycemia was observed starting from the 30th minute and throughout the observation period. Glucose level (7.9±0.4 mmol/liter, p_2<0.001) peaked on the 120th minute and remained high to the 240th minute (7.2±0.4 mmol/liter, p_2<0.01). Hyperglycemic and hypoglycemic indices were significantly increased. Thus, CT impaired glucose tolerance. After combined administration of CT with nifedipine (group 7) or isoptin (group 8), clear antagonism was observed: attenuation of hyperglycemic effect of CT. The pattern of glycemic curves in this case was similar to that after glucose load (Table 3, Fig. 3). In other words, nifedipine and isoptin corrected glucose tolerance impairment caused by CT injection during the oral glucose tolerance test.

Differential effect of CCB drugs on specific types of Ca^{2+}-channels, *i.e.* tropism of nifedipine to slow chemosensitive calcium channels and that of isoptin to L-type slow voltage-dependent calcium channels, as well as their capacity to completely abolish the hyperglycemic effect of CT indicates that the calcium channels of these types are involved in the realization of the activity of this hormone. In this connection, a logical assumption is that the blockade of calcium channels in membranes of non-specific organs by CCB reducing Ca^{2+} transport across the plasma membrane results in inactivation of mechanisms that underlie the hyperglycemic effects of CT. The mechanism of hyperglycemic action of CT preparations is little studied. It can be hypothesized that CT via non-specific receptors and calcium-dependent processes enhances Ca^{2+} entry via L-type Ca^{2+}- channels, thus increasing the intracellular Ca^{2+} concentration. Excessive Ca^{2+} entry or its impaired removal from the cell are known to be accompanied by impairment of intrinsic cell functions. Thus, intracellular Ca^{2+} plays a key role in obesity-related metabolic disorders and insulin resistance [10], in development of a range of pathologies (hypertension, cardiac arrhythmia, diabetes mellitus, encephalopathy, dementia, and others), and can accelerate aging process. Interestingly, long-term rise of intracellular Ca^{2+} was found in skeletal muscle cells and adipocytes of patients with insulin resistance [10,14]. It is believed that endogenous Ca^{2+} can be involved in the development of diabetes by reducing insulin sensitivity [12]. In addition, disturbanced cell Ca^{2+} homeostasis was found in skeletal and cardiac muscle, erythrocytes, liver, adipocytes, and pancreatic β-cells of patients with type 2 diabetes mellitus [13]. In our previous studies

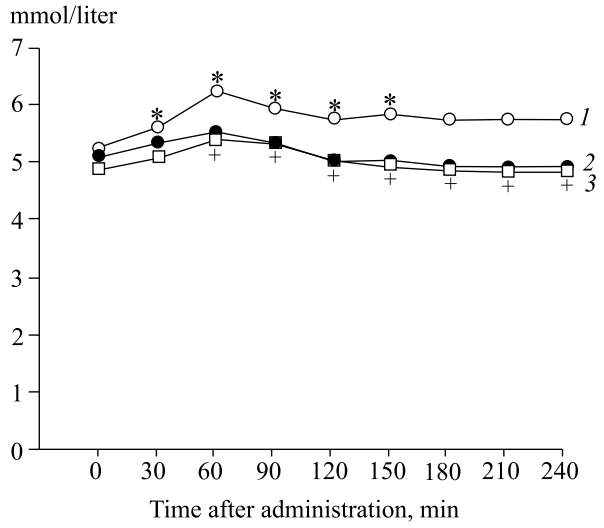

Fig. 2. Effects of CT on glucose levels against the background of isoptin and nifedipine administration. *1*) CT; *2*м CT+nifedipine; *3*) CT+isoptin. **p*<0.05: in comparison with initial values; +*p*<0.02: in comparison with CT administration.

we have shown that isoptin reducing the concentration of intracellular Ca^{2+}, blocks the inhibitory effect of CT on insulin-stimulated muscle and adipose tissue glucose uptake probably due to higher levels of glucose transporters GLUT-4, resulting in increased glucose uptake by peripheral tissues [1], thereby preventing the development of insulin resistance [15]. Thus, the metabolic effect of CCB is enhanced due to their ability to affect glucose utilization at the cellular level via reduction of intracellular Ca^{2+}. We have to assume that this mechanism can underlie the inhibitory effect of CCB on hyperglycemic effect of CT.

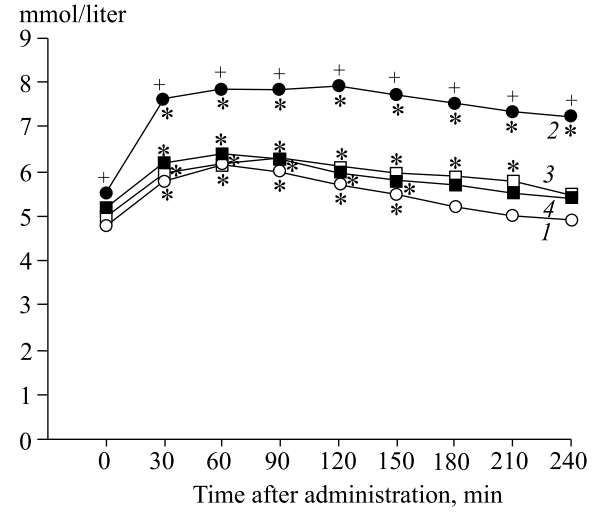

Fig. 3. Effects of nifedipine and isoptin on glucose levels against the background of CT administration during glucose load. Ordinate: glucose levels. *1*) glucose load; *2*) CT+glucose; *3*) CT+glucose+nifedipine; *4*) CT+glucose+isoptin.**p*<0.05: in comparison with initial levels; +*p*<0.02: in comparison with values during glucose load.

TABLE 3. Effects of CT on the Pattern of Alimentary Hyperglycemia against the Background of Isoptin and Nifedipine Administration *(M±m)*

Experimental conditions	Initial level	Glucose level (mmol/liter) after load (min)								K_1	K_2
		30	60	90	120	150	180	210	240		
Glucose load	4.8±0.2 (14)	5.8±0.2 (14)	6.2±0.3 (14)	6.0±0.1 (14)	5.7±0.3 (14)	5.5±0.2 (14)	5.2±0.1 (7)	5.0±0.1 (7)	4.9±0.4 (7)	1.325±0.06 (13)	1.042±0.07 (14)
p_1		<0.01	<0.001	<0.01	<0.02	<0.05	>0.5	>0.5	>0.5	-	-
CT+ glucose	5.5±0.2 (11)	7.6±0.3 (11)	7.8±0.4 (11)	7.8±0.3 (11)	7.9±0.4 (10)	7.7±0.2 (10)	7.5±0.1 (10)	7.3±0.2 (11)	7.2±0.4 (11)	1.605±0.09 (11)	1.23±0.07 (11)
p_1		<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01	<0.01	-	-
p_2	<0.05	<0.001	<0.01	<0.01	<0.01	<0.001	<0.001	<0.001	<0.01	<0.02	0.1<p<0.05
CT+ nifedipine+ glucose	5.0±0.2 (6)	6.0±0.1 (6)	6.2±0.1 (6)	6.3±0.3 (6)	6.1±0.05 (6)	6.0±0.2 (6)	5.9±0.3 (6)	5.8±0.1 (6)	5.5±0.2 (6)	1.46±0.01 (6)	1.1±0.02 (6)
p_1		<0.01	<0.001	<0.01	<0.001	<0.01	<0.05	<0.01	>0.1	-	-
p_2	>0.2	>0.2	-	>0.2	>0.2	>0.2	0.1<p<0.05	<0.001	>0.2	<0.05	>0.2
p_3	<0.01	<0.001	<0.05	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	>0.2	>0.1
CT+ isoptin+ glucose	5.2±0.1 (6)	6.2±0.3 (6)	6.4±0.1 (6)	6.3±0.1 (6)	6.0±0.3 (6)	5.8±0.2 (6)	5.7±0.3 (6)	5.5±0.2 (6)	5.4±0.1 (6)	1.23±0.01 (6)	1.038±0.03 (6)
p_1		<0.01	<0.001	<0.001	<0.05	<0.05	>0.1	>0.2	>0.1	-	-
p_2	>0.1	>0.2	>0.5	>0.1	>0.5	>0.2	>0.1	<0.05	>0.2	>0.1	>0.5
p_3	>0.1	>0.1	<0.01	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	>0.2

Note: p_1: in comparison with initial levels; p_2: in comparison with glucose load; p_3: in comparison with CT administration; K_1: hyperglycemic coefficient; K_2: hypoglycemic coefficient.

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