Effect of Calcium-Regulating Hormones and Calcium Channel Modulators on Glucose Consumption by Muscle and Adipose Tissues *In Vivo* and *In Vitro*

S. S. Butakova and A. D. Nozdrachev

Translated from *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 148, No. 8, pp. 133-136, August, 2009
Original article submitted December 25, 2008

We studied the effects of calcitonin, parathyrin, and Ca^{2+} channel antagonist isoptin and agonist Bay-K-8644 on glucose consumption by muscle (diaphragm) and adipose (epididymal) tissues and insulin-stimulated glucose consumption *in vivo* and *in vitro*. Calcitonin and parathyrin did not alter glucose consumption; parathyrin did not affect, while calcitonin completely abolished the stimulating effect of insulin *in vivo* and *in vitro*. Isoptin significantly increased glucose consumption *in vivo* and *in vitro,* while Bay-K-8644 *in vitro* had no effect glucose consumption. Isoptin did not affect, while Bay-K-8644 significantly reduced the stimulating effect of insulin on glucose consumption by the muscle and adipose tissues. Isoptin did not affect the stimulating effect of insulin against the background of parathyrin administration and completely blocked the inhibitory effect of calcitonin on insulin-stimulated glucose consumption by the muscle and adipose tissues *in vivo* and *in vitro*, while Bay-K-8644 potentiated this effect of calcitonin in vitro.

Key Words: *calcitonin; insulin; isoptin; Bay-K-8644; L-type Ca^{2+} channel*

It is now beyond doubt that impaired transport of glucose and amino acids across the plasmolemma in insulin-dependent tissues is a primary metabolic defect during the development of metabolic syndrome and diabetes mellitus. Investigation of factors responsible for reduced insulin sensitivity of tissues is of crucial importance. Numerous studies proved the role of Ca^{2+} in insulin secretion, therefore it can be hypothesized that this cation is also important for manifestation of biological activity of insulin. For evaluation of the role of Ca^{2+} in the realization of biological effects of insulin on glucose consumption by the muscle and adipose tissues, we studied the effect of Russian-made compounds porcine calcitonin (calcitrin; CT), parathyrin (parathormone; PT), Ca^{2+} channel blocker isoptin (phenylalkylamine derivative), and Ca^{2+} agonist Bay-K-8644 (dihydropyridine derivative) on glucose consumption by the muscle and adipose tissues *in vivo* and *in vitro*.

Department of General Physiology, St. Petersburg State University, Russia. ***Address for correspondence:*** butalana07@list.ru. S. S. Butakova

MATERIALS AND METHODS

In vivo and *in vitro* experiments were conducted on 300 male Wistar rats weighing 100-150 g. In *in vivo* experiments, CT and PT were administered intramuscularly in a dose of 1 U per 100 g of body weight, 60 min later the rats were decapitated. For stimulation of glucose consumption by the muscle and adipose tissues, insulin was injected intramuscularly in a dose of 1 U per 100 g of body weight, the animals were sacrificed 60 min later. For evaluation of the effect of CT and PT on insulin-stimulated glucose consumption by tissues, CT and PT were administered 30 min before insulin injection and the rats were decapitated 1 h after insulin administration. Isoptin was administered intraperitoneally in a dose of 5 mg/kg 1 h before

0007-4888/09/1482-0171

sacrifice. For evaluation of the effect of isoptin on combined effects of CT and insulin or PT and insulin, it was administered 30 min after combined administration of these two agents. The rats were decapitated after 1 h. In *in vitro* experiments, 0.1 U/ml CT, 0.1 U/ml PT, 0.5 U/ml insulin, 5 μg/ml isoptin, and 6 μg/ml Bay-K-8644 were added to the Krebs—Ringer medium (1 ml). For evaluation of the combined effects of preparations, they were simultaneously added to the medium. Cooled muscle and adipose tissue fragments (100-120 mg) were placed in Krebs—Ringer solution containing 11.1 mmol/liter glucose and incubated in Warburg apparatus at 37.7°C and 60-80 shakes per minute. Tissue glucose consumption was estimated by the difference between its content in the medium before and after incubation. Glucose consumption by the muscle and adipose tissue of intact rats was used as the control. Glucose concentration in the medium was detected using Frank and Kirberger method. The data were statistically processed by methods of variation statistics (Student—Fisher test).

RESULTS

Injections of PT and CT to rats (Table 1) had no effect on glucose consumption by diaphragmatic and adipose tissue compared to the control (p_1>0.5). Insulin injection significantly increases glucose consumption by the muscle and adipose tissues (p_1<0.001). Insulin-stimulated glucose consumption by the diaphragm and epididymal fat did not significantly change against the background of PT administration (p_2>0.5). On the contrary, CT completely blocked the stimulating effect of insulin on glucose consumption by the muscle and adipose tissues (p_2<0.001). Being injected *in vivo*, isoptin stimulated glucose consumption by the muscle

TABLE 1. *In Vivo* Glucose Consumption by Muscle and Adipose Tissues under the Effects of Insulin, PT, CT, and Isoptin (*M*±*m*)

Experimental conditions	Number of experiments	Glucose consumption by muscle and adipose tissues during 2-h incubation, μg/100 mg	p_1	p_2	p_3
Muscle tissue					
Control	6	140±5	—	<0.001	
Insulin	6	203±3	<0.001	—	
Isoptin	6	180±6	<0.001	<0.05	
Insulin+isoptin	6	200±2	<0.001	>0.5	
PT	6	150±7	>0.5	<0.001	
PT+insulin	6	206±4	<0.01	>0.5	
PT+insulin+isoptin	6	190±8	<0.01	<0.02	
CT	7	148±10	>0.5	<0.001	
CT+insulin	6	143±7	>0.5	<0.001	
CT+insulin+isoptin	6	187±9	<0.001	<0.05	<0.001
Adipose tissue					
Control	6	100±4	—	<0.001	
Insulin	8	163±8	<0.001	—	
Isoptin	6	120±5	<0.05	<0.05	
Insulin+isoptin	6	158±3	<0.001	>0.5	
PT	6	110±8	>0.5	<0.05	
PT+insulin	6	175±6	<0.001	>0.5	
PT+insulin+isoptin	6	170±7	<0.001	>0.5	
CT	8	101±3	>0.5	<0.001	
CT+insulin	6	101±9	>0.5	<0.001	
CT+insulin+isoptin	6	163±10	<0.001		<0.001

Note. Here and in Tables 2 and 3: p_1: compared to the control; p_2: compared to the effect observed against the background of insulin administration; p_3: compared to the action of insulin against the background of CT administration.

TABLE 2. *In Vitro* Glucose Consumption by Muscle Tissue under the Effects of Insulin, PT, CT, Isoptin, and Bay-K-8644 (*M*±*m*)

Experimental conditions	Number of experiments	Glucose consumption by muscle tissue during 2-h incubation, μg/100 mg	p_1	p_2	p_3
Control	6	140±5	—	<0.001	
Insulin	6	272±8	<0.001	—	
Isoptin	6	200±6	<0.001	<0.001	
Insulin+isoptin	6	280±2	<0.001	>0.5	
PT	6	140±3	--	<0.001	
PT+insulin	6	266±7	<0.001	>0.5	
PT+insulin+isoptin	6	280±8	<0.001	>0.5	
CT	6	135±7	>0.5	<0.001	
CT+insulin	6	218±20	<0.05	<0.05	
CT+insulin+isoptin	6	250±4	<0.001	<0.02	<0.001
Bay-K-8644	6	146±5	>0.5	<0.001	<0.001
Bay-K-8644+insulin	6	206±9	<0.001	<0.05	
Bay-K-8644+PT	6	135±7	>0.5	<0.001	
Bay-K-8644+CT	6	140±3	—	<0.001	
Bay-K-8644+CT+insulin	6	200±2	<0.001	<0.001	<0.05

TABLE 3. *In Vitro* Glucose Consumption by Adipose Tissue under the Effects of Insulin, PT, CT, Isoptin, and Bay-K-8644 (*M*±*m*)

Experimental conditions	Number of experiments	Glucose consumption by adipose tissue during 2-h incubation, μg/100 mg	p_1	p_2	p_3
Control	6	100±4	--	<0.001	
Insulin	6	208±6	<0.001	--	
Isoptin	6	150±8	<0.001	<0.05	
Insulin+isoptin	6	195±3	<0.001	>0.5	
PT	6	103±2	>0.5	<0.001	
PT+insulin	6	206±7	<0.001	>0.5	
PT+insulin+isoptin	6	192±10	<0.001	>0.5	
CT	6	95±12	>0.5	<0.001	
CT+insulin	6	100±9	>0.5	<0.001	
CT+insulin+isoptin	6	213±3	<0.001	>0.5	<0.001
Bay-K-8644	6	106±3	>0.5	<0.02	<0.001
Bay-K-8644+insulin	6	156±7	<0.02	<0.001	
Bay-K-8644+PT	6	100±6	>0.5	<0.001	
Bay-K-8644+CT	6	103±5	>0.5	<0.001	
Bay-K-8644+CT+insulin	6	90±2	>0.5	<0.001	>0.5

and adipose tissues (p_1<0.001 and p_1<0.05, respectively), but did not affect the stimulating effect of insulin on glucose consumption and did not sufficiently influence the effect of insulin against the background of PT administration. However, it blocked the inhibitory effect of CT on insulin-stimulated glucose consumption by the muscle and adipose tissue (p_3<0.001). Similar results were obtained in *in vitro* experiments (Tables 2 and 3). After addition of Bay-K-8644 to the medium, glucose consumption by the diaphragm and adipose tissue did not differ from the control (p_1>0.5). Combined application of Bay-K-8644 and PT or Bay-K-8644 and CT did not modulate glucose consumption by the muscle and adipose tissues either.

Being added to the medium, Bay-K-8644 attenuated the stimulating effect of insulin on glucose consumption by the diaphragm and adipose tissue (p_2<0.05 and p_2<0.001, respectively) and potentiated the inhibitory influence of CT on this process (p_3<0.05 and p_3>0.5, respectively). *In vitro* experiments also showed that isoptin blocked, while Bay-K-8644 enhanced the inhibitory effect of CT on insulin-stimulated glucose consumption in the muscle and adipose tissue. Isoptin, an antagonist of slow voltage-dependent L-type Ca^{2+} channels, reduces Ca^{2+} transport and Bay-K-8644, L-type Ca^{2+} channel agonist, increases Ca^{2+} transport across the sarcolemma. Modulation of the inhibitory effect of CT on insulin-stimulated glucose consumption in the muscle and adipose tissue by isoptin and Bay-K-8644 indicates that Ca^{2+} ions and L-type Ca^{2+} channels participate in the process. After binding to the receptor, insulin induces various cell responses, including Ca^{2+} influx into the cytoplasm and amino acid and sugar consumption. Insulin induces translocation of glucose transporters GLUT-4 from intracellular depots to the plasma membrane, which leads to increased glucose consumption by the muscle and adipose tissues. Thus, we demonstrated the blocking effect of CT on insulin-stimulated glucose consumption in the muscle and adipose tissues; the following mechanism of this nonspecific action of CT can be hypothesized. CT acting on non-specific receptors through Ca^{2+}-dependent processes enhances Ca^{2+} entry through L-type Ca^{2+} channels, thus increasing intracellular Ca^{2+} concentration, and triggers the Ca^{2+} release from depots that inhibits insulin-stimulated mobilization of GLUT-4 from intracellular depots to the plasma membranes. It is known that Ca^{2+} channels were revealed in skeletal muscles, liver, pancreas, neuroendocrine tissue, brain, and smooth muscles of vertebrates and other tissues [3] and in human adipocytes [6]. Intracellular Ca^{2+} plays a key role in metabolic disorders associated with obesity and insulin resistance [2]. According to some authors, endogenous Ca^{2+} can be involved in the development of diabetes mellitus via decreased insulin sensitivity [4]. Moreover, disorders in cell Ca^{2+} homeostasis were revealed in skeletal, cardiac muscles, erythrocytes, liver, adipocytes, and pancreatic β-cells of patients with type 2 diabetes mellitus [5]; negative Ca^{2+} balance was observed in 6-16-year-old children with type 1 diabetes mellitus [1]. Long-term elevation of intracellular calcium concentration was revealed in skeletal muscle cells and adipocytes of patient with insulin resistance [2]. The fact we established, that isoptin decreasing intracellular Ca^{2+} concentration blocks the inhibiting effect of CT on insulin-stimulated glucose consumption by the muscle and adipose tissues, thus preventing the development of insulin resistance, is supported by published reports. Thus, nifedipine therapy (Ca^{2+} channel antagonist) not merely promotes arterial pressure decrease, but also improves insulin resistance in elderly patients with hypertension [7].

REFERENCES

1. I. S. Smiyan and V. B. Furdela, *Pediatriya, Akusherstvo ta Ginekol.*, No. 5, 38-40 (2003).
2. R. L. Byyny, M. Loverde, S. Lloytd, *et al.*, *Am. J. Hypertens.*, **5**, No. 7, 459-464 (1992).
3. E. A. Ertel, K. P. Campbell, M. M. Harpold, *et al.*, *Neuron*, **25**, No. 3, 533-535 (2000).
4. E. Hagstrom, P. Hellman, E. Lundgren, *et al.*, *Diabetologia*, **50**, No. 2, 317-324 (2007).
5. J. Levy, *Endocrine*, **10**, No. 1, 1-6 (1999).
6. W. O. Wilkinson, M. B. Zemel, *Moustaid-Moussa Naima*, Pat. 6569633 USA, MPK 7 GOIN 33/566, GOIN 33/567, Artesel Science Inc., No. 09/592421 (2003).
7. Zhe-hui Zhou, Li-ying Zhuang, Ya-jun Song, *Clin. J. New Drugs Clin. Rem.*, **21**, No. 8, 491-492 (2002).