Variations in Functional Activity of the Hormone-Sensitive Adenylate Cyclase System in Tissues of Gastropod Mollusks with Streptozotocin-Induced Diabetes

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Treatment of gastropod mollusks of pond snail *Lymnaea stagnalis* and orb snail *Coretus corneus* with streptozotocin was followed by an increase in hexose content in the hemolymph and development of the diabetic state (day 1 after treatment). Functional activity of the hormone-sensitive adenylate cyclase system significantly decreased in the muscles and hepatopancreas of mollusks with diabetes. We revealed a decrease in the regulatory effects of biogenic amines and peptide hormones that were realized via stimulatory (octopamine, dopamine, serotonin, tryptamine, and relaxin) and inhibitory G proteins (somatostatin). Disturbances in the hepatopancreas were more pronounced than in the muscle. The severity of disorders in the adenylate cyclase system reached maximum 1 day after streptozotocin treatment. The sensitivity of this system to hormonal and nonhormonal agents was partially restored on days 3 and 5. Hexose content in the hemolymph was elevated after streptozotocin treatment, but returned to normal on day 3. Our results indicate that hyperglycemia is one of the key factors for dysfunction of the adenylate cyclase system in mollusks with the diabetic state.

Key Words: *adenylate cyclase; G protein; hyperglycemia; diabetes; mollusk*

Disorders of hormonal signal systems and, primarily, of the hormone-sensitive adenylate cyclase system (AC system) occur at the early stage of diabetes mellitus and play a major role in the etiology and pathogenesis of this disease [3,5-8,10,11,14]. Multihormonal disorders concern the mechanisms for transduction of signals that are generated by hormones of different chemical nature. Streptozotocin-induced (STZ-induced) diabetes in rats and diabetes mellitus in pregnant women are accompanied by changes in hormonal signal transduction in the typical 3-component AC system and 6-component AC system. We showed that the latter system is regulated by peptides of the insulin superfamily. It should be emphasized that these disorders occur at the stage of functional coupling between heterotrimeric G proteins and other components of the AC system [3,5,6,11,14].

One of the approaches to study the molecular mechanism for diabetes mellitus is induction of experimental diabetes in invertebrate animals [2]. We developed a model of STZ-induced diabetic state (DS) in the bivalve mollusk *Anodonta cygnea* [1]. The progression of DS is accompanied by hyperglycemia and hyposensitivity of the AC system in

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molluscan muscles to insulin and other peptides of the insulin superfamily. Dysfunction of signal transduction in the AC system is observed on day 2 after treatment with STZ. However, the sensitivity of this system to hormones returns to normal on days 4 and 8. In the search for new models of DS in invertebrates, we developed a model of STZ-induced diabetes in gastropod mollusks of the pond snail *Lymnaea stagnalis* and orb snail *Coretus corneus*. The AC system of these mollusks was described previously [4].

Here we compared functional activity and hormone sensitivity of a 3-component AC system (serpentine receptor, heterotrimeric G protein, and AC) in the muscles and hepatopancreas of gastropod mollusks with STZ-induced DS. The goal of our experiments was to evaluate the dynamics of disturbances in the AC system. Moreover, we studied the restoration of signal transduction in this system.

MATERIALS AND METHODS

Experiments were performed on freshwater gastropod mollusks of the pond snail *L. stagnalis* and orb snail *C. corneus*. The animals of 3 groups were examined at various stages of DS (days 1, 3, and 5) after treatment with 1 ml STZ (65 mg/kg, Sigma). Physiological saline was administered to control animals. Severe hyperglycemia in mollusks was observed on day 1 after STZ administration.

The content of hexoses (including glucose) in the hemolymph was measured by *o*-toluidine method with Sintakon reagents. To obtain partially purified membrane fractions of mollusk foot muscles and hepatopancreas, the homogenate was centrifuged at 3000*g* for 15 min. The supernatant was centrifuged at 20,000*g* for 45 min. The pellet was resuspended in 10 mM Tris-HCl buffer (pH 7.5) with 5 mM $MgCl_2$, reprecipitated in the same buffer, and assayed for AC.

Hormonal signal transduction via stimulatory (G_s proteins) and inhibitory G proteins (G_i proteins) was studied to evaluate activity of G protein-coupled AC systems in molluscan tissues under conditions of DS.

Experiments were performed with the following hormones: somatostatin, octopamine, dopamine, serotonin, and tryptamine (Sigma). Pig relaxin-2 was gifted by Prof. O. D. Sherwood (USA). Other reagents were manufactured by Sigma and Reanal. AC activity was measured with [α-^{32}P]ATP (30 Ci/mol, Amersham) as described elsewhere [12,14]. The membrane fraction was incubated in the reaction mixture at 30°C for 10 min. AC activity was evaluated from cAMP formation in the enzymatic reaction.

The results were analyzed by means of ANOVA software. Each experiment was performed in 3 repetitions. The effects of hormones in control and treated animals were considered to be different at $p<0.05$.

RESULTS

The development of hyperglycemia and significant increase in the basal AC activity in muscle tissues of the pond snail *L. stagnalis* and orb snail *C. corneus* were observed 1 day after treatment with STZ (Table 1). These changes were accompanied by a decrease in AC sensitivity to G protein stimulators, GppNHp (nonhydrolyzable GTP analogue) and NaF (Table 2). Basal activity of AC and its regulation by nonhormonal agents under conditions of chronic DS did not differ from the control. Hexose content in the hemolymph significantly decreased, but returned to normal on day 3 (Table 1). These differences were particularly pronounced in the hepatopancreas and persisted by the 3rd day of DS. On day 5 after treatment with STZ, the test parameters practically did not differ from the control (Table 2). Basal activity of AC did not increase, but decreased in the hepatopancreas (as differentiated from muscle tissue). Moreover, forskolin produced less pronounced stimulatory effect on this enzyme in the hepatopancreas. It should be emphasized that forskolin directly modulates AC activity. On days 1 and 3 of DS, the impairment of AC sensitivity to nonhormonal agents in muscles and hepatopancreas of the pond snail were more pronounced than in the orb snail. However, the type of these dis-

TABLE 1. Hexose Content in the Hemolymph of the Pond Snail *L. stagnalis* and Orb Snail *C. corneus* (mmol/liter, $M \pm m$)

Object	Control	Duration of DS, days		
		1	3	5
L. stagnalis	0.49±0.09 (14)	1.32±0.23 (12)	0.60±0.12 (12)	0.51±0.13 (11)
C. corneus	0.77±0.12 (17)	1.96±0.36 (15)	0.88±0.14 (15)	0.74±0.15 (12)

Note. Number of animals is shown in brackets.

TABLE 2. Basal Activity of AC and Its Stimulation with Nonhormonal Agents in Muscles and Hepatopancreas of the Pond Snail *L. stagnalis* and Orb Snail *C. corneus* ($M\pm m$)

Treatment	AC activity, pmol cAMP/mg membrane protein/min			
	control	duration of DS, days		
		1	3	5
L. stagnalis, muscles				
no treatment	42±2 (100)	57±3 (100)	46±3 (100)	40±4 (100)
GppNHp, 10^{-6} M	155±9 (369)	119±8 (209)	134±11 (291)	149±12 (373)
NaF, 10^{-2} M	524±28 (1248)	369±16 (647)	492±29 (1070)	515±27 (1288)
forskolin, 10^{-6} M	120±4 (286)	131±10 (230)	125±7 (272)	113±6 (283)
L. stagnalis, hepatopancreas				
no treatment	75±6 (100)	59±8 (100)	64±3 (100)	70±5 (100)
GppNHp, 10^{-6} M	157±8 (209)	85±4 (144)	105±6 (164)	129±11 (184)
NaF, 10^{-2} M	317±15 (423)	181±16 (307)	208±14 (325)	264±19 (377)
forskolin, 10^{-6} M	210±7 (280)	126±11 (214)	144±10 (225)	186±9 (266)
C. corneus, muscles				
no treatment	25±1 (100)	33±3 (100)	26±3 (100)	23±1 (100)
GppNHp, 10^{-6} M	69±4 (276)	58±2 (176)	62±5 (238)	63±7 (274)
NaF, 10^{-2} M	159±8 (636)	134±4 (406)	149±8 (573)	151±5 (657)
forskolin, 10^{-6} M	60±2 (240)	68±4 (206)	64±7 (246)	61±6 (265)
C. corneus, hepatopancreas				
no treatment	49±3 (100)	37±5 (100)	39±3 (100)	45±4 (100)
GppNHp, 10^{-6} M	117±5 (239)	71±5 (192)	77±4 (197)	94±6 (209)
NaF, 10^{-2} M	220±7 (449)	144±10 (389)	160±7 (410)	200±13 (444)
forskolin, 10^{-6} M	165±13 (337)	108±9 (292)	112±11 (287)	138±10 (307)

Note. AC activity is shown in brackets (%).

orders was similar (no species-specificity). Functional activity of G proteins in the hepatopancreas and muscles of gastropod mollusks decreased 1 day after STZ treatment, but returned to normal on day 5. Moreover, catalytic activity of AC in the hepatopancreas decreased in mollusks with DS.

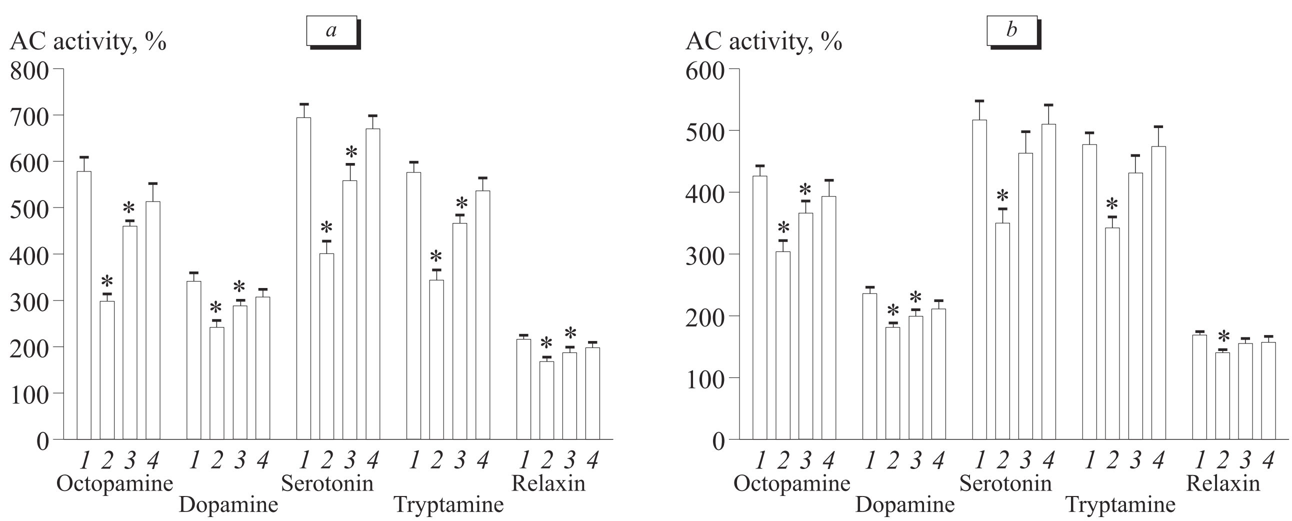

Fig. 1. AC-stimulating effects of biogenic amines in muscle tissue of mollusks *L. stagnalis* (*a*) and *C. corneus* with DS (*b*). Here and in Figs. 2 and 3: control (*1*); and days 1 (*2*), 3 (*3*), and 5 of DS (*4*). Basal AC activity, 100%. $^*p<0.05$ compared to the control.

The AC-stimulating effects of biogenic amines (octopamine, dopamine, serotonin, and tryptamine) and peptide hormone relaxin (modulation of AC via G_s proteins) in molluscan muscles significantly decreased on days 1 and 3 of DS (Fig. 1). However, these effects practically returned to normal on day 5. In this period, the effects of serotonin and tryptamine did not differ from the control. Similar results were obtained in studying the hepatopancreas. The AC-stimulating effects of octopamine, serotonin, and dopamine in the hepatopancreas decreased more significantly than in the muscles. On day 5 after STZ treatment, the effects of these hormones were 60-70% of the control level (Fig. 2). Our results indicate that a change in the sensitivity to hormones that modulate G_s protein-coupled AC were particularly pronounced in the hepatopancreas of mollusks with DS (as compared to muscle tissue).

In muscles of the orb snail, peptide hormone somatostatin inhibited activity of forskolin-stimulated AC. The inhibitory effect of this hormone was less significant in muscles of the pond snail (Fig. 3). The AC-inhibitory effect of somatostatin significantly decreased on day 1 of DS, but did not differ from the control by the 5th day. Our results indicate that the sensitivity of G_i protein-coupled AC in molluscan muscles to somatostatin significantly decreased in the initial stage of DS, but rapidly returned to normal in the follow-up period (partial and complete recovery on days 3 and 5 after STZ treatment, respectively). Dopamine, relaxin, and somatostatin did not modulate AC activity in hepatopancreas (as differentiated from muscle tissue). These data illustrate the tissue-specific effect of peptide hormones and biogenic amines on the AC system in gastropod mollusks.

We conclude that treatment with STZ is followed by initiation of processes restoring sensitivity of the AC system in molluscan tissues to hormones of different chemical nature. The effects of these

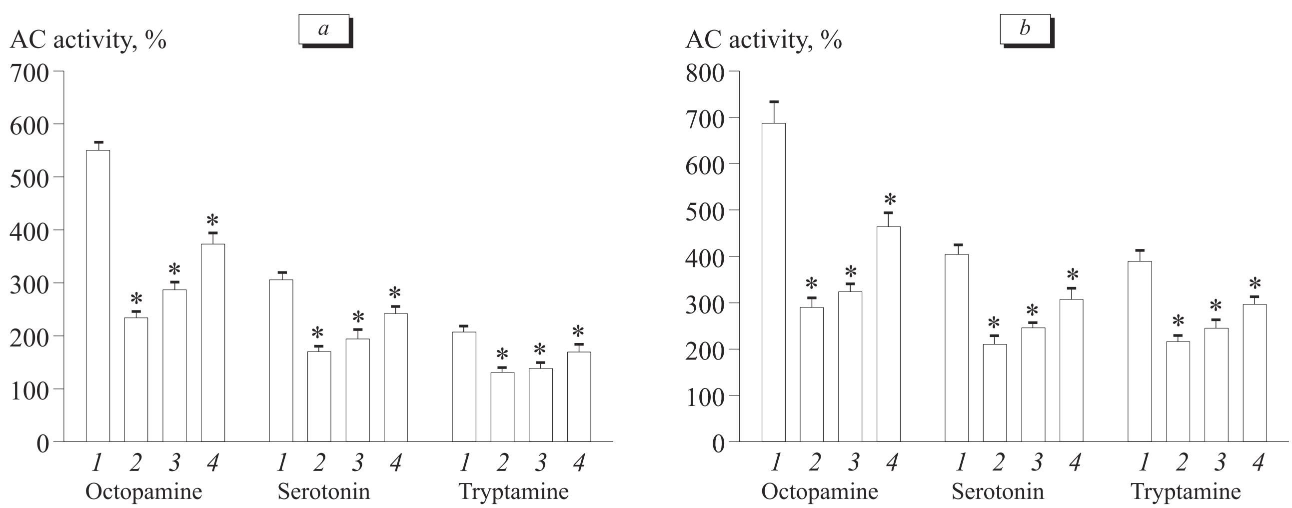

Fig. 2. AC-stimulating effects of biogenic amines in the hepatopancreas of mollusks *L. stagnalis* (*a*) and *C. corneus* with DS (*b*).

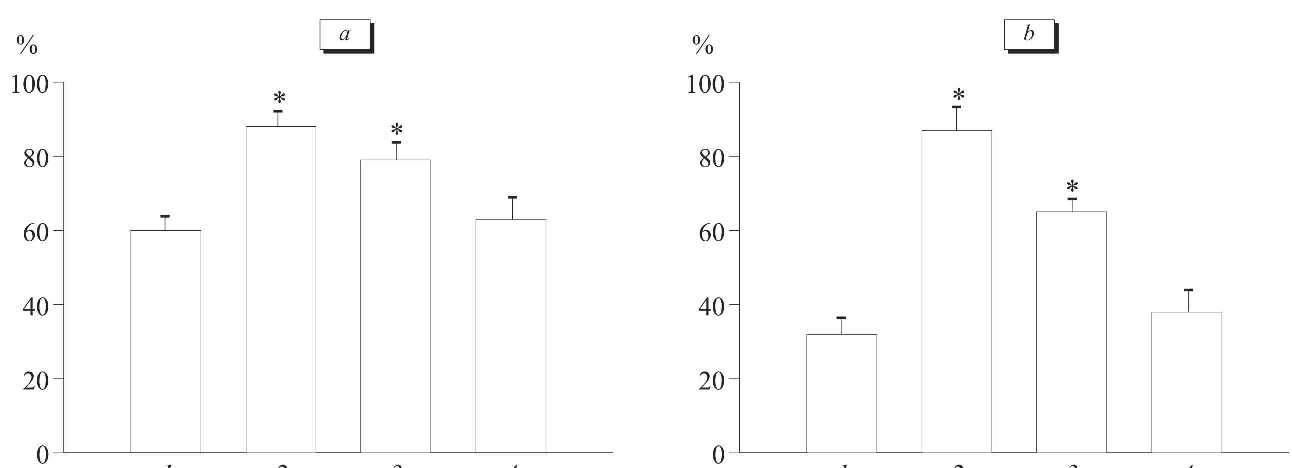

Fig. 3. Somatostatin-induced inhibition of forskolin-stimulated AC activity in muscles of *L. stagnalis* (*a*) and *C. corneus* with DS (*b*).

hormones are realized via G_s and G_i proteins. These changes contribute to tissue regeneration in invertebrate animals (*e.g.*, mollusk tissues that produce insulin-like substances) [9,13]. The increase in the amount of substances with hypoglycemic activity is followed by a decrease in hexose content in the hemolymph. On days 3 and 5 after STZ treatment, hexose content in the hemolymph of mollusks decreases to the control level. These changes illustrate rapid regeneration of tissues, which are involved in the synthesis and secretion of insulin-like substances.

Hyperglycemia is one of the major factors for dysfunction of hormonal signal systems. Therefore, the decrease in hexose content contributes to the restoration of their sensitivity to hormones. However, activity of the AC system can be normalized by various intracellular mechanisms. They include increase in the expression of signal proteins in the AC system and high effectiveness of functional coupling between these proteins, which is impaired under conditions of DS. Study of molecular mechanisms underlying recovery of the sensitivity of tissue AC in bivalve [1] and gastropod mollusks with DS holds much promise for the development of new approaches to the therapy of patients with diabetes mellitus and prevention of diabetes complications.

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REFERENCES

1. L. A. Kuznetsova, S. A. Plesneva, O. V. Chistyakova, *et al.*, *Zh. Evolyuts. Biokhim. Fiziol.*, **43**, No. 6, 460-467 (2007).
2. M. N. Pertseva, *Ibid.*, **42**, No. 5, 401-408 (2006).
3. M. N. Pertseva, L. A. Kuznetsova, A. O. Shpakov, *et al.*, *Patogenez*, **4**, No. 3, 4-10 (2006).
4. A. O. Shpakov and K. V. Derkach, *Zh. Evolyuts. Biokhim. Fiziol.*, **30**, No. 4, 516-524 (1994).
5. A. O. Shpakov, L. A. Kuznetsova, S. A. Plesneva, *et al.*, *Byull. Eksp. Biol. Med.*, **142**, No. 12, 641-645 (2006).
6. A. O. Shpakov, L. A. Kuznetsova, S. A. Plesneva, *et al.*, *Tekhnologii Zhivykh Sistem*, **4**, No. 5-6, 96-108 (2007).
7. A. O. Shpakov, L. A. Kuznetsova, S. A. Plesneva, *et al.*, *Tsitologiya*, **47**, No. 6, 540-548 (2005).
8. A. O. Shpakov, L. A. Kuznetsova, S. A. Plesneva, and M. N. Pertseva, *Byull. Eksp. Biol. Med.*, **140**, No. 9, 286-290 (2005).
9. B. Egger, R. Gschwentner, and R. Rieger, *Dev. Genes Evol.*, **217**, No. 2, 89-104 (2007).
10. S. Hashim, Y. Li, A. Nagakura, *et al.*, *Cardiovasc. Res.*, **63**, No. 4, 709-718 (2004).
11. L. Kuznetsova, S. Plesneva, A. Shpakov, and M. Pertseva, *Ann. N. Y. Acad. Sci.*, **1041**, 446-448 (2005).
12. M. Pertseva, A. Shpakov, L. Kuznetsova, *et al.*, *Cell Biol. Int.*, **30**, No. 6, 533-540 (2006).
13. A. Sancher Alavardo, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **359**, No. 1445, 759-763 (2004).
14. A. O. Shpakov, L. A. Kuznetsova, S. A. Plesneva, *et al.*, *Cent. Eur. J. Biol.*, **1**, 530-544 (2006).