Functional Coupling of Hormone Receptors with G Proteins in the Adenylate Cyclase System of the Rat Muscle Tissues and Brain under Conditions of Short-Term Hyperglycemia

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The sensitivity of components of the adenylate cyclase signaling system (heterotrimer G proteins and adenylate cyclase enzyme) to the regulatory effects of hormones mediated through G proteins (stimulatory effect of isoproterenol and relaxin and inhibitory effects of somatostatin) was decreased in the myocardium of hyperglycemic rats under conditions of transitory hyperglycemia caused by intravenous glucose and in hyperglycemia associated with insulin insufficiency in 24-h type 1 streptozotocin-induced diabetes mellitus. Changes in hormone sensitivity of the adenylate cyclase system were tissue-specific: clearly manifest in the myocardium, minor in skeletal muscles, and virtually absent in the brain of hyperglycemic rats. The main disorders of this system in the myocardium were observed at the stage of hormone receptor coupling with G proteins, which was seen from reduced stimulatory effect of GppNHp on adenylate cyclase activity and attenuation of the regulatory effect of hormones on adenylate cyclase enzyme and G proteins functionally coupled with it.

Key Words: *adenylate cyclase; G protein; hyperglycemia; myocardium; brain*

Elevated blood glucose level (hyperglycemia) can result from excessive consumption of carbohydrates, particularly glucose and sucrose, and from the development of diseases associated with disorders in carbohydrate homeostasis, the main of which is diabetes mellitus (DM). Hyperglycemia is one of the hypothetical pathogenetic factors leading to numerous DM complications. However, the molecular mechanisms underlying the negative effects of high glucose levels on organ and tissue functions remain not quite clear.

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Recent studies showed that the sensitivity of the hormonal signal systems to the regulatory effects of hormones changed under conditions of hyperglycemia [7]. One of the possible causes is changed expression of signal proteins (components of the signal systems), primarily heterotrimer G proteins. If a culture of smooth-muscle cells is placed into the medium containing glucose in a concentration 5-fold higher than its normal concentration in the plasma, the expression of inhibitory G protein α-subunits ($G\alpha_i$ subunits) decreases, while the expression of stimulatory G protein α-subunits ($G\alpha_s$ subunits) remains unchanged [7,8]. Similar changes in the expression of $G\alpha_i$ and $G\alpha_s$ subunits are detected in experimental streptozotocin DM, which is also paralleled by an increase of blood glucose

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level [6, 9,14]. Decreased functional activity of signal protein and weaker functional coupling between them can be another cause of changed sensitivity of the signal systems to hormones in hyperglycemia. This is seen from disorders in the functional reactions between the components of the adenylate cyclase (AC) signal system: receptors, G proteins, and AC enzyme in tissues of rats with DM, which was associated with a clear-cut elevation of blood glucose level in diabetic animals [2-4,10,12].

We compared the effects of transitory (1 h) hyperglycemia induced by intravenous injection of glucose to normal rats and of hyperglycemia resulting from the development of 24-h streptozotocin type 1 DM on the function of AC system components in the myocardium, skeletal muscles, and brain of rats. Biogenic amines and their analogs served as hormones; peptide hormones (relaxin and somatostatin) stimulating (through G_s proteins) and inhibiting (through G_i proteins) AC were used.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (230±25 g). Group 1 (n=15) consisted of intact animals (control); group 2 (n=13) were healthy animals receiving 2 intravenous injections (2 ml) of 40% glucose at 30-min interval (the animals were sacrificed 30 min after the second injection of glucose); group 3 (n=15) were rats with 24-h DM induced by injection of streptozotocin in a dose of 65 mg/kg. Blood glucose level was evaluated by ortho-toluidine method. It was 4.8±0.5 mmol/liter in the control, 19.3±4.1 in group 2, and 12.4±1.9 mmol/liter in group 3. The methods for isolation of the plasma membrane fraction from rat myocardium, skeletal muscles, and brain tissue were described previously [1,11]. Each fraction was pooled from 4-5 animals.

The following hormones were used: somatostatin, serotonin, isoproterenol, and bromocryptin (Sigma). Porcine relaxin-2 was a kind gift from Prof. O. D. Sherwood (USA). Other reagents were from Sigma and Reanal. Activity of AC was measured using [α-^{32}P]ATP (30 Ci/mol; Amersham). The GTP binding of G proteins was evaluated using ammonium salt of β,γ-imido[8-^{3}H]-guanosine-5'-triphosphate ([8-^{3}H]-GppNHp; 5 Ci/mol; Amersham) and HA nitrocellulose filters with 0.45-μ pores (Millipore).

Activity of AC was measured as described previously [11]. Plasma membrane fractions in reaction mixture were incubated at 37°C for 10 min. AC activity was evaluated by the formation of cyclic AMP in the enzymatic reaction. Specific GTP binding of heterotrimer G proteins was determined as the difference between the binding of labeled [8-^{3}H]GppNHp in the sample without GTP and in the presence of 10 mM GTP [5].

Each experiment was repeated 3 times. The differences between control samples and samples treated with hormonal and non-hormonal agents were considered significant at $p<0.05$ (ANOVA test).

RESULTS

Basal AC activity in the myocardium, but not in the skeletal muscles and brain, was somewhat higher in both groups of rats with hyperglycemia caused by glucose load and DM in comparison with intact controls (Table 1). The stimulatory effect of GppNHp (non-hydrolyzed analog of guanine nucleotides), activator of enzyme-coupled heterotrimer G proteins, on myocardial AC of hyperglycemic rats was lower than in the control. Reduction of this effect was particularly demonstrative in the myocardium of diabetic animals. The stimulatory effect of GppNHp on AC activity in the skeletal muscles decreased to a lesser extent than in the myocardium and virtually did not change in the brain. The AC-stimulating effect of forskolin directly reacting with the catalytic center of AC molecule did not differ from that in the control in all studied tissues of hyperglycemic rats (Table 1). Basal level of GTP binding (indicator of functional activity of heterotrimer G proteins) was virtually the same in tissues of control and hyperglycemic animals and just slightly decreased in the muscles of diabetic rats (Table 2). These data indicate reduced coupling between G_s proteins and AC enzyme in the myocardium and less so in the skeletal muscles of hyperglycemic rats, which was confirmed in experiments on evaluation of the effects of AC-stimulating hormones on enzyme activity and GTP binding of G proteins.

Biogenic amines (isoproterenol in the muscles and serotonin in the brain) and peptide hormone relaxin (in the myocardium and brain) modulating AC through receptors functionally coupled with G_s proteins stimulated enzyme activity and elevated the level of GTP binding of G proteins in tissues of control animals (Tables 1, 2). The stimulatory effects of isoproterenol and relaxin on activities of AC system components clearly decreased in the myocardium of hyperglycemic rats of both groups. In skeletal muscles, the decrease in the stimulatory effect of isoproterenol on AC system was detected only in diabetic rats, this decrease being less pronounced than in the myocardium. No appreciable changes in the sensitivity of AC and G proteins to the

TABLE 1. Stimulatory Effect of Hormones and Non-Hormonal Agents on AC Activity of the Myocardium, Skeletal Muscles, and Brain of Control and Hyperglycemic Rats with Glucose Load (1 h) and 24-Hour Diabetes Mellitus (*M*±*m*)

Treatment	AC activity, pmol cAMP/min/mg membrane protein		
	control	glucose load	diabetes, 24 h
Myocardium			
Intact	17.0±0.6	19.9±1.2	21.1±1.3
GppNHp, 10^{-5} M	46.3±2.6 (172)	40.1±3.3 (102)	36.7±1.8 (74)
Forskolin, 10^{-5} M	72.7±4.9 (328)	82.4±3.8 (314)	86.8±6.4 (311)
Isoproterenol, 10^{-5} M	48.4±2.3 (185)	41.6±2.5 (109)	38.7±3.2 (83)
Relaxin, 10^{-8} M	71.0±3.7 (318)	55.8±3.9 (180)	49.5±4.6 (135)
Skeletal muscles			
Intact	16.8±1.0	17.6±1.7	17.8±1.1
GppNHp, 10^{-5} M	39.4±2.1 (135)	36.3±1.5 (106)	35.8±3.0 (101)
Forskolin, 10^{-5} M	65.9±4.8 (292)	68.0±3.7 (286)	67.2±5.2 (278)
Isoproterenol, 10^{-5} M	41.2±2.6 (145)	39.6±1.8 (125)	36.7±2.3 (106)
Brain			
Intact	75±3	80±7	77±8
GppNHp, 10^{-5} M	146±8 (95)	144±10 (80)	143±13 (86)
Forskolin, 10^{-5} M	214±6 (185)	216±15 (170)	206±17 (168)
Serotonin, 10^{-5} M	189±7 (152)	204±16 (155)	183±14 (138)
Relaxin, 10^{-8} M	338±17 (351)	328±22 (310)	321±19 (317)

Note. Here and in Table 2: the percentage of AC-stimulating effect of hormones and non-hormonal agents is shown in parentheses.

TABLE 2. Basal and Hormone-Stimulated Level of GTP Binding in the Myocardium, Skeletal Muscles, and Brain of Intact and Hyperglycemic Rats with Glucose Load (1 h) and 24-Hour Diabetes Mellitus (*M*±*m*)

Treatment	GTP binding, pmol [8-^{3}H]-GppNHp/mg membrane protein		
	control	glucose load	diabetes, 24 h
Myocardium			
Intact	2.6±0.1	2.5±0.4	2.2±0.2
Isoproterenol, 10^{-5} M	5.8±0.3 (123)	4.7±0.3 (88)	4.0±0.2 (82)
Relaxin, 10^{-8} M	8.4±0.5 (223)	6.0±0.4 (140)	5.1±0.6 (132)
Somatostatin, 10^{-7} M	8.2±0.5 (215)	5.5±0.6 (120)	4.6±0.2 (109)
Skeletal muscles			
Intact	1.9±0.2	2.0±0.2	1.6±0.1
Isoproterenol, 10^{-5} M	4.2±0.2 (121)	4.1±0.2 (105)	3.3±0.4 (106)
Brain			
Intact	7.1±0.4	6.9±0.5	6.9±0.5
Serotonin, 10^{-5} M	16.6±0.7 (134)	15.2±0.9 (120)	14.4±0.7 (109)
Relaxin, 10^{-8} M	20.5±1.3 (189)	18.2±0.8 (164)	17.6±1.8 (155)
Bromocryptin, 10^{-5} M	17.8±1.2 (151)	14.0±1.3 (103)	12.1±1.0 (75)
Somatostatin, 10^{-7} M	15.8±0.3 (123)	12.4±0.6 (80)	12.9±1.1 (87)

regulatory effects of hormones under conditions of hyperglycemia were detected in the brain (Table 1, 2).

The next step of the study was evaluation of the effect of somatostatin (peptide hormone) and bromocryptin (specific agonist of D_2 dopamine receptors) on AC system components. The effect of these hormones is realized through receptors coupled with G_i proteins [1,2]. Somatostatin (in the myo-

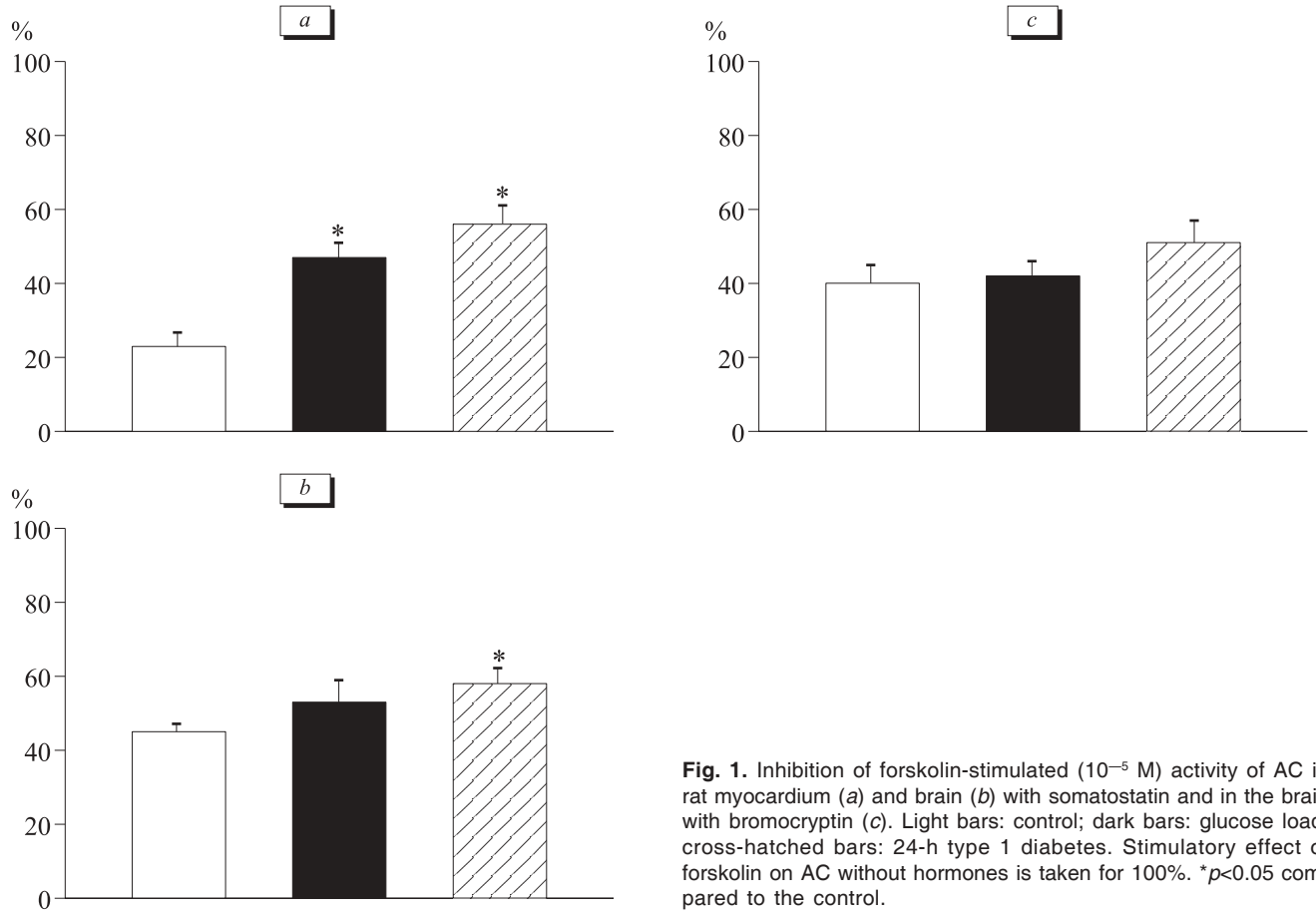

Fig. 1. Inhibition of forskolin-stimulated (10^{-5} M) activity of AC in rat myocardium (*a*) and brain (*b*) with somatostatin and in the brain with bromocryptin (*c*). Light bars: control; dark bars: glucose load; cross-hatched bars: 24-h type 1 diabetes. Stimulatory effect of forskolin on AC without hormones is taken for 100%. *$p<0.05$ compared to the control.

cardium and brain) and bromocryptin (in the brain) reduced forskolin-stimulated activity (Fig. 1) and increased GTP binding due to activation of G proteins (Table 2). The regulatory effect of somatostatin on the myocardial AC system was significantly weaker under conditions of hyperglycemia, particularly in diabetic animals, which manifested in a decrease of the inhibitory effect of the hormone on forskolin-stimulated AC activity and reduction of the hormone stimulation of GTP binding of G_i proteins. On the other hand, changes in hormone sensitivity of AC system coupled with G_i proteins were minor or absent in the brain (Fig. 1, Table 2).

Hence, the sensitivity of myocardial AC system components to the regulatory effects of hormones decreases under conditions of transitory hyperglycemia induced by intravenous glucose and in hyperglycemia caused by insulin insufficiency in animals with DM. The major disorders in the AC system are observed at the stage of hormone receptor coupling with G proteins, which is seen from reduced AC-stimulating effect of GppNHp and attenuation of the hormone effects on AC activity and coupled G proteins in the myocardium of hyperglycemic rats. On the other hand, functional activity of AC evaluated by the effect of forskolin virtually did not change.

The sensitivity of AC system under conditions of transitory hyperglycemia decreased for hormones of different chemical nature, meadiating their effects on AC through G_s and G_i proteins. This indicates that disorders in the process of hormonal signal transmission through the AC system involve universal molecular mechanisms underlying the interrelationships between different types of G proteins and the serpentine type receptors. Study of the AC system functioning under conditions of type 1 and 2 DM lasting for 7 days and more showed that the main disorders appeared in the signal cascades mediated through G_i proteins, presumably because of reduced expression of this type of G proteins in tissues of diabetic animals [2-4]. The absence of specificity of disorders in the signal cascades coupled with G_s and G_i proteins and basal level of GTP binding in the myocardium of hyperglycemic rats, close to the control level, indicate that changed expression of G proteins is most likely not the key cause of reduction of the AC system sensitivity to hormones under conditions of transitory hypergly-

cemia, as was shown for hyperglycemia of more than 2 days duration induced by DM and on a model of hyperglycemia during incubation of cell cultures in a medium with high glucose concentration for 3 days [6-9,14].

Changes in the function of the AC system in hyperglycemia resulting from the development of insulin insufficiency under conditions of DM are qualitatively similar to those in short-term hyperglycemia caused by glucose load, though are more pronounced. Based on this observation, we hypothesized that hyperglycemia is the key, if not the only, pathogenetic factor at the initial stages of DM development, leading to the development of tissue resistance to hormone regulation. The detected shifts in hormone sensitivity of the AC system under conditions of transitory hyperglycemia are tissue-specific: clear-cut in the myocardium, minor in skeletal muscles, and virtually absent in the brain. The absence of appreciable changes in the functioning of the AC system in cerebral tissue of hyperglycemic rats can be explained by certain autonomy of the cerebral hormonal systems and their lesser availability for peripheral effects, specifically, glucose and insulin fluctuations in the blood [13]. High sensitivity of the myocardial AC system indicates that the heart is the organ where short-term elevation of blood glucose produces maximum changes in hormonal regulation.

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