Decrease in Functional Activity of G-Proteins Hormone-Sensitive Adenylate Cyclase Signaling System, during Experimental Type II Diabetes Mellitus

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The development of experimental type II diabetes mellitus in rats was accompanied by dysfunction of inhibitory and stimulatory heterotrimeric G-proteins, components of hormone-sensitive adenylate cyclase signal system. The function of inhibitory G-proteins decreased most significantly under these conditions, which is seen from weakened regulatory effects of somatostatin (in the myocardium) and bromocriptine (in the brain striatum) realized via inhibitory G-proteins in diabetic rats compared to controls. These hormones produce less pronounced inhibitory effect on forskolin-induced activation of adenylate cyclase. In the myocardium of diabetic rats, the stimulatory effects of isoproterenol and relaxin on adenylate cyclase realized via stimulatory G-proteins were decreased to a lesser extent. In the striatum of diabetic rats the stimulatory effect of serotonin and relaxin did not differ from the control. Therefore, dysfunction of stimulatory G-proteins during type II diabetes mellitus is characterized by tissue specificity. Synthetic peptides corresponding to functionally important regions in α-subunits of G-proteins and relaxin receptor LGR7 less effectively inhibited hormone signal transduction via the adenylate cyclase system in rats with type II diabetes. These changes reflect abnormal coupling between receptors and G-proteins in tissues of diabetic rats.

Key Words: *adenylate cyclase; G-protein; diabetes; myocardium; relaxin*

Dysfunction of hormone signaling systems in diabetes mellitus is an urgent problem of molecular endocrinology. Our previous studies showed that the development of streptozotocin-induced type I diabetes mellitus in rats is followed by a decrease in functional activity of heterotrimeric G-proteins and adenylate cyclase (AC) and impairment of coupling between components of the AC system [2,3,8]. A significant increase in basal enzyme activity and decrease in the influence of hormonal (biogenic amines and peptide hormones) and nonhormonal agents (NaF, guanine nucleotides, and forskolin) on the enzyme were noted. Expression and functional activity of signal proteins, components of the hormone-sensitive AC signal system, are changed during type I insulin-dependent diabetes mellitus [5,6]. As distinct from type I diabetes, dysfunction of hormone signaling systems (*e.g.*, AC system) during type II non-insulin-dependent diabetes mellitus remains unknown. It should be emphasized that more than 90% diabetic patients have type II diabetes.

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Here we studied functional changes in the hormone-sensitive AC system during experimental type II diabetes. To this end we compared the regulatory effects of chemically different hormones (biogenic amines and peptide) and nonhormonal agents (NaF, nonhydrolyzed analogue of guanine nucleotides GppNHp, and forskolin) on AC activity in the myocardium and brain striatum of control and diabetic rats. Previous studies revealed changes in the expression and function of various G-proteins during type II diabetes mellitus [9,10]. Taking into account these data, we evaluated functional changes in hormone signal transduction via stimulatory (G_s) and inhibitory G-proteins (G_i). For studying the dysfunction of the AC system we used a new approach consisting in the use of peptides corresponding by their primary structure to functionally important regions of G_s- and G_i-protein α-subunits and relaxin receptor LGR7 as functional probes [1,4,12].

MATERIALS AND METHODS

Experiments were performed on the model of streptozotocin-induced type II diabetes mellitus [7]. Streptozotocin (Sigma) in a dose of 80 mg/kg was administered to newborn (1-2-day-old) Wistar rats to induce type II diabetes. The animals were euthanized 80 days after streptozotocin administration. The fractions of sarcolemmal and synaptosomal membranes were isolated from rat myocardium and brain striatum [1,2]. Each fraction was obtained from 5-6 control or diabetic rats.

Radioisotope studies were performed with [α-^{32}P]ATP (30 Ci/mmol, Amersham). The peptides corresponding to C-terminal regions 385-394 (α_S-subunit) and 346-355 (α_{i2}-subunit) of mammalian G-proteins and peptides 619-629-Lys(Palm) and 615-629 (derivatives of the C-terminal region in the third cytoplasmic loop of the relaxin receptor LGR7) were synthesized as described previously [1,4].

AC activity was measured as described elsewhere [11]. Membrane fractions were incubated in the reaction mixture at 37°C for 10 min. AC activity was estimated from the amount of cAMP formed in the enzymatic reaction. Synthetic peptides were preincubated with the samples at 4°C for 10 min and then hormones were added. Control samples were incubated with solvents.

The results were analyzed by means of ANOVA software. Each experiment was performed in 3 repetitions. The data were expressed as means± standard errors (several independent experiments). The differences between the control samples and samples incubated with hormones and nonhormonal agents were significant at $p<0.05$.

RESULTS

Basal AC activity in the myocardium and brain striatum of diabetic rats (19.9±1.7 and 68.3±4.0 pmol cAMP/mg protein/min, respectively) insignificantly differed from that in control animals (16.6±1.1 and 76.9±5.2 pmol cAMP/mg protein/min, respectively). G-protein activators NaF (10^{-2} M) and GppNHp (10^{-5} M) increased myocardial AC activity by 1385 and 123%, respectively. The stimulatory effect of these compounds in diabetic rats was lower than in control animals (1845 and 179%, respectively). Small differences were revealed in the stimulatory effect of NaF and GppNHp on striatal AC in diabetic (770 and 74%, respectively) and control animals (855 and 91%, respectively). No differences were found in the influence of forskolin (10^{-5} M), which directly affects the catalytic site of the enzyme. In the myocardium and striatum of diabetic rats forskolin increased AC activity by 289 and 210%, respectively, and in control rats the corresponding values were 305 and 197%. These data show that type II diabetes mellitus is associated with a decrease in the sensitivity of G_s-proteins to nonhormonal activators and disturbances in their interaction with AC, while catalytic activity of AC remains unchanged.

At the next stage we studied the G_s-protein-mediated regulatory effect of hormones on AC activity. Peptide hormone relaxin and specific β-adrenoceptor agonist isoproterenol dose-dependently increased AC activity in the myocardium of diabetic and control animals (Fig. 1, *a*, *b*). However, the stimulatory effects of hormones in diabetic rats were less significant than in control animals. These differences were most pronounced in experiments with relaxin.

C-terminal peptide 385-394 of G-protein α_S-subunit (10^{-4} M) violating hormone signal transduction via G_s-protein-coupled receptors suppressed the effects of relaxin and isoproterenol on myocardial AC in diabetic and control rats (Fig. 2, *a*). C-terminal peptide 346-355 of α_{i2}-subunit (derivative of the primary structure of α-subunit from inhibitory G-proteins) was ineffective under these conditions. In the myocardium of diabetic rats (compared to controls) peptide 385-394 of the α_S-subunit was less potent in inhibiting the effect of hormones on AC. These differences were probably related to lower effectiveness of coupling of relaxin receptors and β-adrenoceptors to G_s-protein during type II diabetes mellitus. The stimulatory effect of relaxin on AC was less pronounced in the presence of peptides 619-629-Lys(Palm) and 615-629 (derivatives of the third cytoplasmic loop of the relaxin receptor LGR7). Our previous studies showed that

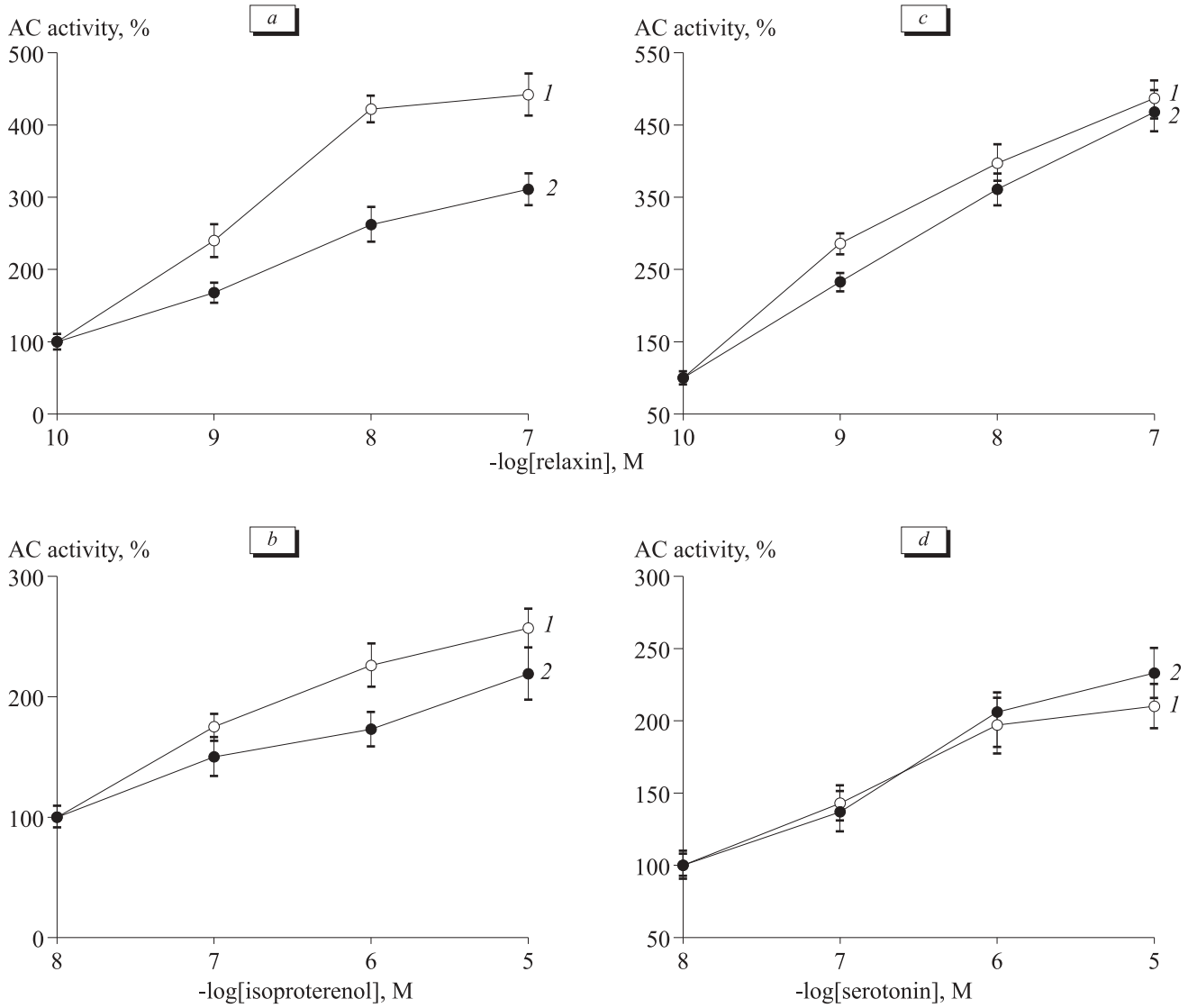

Fig. 1. Stimulatory effect of relaxin (*a*, *c*) and biogenic amines isoproterenol (*b*) and serotonin (*d*) on AC in rat myocardium (*a*, *b*) and brain striatum (*c*, *d*) in control (*1*) and diabetic rats (*2*). Basal AC activity, 100%. Here and in Figs. 2 and 3: confidence intervals at $p<0.05$.

these peptides competitively inhibit relaxin signal transduction from the receptor to AC [4]. Similarly to peptide 385-394 of the α_s-subunit, these peptides had a more potent inhibitory effect in the myocardium of control animals, which attested to weakening of relaxin receptor coupling to G_s-protein in the myocardium of rats with type II diabetes. Peptides 619-629-Lys(Palm) and 615-629 (10^{-5} M) decreased the stimulatory effect of relaxin (10^{-8} M) on myocardial AC in control rats to 28 and 29% of that observed in the absence of peptides, respectively, while in the myocardium of diabetic animals this stimulatory effect decreased only to 45 and 48%, respectively.

The stimulatory effect of relaxin and serotonin on striatal AC was similar in diabetic and control rats (Fig. 1, *c*, *d*). No differences were revealed in the influence of peptide 385-394 of α_s-subunit on AC-effects of hormones. These data attest to tissue-specific changes in functional activity of G_s-proteins and their coupling to hormone receptors in type II diabetes.

The influence of peptide hormone somatostatin and D_2 receptor agonist bromocriptine on AC in tissues of control and diabetic rats was studied to evaluate changes in G_i-protein-coupled signal systems. Previous studies showed that somatostatin and bromocriptine decreased forskolin-induced enzyme activity in the myocardium and brain striatum, respectively, via activation of G_i-proteins [1].

The inhibitory effect of hormones on forskolin-induced activation of AC in the myocardium and

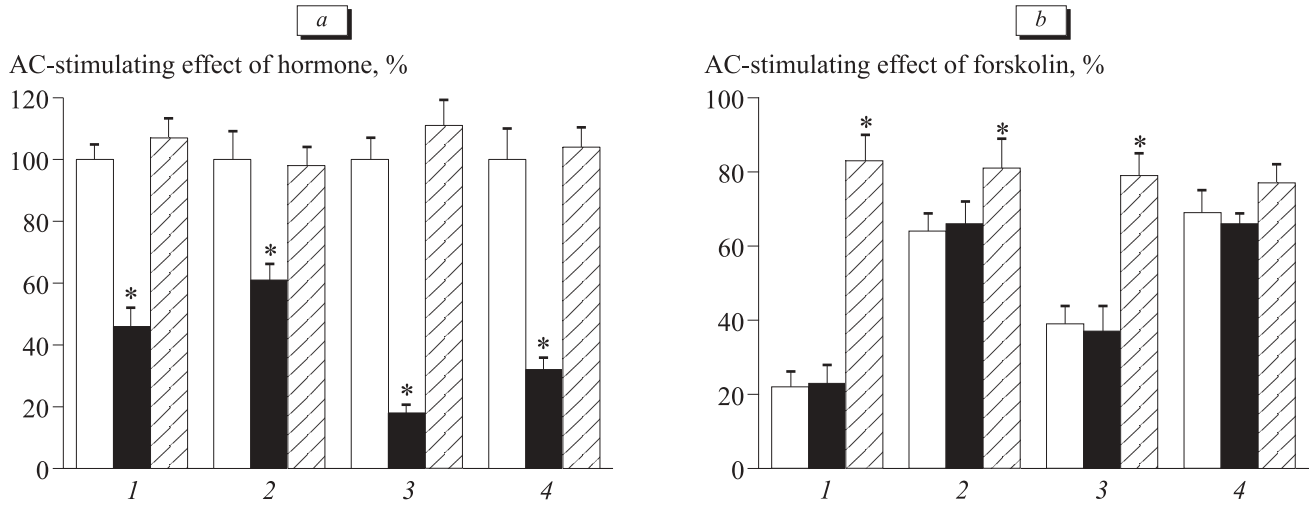

Fig. 2. Effect of C-terminal peptides 385-394 (α_S-subunit, 10^{-4} M) and 346-355 (α_{i2}-subunit, 10^{-4} M) on stimulation of AC with relaxin (10^{-8} M; *1*, *2*) and isoproterenol (10^{-5} M; *3*, *4*) in the myocardium (*a*) and on inhibition of forskolin-induced activation of AC with somatostatin (10^{-7} M; *1*, *2*) and bromocriptine (10^{-5} M; *3*, *4*) in the myocardium and striatum of control and diabetic rats (*b*). Control (*1*, *3*) and diabetic rats (*2*, *4*). Light bars, without peptide; dark bars, in the presence of peptide 385-394 (α_S-subunit); shaded bars in the presence of peptide 346-355 (α_{i2}-subunit). Stimulation of AC with hormones (*a*) or forskolin in the absence of hormones (*b*) is taken as 100%. **p*<0.05 compared to AC activity in the absence of peptides.

brain striatum was much less pronounced in diabetic rats (Fig. 3). The inhibitory effect of somatostatin and bromocriptine on forskolin-induced activation of AC in tissues of diabetic rats slightly decreased in the presence of the C-terminal peptide 346-355 from the α_{i2}-subunit of G-protein (10^{-4} M). The protein impairs hormone signal transduction via G_i-protein-coupled receptors. However, this peptide completely blocked the inhibitory effect of hormones in tissues of control animals (Fig. 2, *b*).

We conclude that type II diabetes mellitus is accompanied by more significant changes in the AC system with inhibitory G-proteins compared to the G_s-protein-coupled AC system. Tissue-specific changes were not found in the G_i-protein-coupled AC system: the sensitivity of this system to hormones decreased in both myocardium and striatum of diabetic rats. Similarly to the AC system with G_s-proteins, functional inactivation of the G_i-protein-coupled AC system during type II diabetes mellitus is related to dysfunction and decrease in coupling of G_i-proteins to hormone receptors. Our results are consistent with published data that the content and functional activity of various isoforms of G_i-pro-

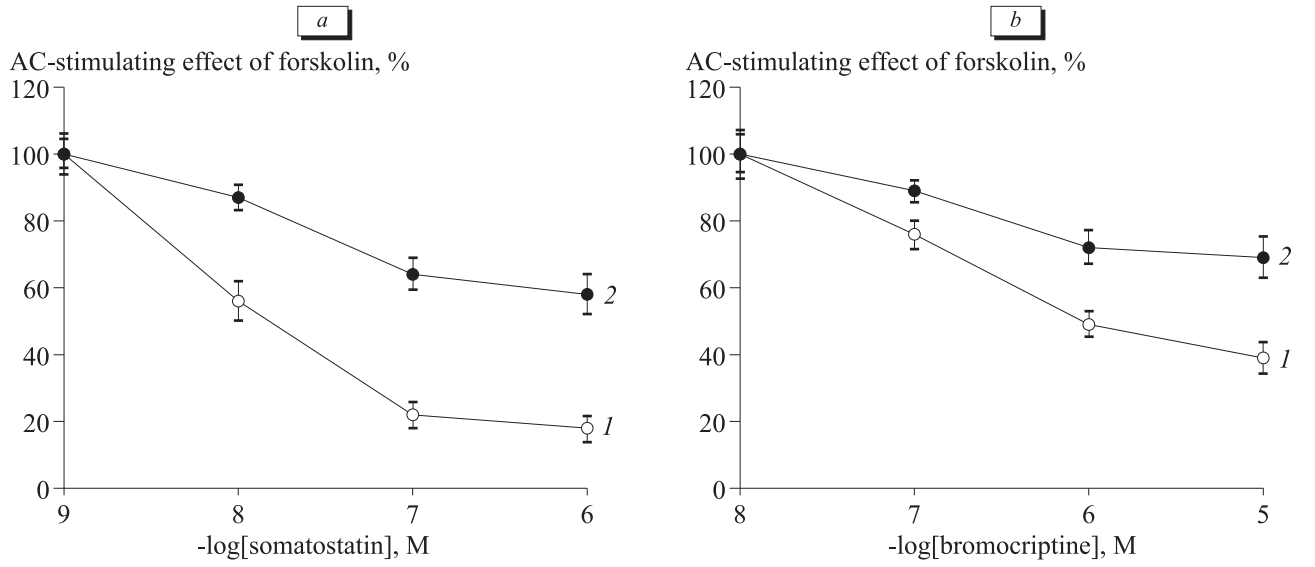

Fig. 3. Inhibition of forskolin-induced (10^{-5} M) activation of AC in the myocardium and brain striatum with somatostatin (*a*) and bromocriptine (*b*), respectively in control (*1*) and diabetic rats (*2*). AC-stimulating effect of forskolin in the absence of hormones is taken as 100%.

teins decrease during non-insulin-dependent diabetes mellitus (experimental diabetes mellitus and type II diabetes mellitus in humans) [9,10].

These data indicate that type II non-insulin-dependent diabetes mellitus is accompanied by impairment of functional coupling of heterotrimeric G-proteins with receptors for chemically different hormones and with AC. The most pronounced changes are observed in hormone signal transduction via G_i-proteins. Our results are consistent with published data that function of G_i-proteins decreases during type II diabetes. As distinct from streptozotocin-induced type I diabetes, basal AC activity in the myocardium and brain striatum remains practically unchanged during experimental type II diabetes. We revealed tissue-specific changes in functional activity of the G_s-coupled AC system during type II diabetes. The sensitivity of this system to hormones decreases in the myocardium, but remains practically unchanged in the striatum of diabetic rats.

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