Molecular Mechanisms of Modified Sensitivity of the Adenylate Cyclase Signaling System to Biogenic Amines during Streptozotocin-Induced Diabetes

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We demonstrated changes in the sensitivity of the adenylate cyclase signaling system to biogenic amines (adrenoceptor agonists and serotonin) underwent a change in skeletal muscles of rats with 30-day streptozotocin-induced diabetes. Isoproterenol had a less significant stimulatory effect on adenylate cyclase in diabetic rats. Hormonal signals via G_i proteins were suppressed in animals with diabetes, which determined a greater stimulatory effect of norepinephrine and serotonin on adenylate cyclase. Hormones less significantly increased guanosine triphosphate-binding activity of G proteins in diabetic rats, which reflects the impairment of their functional coupling with receptors.

Key Words: *adenylate cyclase; G protein; diabetes; norepinephrine*

Hormone-sensitive adenylate cyclase signaling system (ACS) is one of the major systems responsible for signal transduction in cells. Function of this system under pathological conditions is an important problem in modern molecular endocrinology. Recent studies showed that molecular changes in functional activity of this system during diabetes are accompanied by variations in tissue sensitivity to the regulatory effect of hormones (*e.g.*, biogenic amines). These data are consistent with the hypothesis that molecular defects in hormonal signaling systems serve as a cause of endocrine diseases. The development of experimental streptozotocin-induced diabetes is accompanied by changes in the expression of signal proteins (components of ACS). Expression of β_1-adrenergic receptors (AR) significantly decreases, while expression of β_2-AR and β_3-AR increases in the heart of diabetic rats [4,11]. Moreover, expression of some G proteins varies in diabetes. The concentration of G_{i2} and G_o proteins in the heart decreases, while the content of G_S proteins remains unchanged under these conditions [5,15]. However, there are no data on changes in overall activity of ACS during diabetes. Moreover, the molecular mechanisms of these disturbances are poorly understood. Understanding of the mechanism of abnormal sensitivity of ACS to biogenic amines would allow us to develop new approaches to therapeutic treatment of this disease and associated disorders. It is based on the interaction (cross-talk) between signaling systems regulated by biogenic amines and insulin [3,7,10].

Here we compared the sensitivity of ACS to biogenic amines in skeletal muscles of intact animals (control) and rats with 30-day streptozotocin-induced diabetes. The peptide strategy was used to study functional coupling between receptors for biogenic amines and G proteins under normal and pathological conditions. This approach is based on the treatment with synthetic peptides, whose primary structure corre-

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sponds to C-terminal regions in α-subunits of stimulatory (G_S) and inhibitory G proteins (G_i). Previous studies showed that these peptides competitively inhibit hormonal signal transduction from the receptor to adenylate cyclase (AC), which is realized via α-subunits [1,2,9,12, 13]. Study peptides are derived from the primary structure of α-subunits and, therefore, can be used to evaluate the role of certain G proteins in hormonal regulation of AC. Peptide treatment selectively inhibits and/or modulates the stimulatory and inhibitory effect of hormones on AC. These hormones bind to G_S protein-coupled specific receptors and increase AC activity. Binding of hormones to other receptors coupled to G_i proteins leads to inhibition of AC. Our experiments were performed with norepinephrine that can stimulate and inhibit AC through β-AR and α_2-AR, respectively. We also used serotonin producing a stimulatory (via type 4, 6, and 7 serotonin receptors) or inhibitory effect on AC (via type 1 serotonin receptors).

MATERIALS AND METHODS

Fraction of plasma membranes was isolated from hindlimb muscles of *Rattus norvegicus* [8]. Each fraction was obtained from 5-7 control and diabetic animals. Type I diabetes was produced by administration of streptozotocin in a dose of 65 mg/kg (30 days). The disease manifested in a significant increase in blood glucose concentration and glucosuria.

Radioisotope assay involved [α-^{32}P]ATP (30 Ci/mM) and β,γ-imido[8-^{3}H]-guanosine-5'-triphosphate ammonium salt ([8-^{3}H]-GppNHp, 5 Ci/mM, Amersham). Guanosine triphosphate-binding (GTP-binding) activity of G proteins was determined using type HA nitrocellulose filters (0.45 μ, Millipore). Peptides corresponding to C-terminal regions 385-394 and 346-355 of α_S and α_{i2} subunits from human G proteins, respectively, were presented by I. A. Gur'yanov (Institute of High-Molecular-Weight Compounds, Russian Academy of Sciences, St. Petersburg).

AC activity was measured as described elsewhere [14]. The fraction of plasma membranes was incubated in the reaction mixture at 37°C for 10 min. AC activity was determined by the amount of cAMP formed in the enzymatic reaction. C-terminal peptides were preincubated with the sample at 4°C for 10 min. The samples were mixed with hormones. Solvents of hormones and peptides were added to control samples (instead of the test agents).

GTP-binding activity of G proteins was studied as described previously [1]. Specific GTP-binding activity of heterotrimeric G proteins was calculated as the difference between binding of labeled [8-^{3}H]Gpp[NH]p in the absence or presence of 10 mM GTP.

The results were analyzed by means of ANOVA software. Each experiment was performed in 3 repetitions. The data are expressed as means and standard errors estimated in several independent experiments. Differences between control samples and samples exposed to the effect of hormonal and nonhormonal agents were significant at $p<0.05$.

RESULTS

Basal AC activity in skeletal muscles of diabetic rats was much higher compared to control animals (18.5± 0.9 and 13.7±0.4 pmol cAMP/mg protein/min, respectively). A β-AR agonist isoproterenol stimulated AC and increased GTP-binding activity in muscles of control and diabetic rats. Isoproterenol in various concentrations (10^{-8}-10^{-5} M) had a greater stimulatory effect on AC in control animals (as compared to diabetic rats, Fig. 1, *a*). Significant differences were revealed in the influence of 10^{-6} M isoproterenol on GTP binding. Isoproterenol increased GTP-binding activity in control and diabetic rats by 97 and 42%, respectively. The effect of isoproterenol (10^{-6} M) on AC activity dose-dependently decreased in the presence of peptide 385-394 from the G_S protein α_S subunit (Fig. 2, *a*). This peptide blocks transduction of stimulatory signals to AC. It should be emphasized that the shape of curves for an inhibitory effect of peptide 385-394 α_S on stimulation of AC with isoproterenol did not differ in control and diabetic animals. The stimulatory effect of this hormone on GTP binding decreased in control and diabetic rats (by 39 and 27%, respectively). Peptide 346-355 of the G_i protein α_{i2} subunit impairs coupling between receptor and G protein. This peptide had little effect on stimulation of AC with the hormone (slight potentiation in diabetic rats, Fig. 2, *a*). This can be associated with blockade of the signal pathway via G_i protein. This pathway triggers β_3-AR, which is activated by isoproterenol and capable of coupling to G_i proteins in muscle tissues [6]. The stimulatory effect of isoproterenol on GTP-binding activity of G proteins slightly decreased in diabetic rats (by 16%), but remained unchanged in control animals. These changes reflect the decrease in reactivity of G_S-coupled ACS to isoproterenol in skeletal muscles of rats with diabetes.

Norepinephrine in concentrations of 10^{-8}-10^{-5} M stimulated AC. It should be emphasized that this hormone in concentrations of 10^{-6}-10^{-5} M had a more significant effect in muscles of diabetic rats (Fig. 1, *b*). By contrast, the stimulatory effect of 10^{-6} M norepinephrine on GTP binding in control animals was more pronounced than in diabetic rats (by 122 and 69%, respectively). The stimulatory effect of norepinephrine on AC sharply decreased in the presence of peptide 385-394 α_S (particularly in control animals,

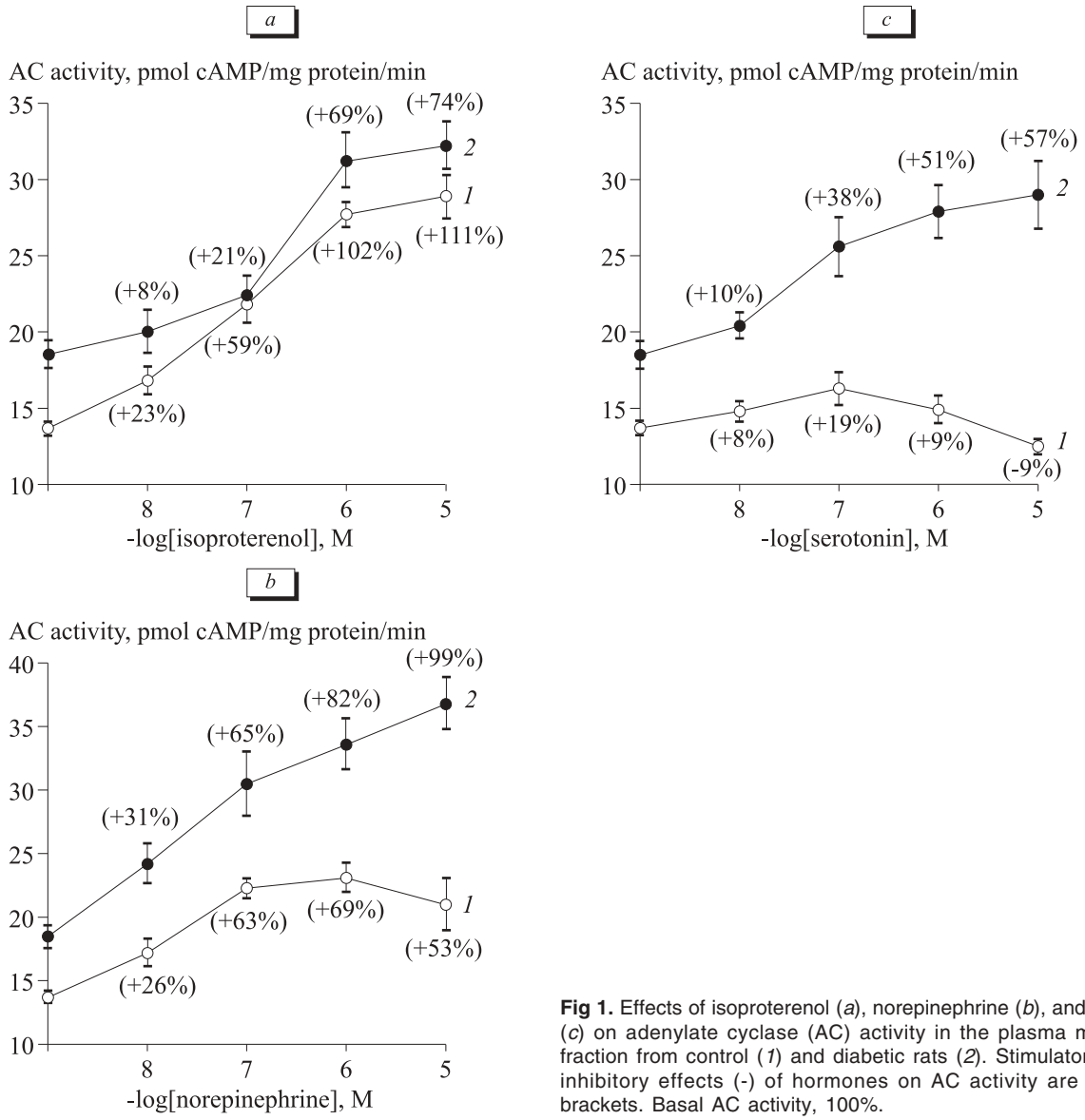

Fig 1. Effects of isoproterenol (*a*), norepinephrine (*b*), and serotonin (*c*) on adenylate cyclase (AC) activity in the plasma membrane fraction from control (*1*) and diabetic rats (*2*). Stimulatory (+) and inhibitory effects (-) of hormones on AC activity are shown in brackets. Basal AC activity, 100%.

Fig. 2, *b*), but increased after treatment with peptide 346-355 α_{i2}. The degree of stimulation increased by 1.5 times and by 20% in muscles of control and diabetic rats, respectively. Our results are consistent with published data on the impairment of G_i protein function during diabetes [5,15]. Previous studies showed that the concentration of G_i proteins in control rats is higher than in diabetic animals. Therefore, the inhibitory pathway via α_2-AR plays a greater role in the regulation of AC activity by norepinephrine. These data explain the results of our study. We showed that peptide 346-355 α_{i2} decreases the inhibitory effect of the hormone. Hence, the stimulatory effect of this hormone in control animals is more significant than in diabetic rats. Our assumption is supported by a more significant decrease in the stimulatory effect of 10^{-6} M norepinephrine on GTP-binding activity of G proteins from control animals in the presence of peptide 346-355 α_{i2} (41 *vs*. 11% in diabetic rats). However, the effect of peptide 385-394 α_s decreased similarly in control and diabetic animals (by 35 and 27%, respectively).

After serotonin treatment AC activity remained practically unchanged in muscles of control rats, but increased in diabetic animals (Fig. 1, *c*). The stimulatory effect of 10^{-6} M serotonin on GTP binding in control rats was more pronounced than in diabetic animals (by 75 and 49%, respectively). In the presence of peptide 385-394 α_s, serotonin inhibited AC in control rats. The stimulatory effect of this hormone in diabetic animals was less pronounced under these conditions (Fig. 2, *c*). Peptide 346-355 α_{i2} partially blocked the inhibitory effect of serotonin in control animals. We revealed a stimulatory effect of serotonin on AC in these rats. It should be emphasized that this

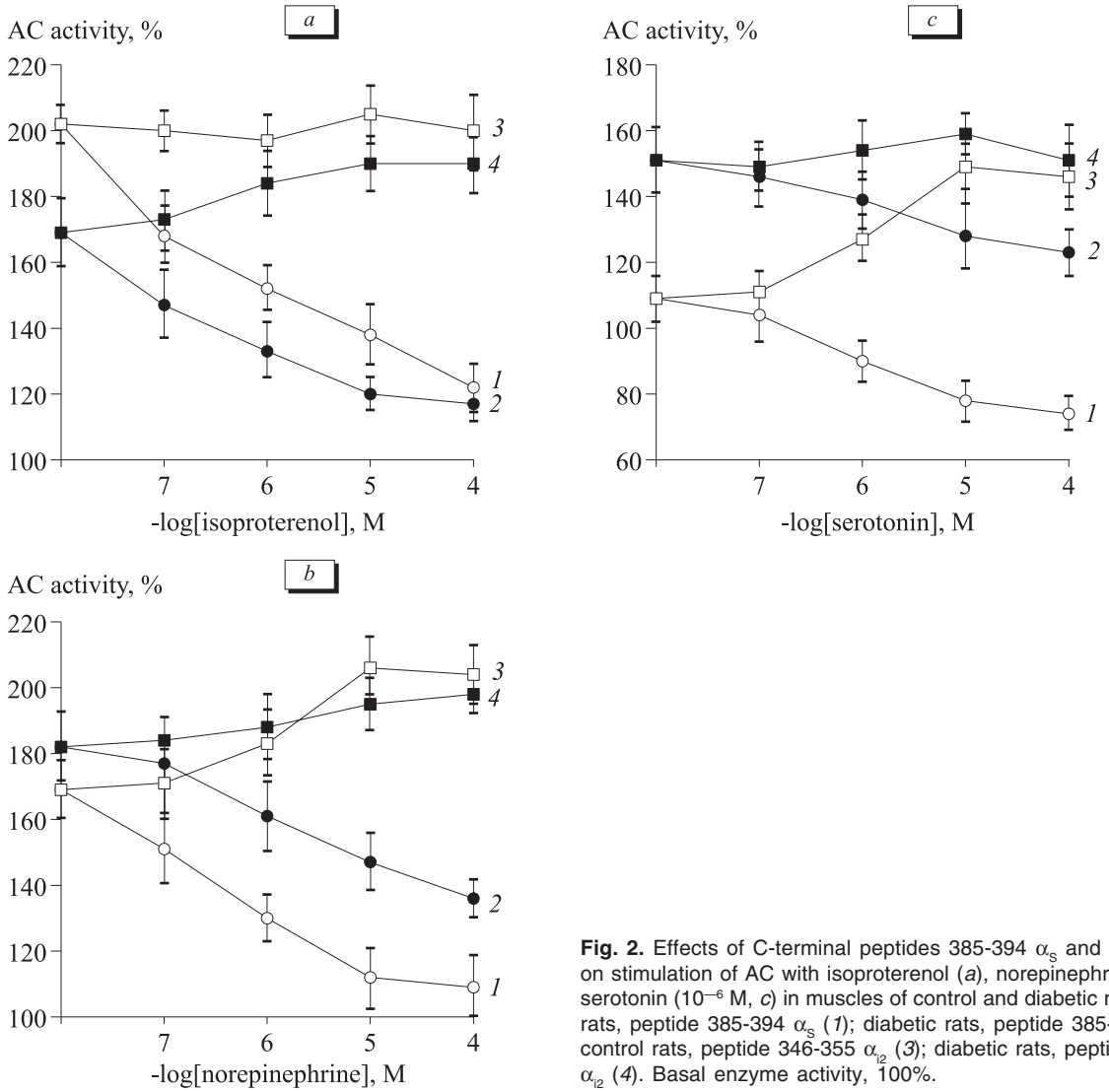

Fig. 2. Effects of C-terminal peptides 385-394 α_S and 346-355 α_{i2} on stimulation of AC with isoproterenol (*a*), norepinephrine (*b*), and serotonin (10^{-6} M, *c*) in muscles of control and diabetic rats. Control rats, peptide 385-394 α_S (*1*); diabetic rats, peptide 385-394 α_S (*2*); control rats, peptide 346-355 α_{i2} (*3*); diabetic rats, peptide 346-355 α_{i2} (*4*). Basal enzyme activity, 100%.

peptide did not modulate the influence of serotonin in diabetic animals. These data are consistent with the results of experiments with norepinephrine. Our findings indicate that coupling of type 1 serotonin receptors to $G_{i/o}$ receptors is impaired during diabetes. Thus, the effect of serotonin is mainly realized via G_S proteins and manifested in activation of AC. We showed that peptide 346-355 α_{i2} does not modulate the stimulatory effect of serotonin on GTB binding in diabetic rats, but blocks this action in control animals (by 31%). In the presence of peptide 385-394 α_S, this effect decreased similarly in control and diabetic rats (by 22 and 20%, respectively).

Our results show that the sensitivity of skeletal muscle ACS to AR ligands and serotonin is modified in rats with experimental diabetes. It is particularly pronounced in ACS coupled to G_i proteins. The observed changes are probably related to the decrease in G_i protein expression and impairment of their functional interaction with receptors.

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