Changed Sensitivity of Adenylate Cyclase Signaling System to Biogenic Amines and Peptide Hormones in Tissues of Starving Rats

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In the myocardium and skeletal muscles of rats deprived of food for 2 days, basal activity of adenylate cyclase decreased, while the sensitivity of adenylate cyclase signaling system to the stimulating effects of non-hormonal agents (guanine nucleotides and NaF) and β-agonist isoproterinol modulating adenylate cyclase through stimulating G proteins increased. In starving organism, the regulatory effects of hormones realizing their effects through inhibitory G proteins (somatostatin in the myocardium and bromocryptin in the brain) weakened. Their inhibitory effects on forskolin-stimulated adenylate cyclase activity and stimulating effects on binding of guanosine triphosphate decreased. In the brain of starving rats, the differences in the sensitivity of the adenylate cyclase signaling system to hormones and nonhormonal agents were less pronounced than in the muscle tissues, which attested to tissue-specific changes in the functional state of this system under conditions of 2-day starvation.

Key Words: *adenylate cyclase; biogenic amine; serotonin; somatostatin*

Food deprivation induces biochemical and physiological shifts in the organism related to changes in functional activity of hormone signaling systems, *e.g.* their sensitivity to the regulatory effects of hormones. Studies of adenylate cyclase (AC) signaling system under conditions of food deprivation [6,8,9] showed that the stimulating effects of hormones on activity of AC, the catalytic component of this system, realized via stimulatory G proteins (G_s proteins) increased, while the inhibiting effects of hormones realized via inhibitory G proteins (G_i proteins) decreased. Stimulation of AC activity in the adipose tissue by adrenergic receptor ligands was observed as soon as after 10-h food deprivation [6]. Starvation for 1-3 days 2-fold increased AC sensitivity in rat kidneys to the stimulating effect of parathyroid hormone [9]. Food deprivation for 24 h considerably potentiated the stimulating effect of neuropeptide orexin mediated via receptors coupled with G_s-proteins on AC activity in the hypothalamus of starving rats and weakened the inhibitory effect of the hormone mediated though receptors coupled with G_i proteins [8].

The overall changes in the sensitivity of AC system in humans and vertebrates to hormones and non-hormonal agents during food deprivation remain unclear. Study of changes in the functioning of the AC system under conditions of nutrient deficiency is necessary for understanding of the molecular mechanisms of hormonal regulation of energy homeostasis at the cellular and organ levels and for the therapy of some diseases accompanied by exhausting of energy resources (*e.g.* anorexia).

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Here were studied the regulatory effect of biogenic amines and peptide hormone somatostatin on functional activity of AC system in the myocardium, skeletal muscles, and striatum of rats deprived of food for 2 days.

MATERIALS AND METHODS

Experiments were performed on male albino Wistar rats. Group 1 rats received standard ration (control) and group 2 animals were deprived of food for 2 days, but had free access to water (experimental group). Fraction of plasma membranes were isolated from tissue samples of the myocardium, skeletal muscles, and striatum [1,4,10]. Each fraction was isolated from 5-6 animals. Glucose concentration in blood plasma was measured by *o*-toluidine method.

For evaluation of changes in the efficiency of transmission of the stimulatory and inhibitory signals to AC during starvation we used isoproterinol and serotonin (5-HT), *i.e.* hormones stimulating AC activity via G_s proteins, and bromocryptin and somatostatin, *i.e.* hormones inhibiting AC activity via G_i proteins. All hormones were purchased from Sigma. The regulatory effects of these hormones on AC activity and binding of guanosine triphosphate (GTP) by G proteins in the myocardium, skeletal muscles, and brain of rats receiving standard ration were studied in our previous experiments [1-5].

For evaluation of AC activity we used (30 Ci/mmol) [α-^{32}P]-ATP (Amersham). For evaluation of GTP binding to G proteins we used nonhydrolysable GTP analog β,γ-imido[8-^{3}H]-guanosine 5'-triphosphate ammonium salt ([8-^{3}H]-GppNHp; 5 Ci/mmol, Amersham) and nitrocellulose filters HA, 0.45 μ (Millipore).

Activity of AC was measured as follows [11]: fractions of plasma membranes were incubated in a reaction mixture at 37°C for 10 min and AC acti-

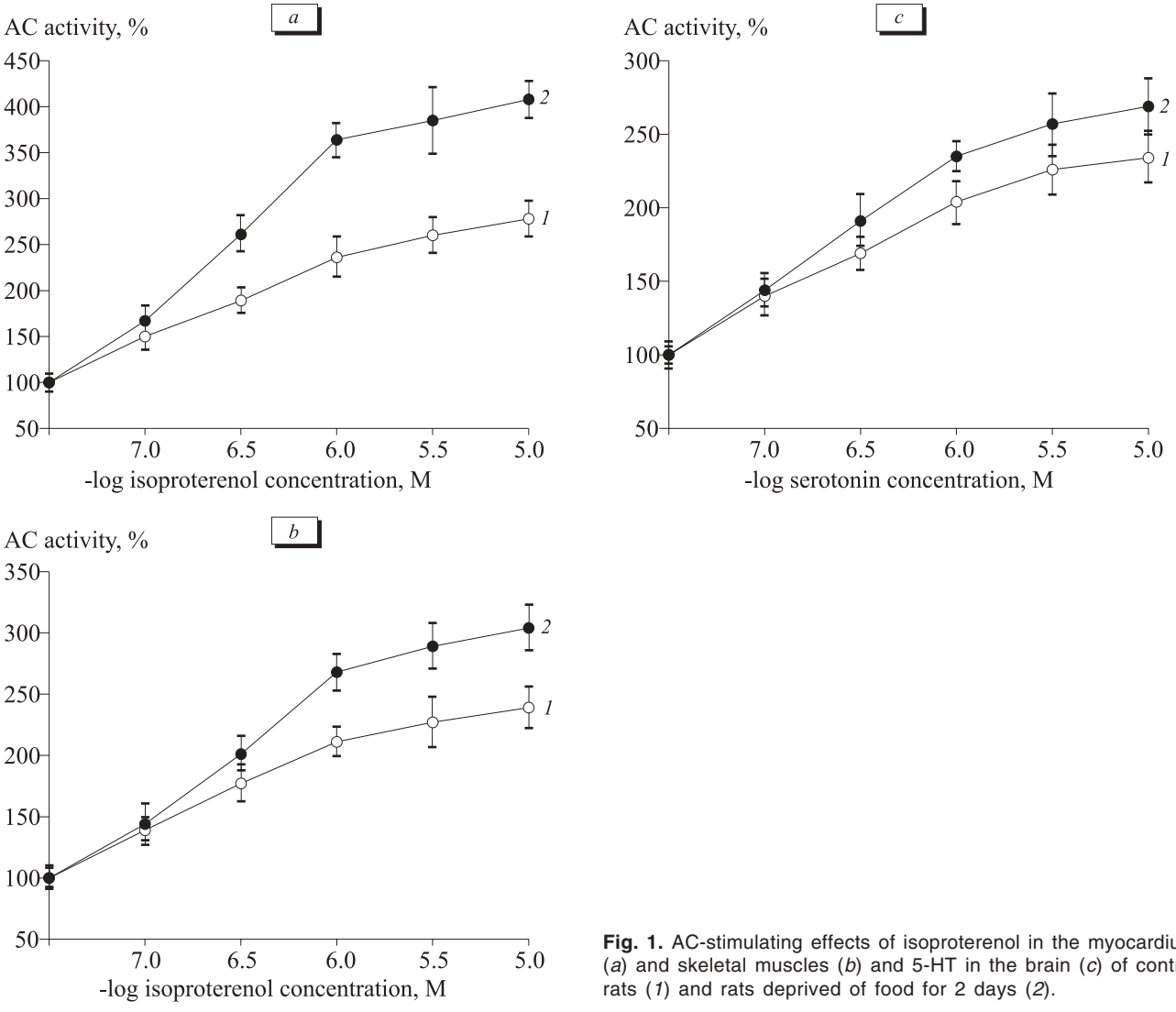

Fig. 1. AC-stimulating effects of isoproterenol in the myocardium (*a*) and skeletal muscles (*b*) and 5-HT in the brain (*c*) of control rats (*1*) and rats deprived of food for 2 days (*2*).

vity was determined by the amount of formed cyclic AMP (cAMP). Specific binding of GTP to heterotrimeric G proteins was determined by the difference between binding of [8-^{3}H]-GppNHp in the absence and presence of 10 mM GTP [1].

The data were processed using ANOVA software. Each experiment was performed in 3 repetitions. The differences between control samples and samples exposed to hormones and non-hormonal agents were considered significant at $p<0.05$.

RESULTS

Basal activity of AC in the myocardium, skeletal muscles, and striatum of control rats was 16.6±1.1, 16.2±0.8, and 76.9±5.2 pmol cAMP/min/mg membrane protein. In rats deprived of food for 2 days, basal activity of the enzyme in the myocardium and skeletal muscles decreased to 12.2±1.0 and 13.4±0.5 pmol cAMP/min/mg, respectively, but remained practically unchanged in the striatum (71.3±6.5 pmol cAMP/min/mg membrane protein). The AC-stimulating effect of GppNHp (10^{-5} M) in the myocardium of control animals was 179 and 98%, respectively, while in starving rats it increased by 1.5 times (to 295 and 142%, respectively). The AC-stimulating effect of another G protein activator NaF (10^{-2} M) also increased during food deprivation, but to a lesser extent. In the myocardium and skeletal muscles of controls it was 1845 and 1290%, respectively, while in starving rats the corresponding values were 2125 and 1440%. The AC-stimulating effect of forskolin (10^{-5} M) that directly interacts with the catalytic site of the enzyme was considerably higher in the myocardium of starving rats (369%) than in controls (305%), whereas in skeletal muscles the AC-effects of forskolin differed insignificantly (330% during food deprivation *vs.* 318% in the control). In the brain of starving and control rats, no reliable differences in the effect of

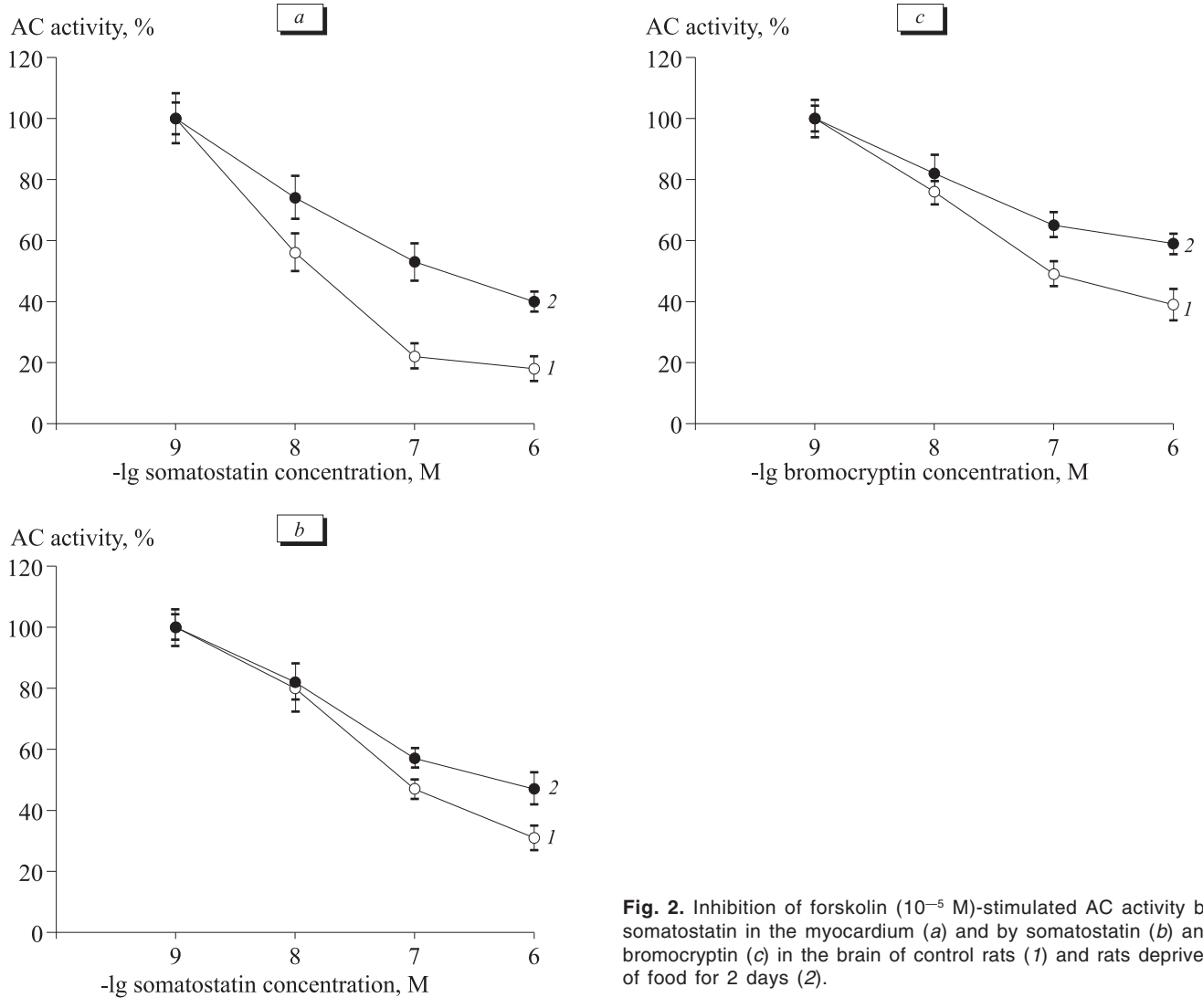

Fig. 2. Inhibition of forskolin (10^{-5} M)-stimulated AC activity by somatostatin in the myocardium (*a*) and by somatostatin (*b*) and bromocryptin (*c*) in the brain of control rats (*1*) and rats deprived of food for 2 days (*2*).

non-hormonal activators on AC activity were observed (data not presented).

Then, we compared the AC-stimulating effects of biogenic amines realizing their effects in tissues of starving and control rats via specific receptors coupled with G_s proteins. The AC-effects of isoproterenol, a specific β-adrenoceptor agonist, in muscles of starving rats far surpassed the control (Fig. 1, *a*, *b*). In the brain of starving rats, the AC-stimulating effect of 5-HT only slightly surpassed the effect of the hormone in the brain of control animals (Fig. 1, *c*).

Evaluation of the sensitivity of the AC system to hormones acting via receptors coupled with G_i proteins showed that the inhibitory effects of somatostatin in the myocardium and D_2-agonist bromocryptin in the brain on forskolin-prestimulated AC activity considerably decreased in rats deprived of food in comparison with the control (Fig. 2). The AC-inhibiting effect of somatostatin in the brain little changed. Thus, transmission of hormonal signals inhibiting AC is suppressed in the myocardium and, to a lesser extent, in the brain of starving rats.

Binding of GTP to G proteins is a parameter reflecting their functional activity. In plasma membrane fraction of the myocardium, skeletal muscles, and striatum of control rats, this parameter was 2.37±0.14, 1.45±0.13 and 6.93±0.35 pmol [8-^{3}H]-GppNHp/mg membrane protein, respectively. During starvation, GTP binding decreased in all tissues (maximum decrease was observed in the myocardium) and was 1.75±0.19, 1.22±0.15 and 5.57±0.41 [8-^{3}H]-GppNHp/mg membrane protein, respectively. Under conditions of food deprivation, stimulation of GTP binding by hormones realizing their regulatory effects on AC via G_s proteins increased, while stimulation of GTP binding by hormones inhibiting AC activity via G_i proteins decreased (Fig. 3). The differences in the sensitivity of G proteins to hormones were most pronounced in the myocardium.

Thus, transmission of AC-stimulating hormonal signals via the AC system in muscle tissues of rats deprived of food for 2 days became more intensive, whereas transmission of AC-inhibiting hormonal signals weakened. The observed changes in functional activity of the AC-system under conditions of 2-day starvation were tissue-specific. In the striatum of control and starving rats, the differences in the sensitivity of the AC system to hormones and non-hormonal agents were less pronounced then in muscle tissues, which attests to tissue-specific changes in the functional state of this system under conditions of 2-day starvation.

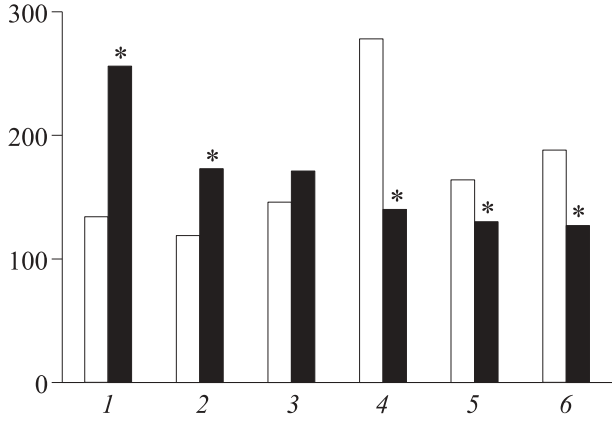

Fig. 3. Simulation of GTP binding by hormones in membrane fraction from the myocardium (*1, 4*), skeletal muscles (*2*) and brain (*3, 5, 6*) of control rats (light bars) and rats deprived of food for 2 days (dark bars). *1*, *2*) isoproterenol, 10^{-5} M; *3*) 5-HT, 10^{-5} M; *4*, 5) somatostatin, 10^{-7} M; *6*) bromocryptin, 10^{-5} Ì. **p*<0.05 compared to the control.

We hypothesized that changes in the sensitivity of the AC system to hormones are an effective adaptation mechanism aimed at optimization of the energy metabolism at the cellular and organ levels under conditions of starvation. Changes in hormonal sensitivity of the AC system can be triggered by hypoglycemia, *i.e.* a decrease in blood glucose concentration in starving rats. In rats deprived of food for 2 days, blood glucose concentration was 2.7±0.7 mM, *i.e.* almost 2-fold below the control (5.1±0.5 mM). A clear-cur relationship between blood glucose concentration and functional activity of hormonal signals was demonstrated in studies of hypoglycemia [7]. It was found that the increase in glucose concentration in insulin-dependent diabetes mellitus and incubation of cell cultures in a medium containing glucose in concentrations considerably surpassing its normal level in blood plasma directly affect functional activity of different G proteins and, consequently, on the efficiency of transmission of the hormonal signal through the AC system.

Thus, modulation of functional activity of hormonal signaling systems, in particular, AC system, and its sensitivity to hormonal signals can be a primary cause of biochemical and physiological changes in the organism during starvation.

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