Changes in Hormone Sensitivity of the Adenylate Cyclase Signaling System in the Testicular Tissue of Rats with Neonatal Streptozotocin-Induced Diabetes

A. O. Shpakov, K. V. Derkach, and V. M. Bondareva

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Activity of the adenylate cyclase signaling system was evaluated in the testicular tissue of rats with neonatal streptozotocin-induced diabetes (120 and 180 days duration). This state is similar to type 2 diabetes in humans. The regulation of this system by polypeptide hormones and biogenic amines was studied. Sensitivity of the adenylate cyclase signaling system to the regulatory effect of human chorionic gonadotropin and PACAP (pituitary adenylyl cyclase-activating polypeptide) was significantly reduced. The effects of these agents are realized via stimulatory G proteins. Somatostatin, acting through inhibitory G proteins, produced less pronounced effect on the adenylate cyclase signaling system. The increase in the duration of diabetes was accompanied by a decrease in the stimulatory effects of human chorionic gonadotropin and PACAP on adenylate cyclase. Sensitivity of the adenylate cyclase signaling system to biogenic amines remained unchanged (serotonin) or increased under these conditions (epinephrine). Our results indicate that changes in hormonal regulation of the adenylate cyclase signaling system and functional activity of cAMP-dependent signaling cascades are important factors for dysfunction of spermatogenesis and steroidogenesis during insulin-independent diabetes.

Key Words: *adenylate cyclase; G protein; diabetes; serotonin; testicular tissue; chorionic gonadotropin; PACAP*

Type 1 insulin-dependent diabetes mellitus and type 2 non-insulin-dependent diabetes mellitus are accompanied by a wide range of functional abnormalities in the human reproductive system [14]. Similar changes were found in reproductive tissues (*e.g.*, in the testes) of rats with experimental streptozotocin-induced (STZ-induced) diabetes [5]. The results of our previous studies and published data show that dysfunction of hormone signaling systems, including the adenylate cyclase signaling system (AC system), can be a cause of diabetes-related disorders [1-4,8,15]. Experimental diabetes and acute hyperglycemia in rats are accompanied by a decrease in reactivity of the muscle AC system to the effect of hormones that act through heterotrimeric G proteins of the stimulatory (G_s) or inhibitory type (G_i). Hormone signal transduction through G_i proteins is impaired to a greater extent, which is typical of not only muscle tissues, but also brain tissues, where G_s protein-coupled signaling cascades remain practically unchanged in diabetes mellitus [1-4,15].

This work was designed to evaluate functional activity of the AC system in the testicular tissue of rats with neonatal STZ-induced diabetes. This state is similar to type 2 non-insulin-dependent diabetes mellitus in humans [9]. Human chorionic gonadotropin (hCG), PACAP (pituitary adenylyl cyclase-activating polypeptide), relaxin, and somatostatin were studied as regulators of the AC system. The testicular tissue

Laboratory of Molecular Endocrinology, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia. ***Address for correspondence:*** alex_shpakov@list.ru. A. O. Shpakov

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serves as the target for these compounds. The regulatory effects of test compounds are realized via serpentine receptors. These receptors are functionally coupled to G_s proteins (hCG, PACAP, and relaxin) and G_i proteins (somatostatin) [6,7,11,13]. Experiments were performed with biogenic amines serotonin and epinephrine that play a role in functional activity of the testes [10,12]. The dynamics of dysfunction in the testicular AC system was evaluated at various stages of diabetes (120 and 180 days).

MATERIALS AND METHODS

Male Wistar rats were divided into 4 groups. Groups 1 and 2 consisted of control animals aging 120 days (n=12, 215±25 g) and 180 days (n=12, 320±40 g), respectively. Groups 3 and 4 included the animals with neonatal STZ-induced diabetes. The age of these specimens was 120 days (n=9, 245±35 g) and 180 days (n=9, 395±45 g), respectively. Neonatal diabetes was induced by administration of STZ (Sigma) in a dose of 80 mg/kg to 1-2-day-old males [9]. The development of diabetes was confirmed by the glucose tolerance test (blood sugar curve) and glucosuria. Two hours after administration of 20% glucose to fasting animals, blood glucose concentration was 11.3±1.2 (group 3) and 14.0±2.7 mM (group 4). Glucosuria was observed in diabetic rats.

Experiments were performed with hCG, somatostatin-14, epinephrine, serotonin, yohimbine, idazoxan, and cyanopindolol (Sigma). PACAP-38 was manufactured by Calbiochem. Pig relaxin-2 was gifted by Prof. O. D. Sherwood (USA). Other reagents were manufactured by Sigma and Reanal. [α-^{32}P]ATP (30 Ci/mol, Amersham) was used to evaluate AC activity.

Partially purified fractions of plasma membranes were obtained from rat testes. The testes were ground and homogenized in cold 40 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$ and 0.32 M sucrose (buffer A). The homogenate was centrifuged at 1500g for 10 min. The supernatant was centrifuged at 20,000g for 30 min. The pellet was resuspended in sucrose-free buffer A and repeatedly centrifuged under the same conditions. The pellet with plasma membranes was resuspended in sucrose-free buffer A and subjected to further analysis.

AC activity was estimated as described previously [15]. The plasma membrane fraction was incubated in the reaction mixture at 37°C for 10 min. AC activity was evaluated form the amount of cAMP (enzyme reaction product).

The data were analyzed by Student's t test. Each experiment was performed in 3 repetitions. The results of several independent experiments are expressed as M±m. The differences between control and hormone-treated samples were significant at p<0.05.

RESULTS

Basal AC activity in the testes of control rats was 96±8 (group 1, 120-day-old males) and 103±7 pmol cAMP/min/mg membrane protein (group 2, 180-day-old males). Basal AC activity in the testes of diabetic rats decreased to 80±9 (group 3, 120-day-old males) and 84±9 pmol cAMP/min/mg membrane protein (group 4, 180-day-old males). Sensitivity of the AC system to sodium fluoride and nonhydrolyzed analogue of guanine nucleotides (GppNHp, activators of heterotrimeric G proteins) was reduced in the testes of diabetic rats. GppNHp and NaF had a stimulatory effect on testicular AC in control animals of groups 1 and 2 (103-111 and 655-725%, respectively). These compounds stimulated the AC system in diabetic rats aging 120 days (group 3; 87 and 565%, respectively) and 180 days (group 4; 83 and 530%, respectively). Forskolin produces a direct effect on the catalytic site of this enzyme. The stimulatory effect of forskolin did not differ in diabetic and control animals. Our results indicate that basal AC activity and enzyme regulation by activators of heterotrimeric G proteins are reduced in the testes of rats with neonatal STZ-induced diabetes.

hCG (functional homologue of luteinizing and follicle-stimulating hormones) and PACAP-38 had a strong stimulatory effect on testicular AC in control rats (Fig. 1, *a*, *b*). The stimulatory effect of hormones on AC was much less pronounced in diabetic animals. Increasing the duration of diabetes from 120 to 180 days was accompanied by the reduction of this effect. Relaxin was less potent than hCG and PACAP in increasing activity of testicular AC in control rats (Fig. 1, *c*). It should be emphasized that relaxin had a similar effect on AC in diabetic animals. Somatostatin abolished the stimulatory effect of forskolin and hCG in the testes of control rats (Fig. 2). However, basal enzyme activity in the testes decreased insignificantly after treatment with somatostatin (data not shown). The inhibitory effect of this hormone on AC was less pronounced in diabetic rats aging 120 and 180 days. Our results indicate that signal transduction from polypeptide hormones is impaired in the testes of diabetic animals. These signals are realized through G_s proteins (hCG and PACAP) and G_i proteins (somatostatin). The exception was stimulatory effect of relaxin on AC. As differentiated from hCG and PACAP, the testes are not the major target for regulatory activity of relaxin. Somatostatin probably attenuates the inhibitory regulation of AC in various tissues of diabetic animals. It can be related to the decrease in the expression of G_i proteins and changes in functional coupling with the receptor [8]. This assumption is confirmed by

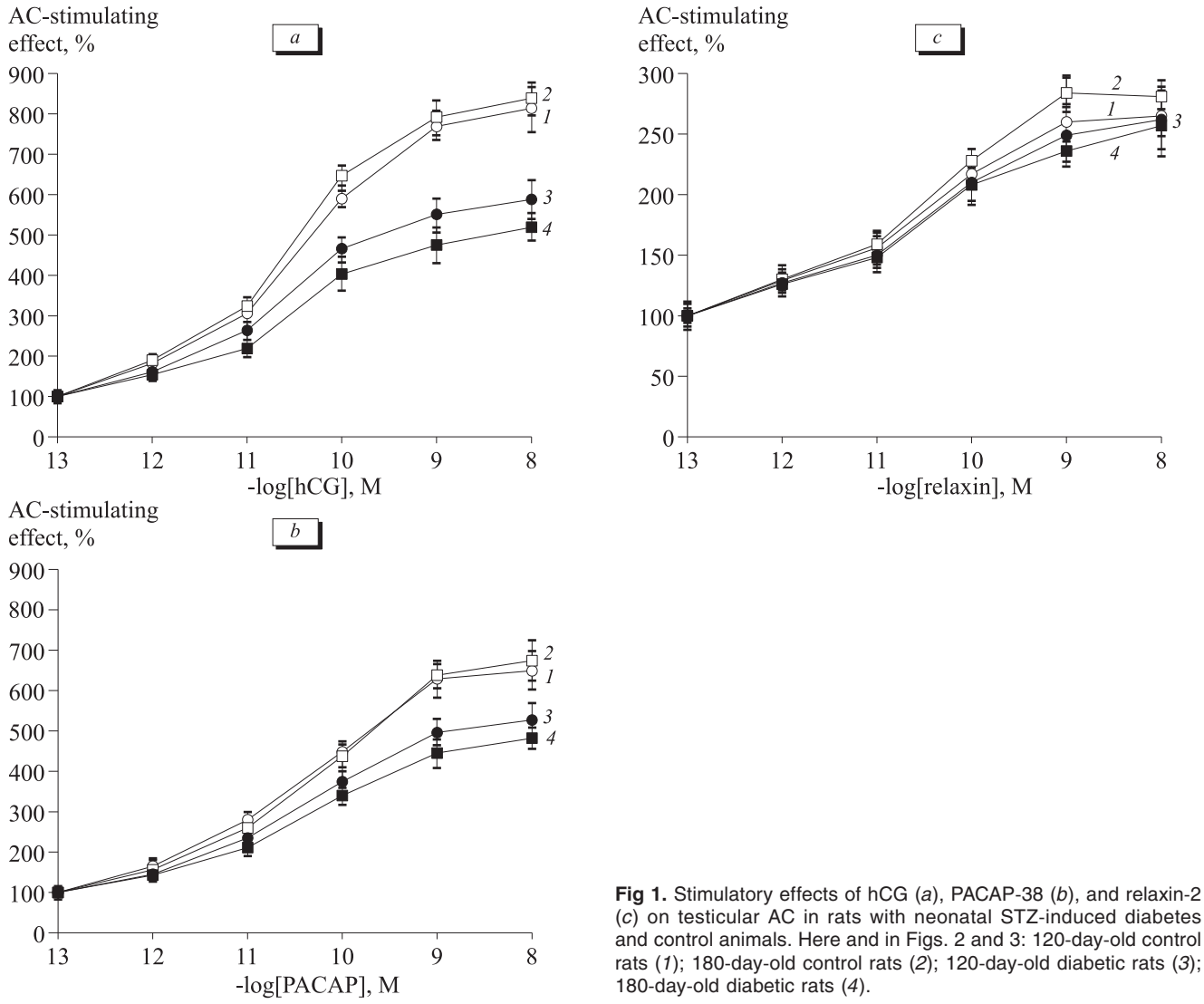

Fig 1. Stimulatory effects of hCG (*a*), PACAP-38 (*b*), and relaxin-2 (*c*) on testicular AC in rats with neonatal STZ-induced diabetes and control animals. Here and in Figs. 2 and 3: 120-day-old control rats (*1*); 180-day-old control rats (*2*); 120-day-old diabetic rats (*3*); 180-day-old diabetic rats (*4*).

the results of our previous experiments. We showed that functional activity of G_i protein-coupled signaling pathways in the myocardium, skeletal muscles, and brain decreases significantly in rats with neonatal STZ-induced diabetes [1,3,4].

Biogenic amines epinephrine and serotonin had a stimulatory effect on enzyme activity in the testes of control animals. Serotonin was more potent than epinephrine in this respect (Fig. 3). Epinephrine had a greater stimulatory effect on AC in diabetic rats. It is probably associated with the blockade of AC-inhibiting signaling pathways. They are triggered by epinephrine due to activation of α_2-adrenoceptors. These receptors are functionally coupled to AC through G_i protein (Fig. 3, *a*). For example, α_2-receptor antagonists yohimbine and idazoxan (10^{-5} M) block binding of epinephrine to α_2-adrenoceptors and prevent the epinephrine-induced inhibition of AC. Test compounds potentiate the stimulatory effect of epinephrine (10^{-6} M) on enzyme activity in the testes of control rats. The testicular tissue is characterized by high functional activity of AC-inhibiting signaling pathways. Epinephrine had a stimulatory effect on testicular AC in 120-day-old control rats (166%), which became more pronounced in the presence of yohimbine and idazoxan (209 and 197%, respectively). These antagonists did not modify the effect of epinephrine on AC during diabetes, since the inhibitory mechanisms of enzyme regulation are significantly reduced in the testes of diabetic animals.

The stimulatory effect of serotonin on testicular AC remained practically unchanged in diabetic animals, which is probably associated with the absence of serotonin-dependent signaling pathways for AC inhibition in the testes (Fig. 3, *b*). This assumption is confirmed by published data that cyanopindolol (10^{-5} M, specific antagonist of subtype 1A/1B serotonin receptors coupled to AC through G_i proteins) does not modify the effect of serotonin in control and diabetic animals. Therefore, biogenic amines have the

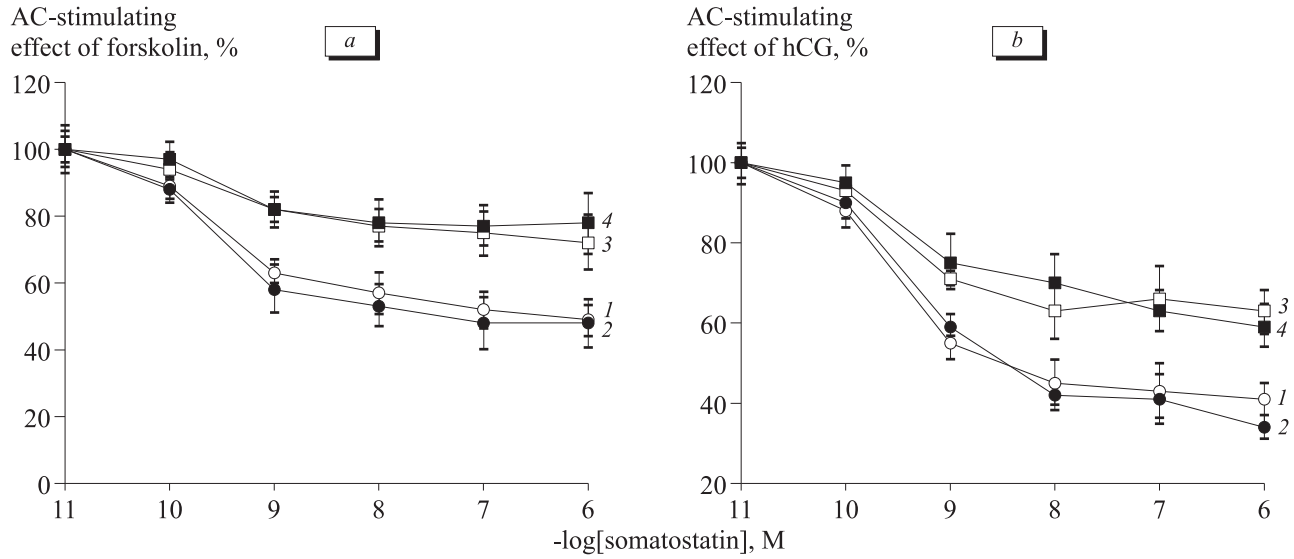

Fig. 2. Somatostatin-induced inhibition of the stimulatory effect of forskolin (*a*) and hCG (*b*) on testicular AC in diabetic and control rats. Ordinate: AC-stimulating effects of forskolin (10^{-5} M, *a*) and hCG (10^{-9} M, *b*), taken as 100%.

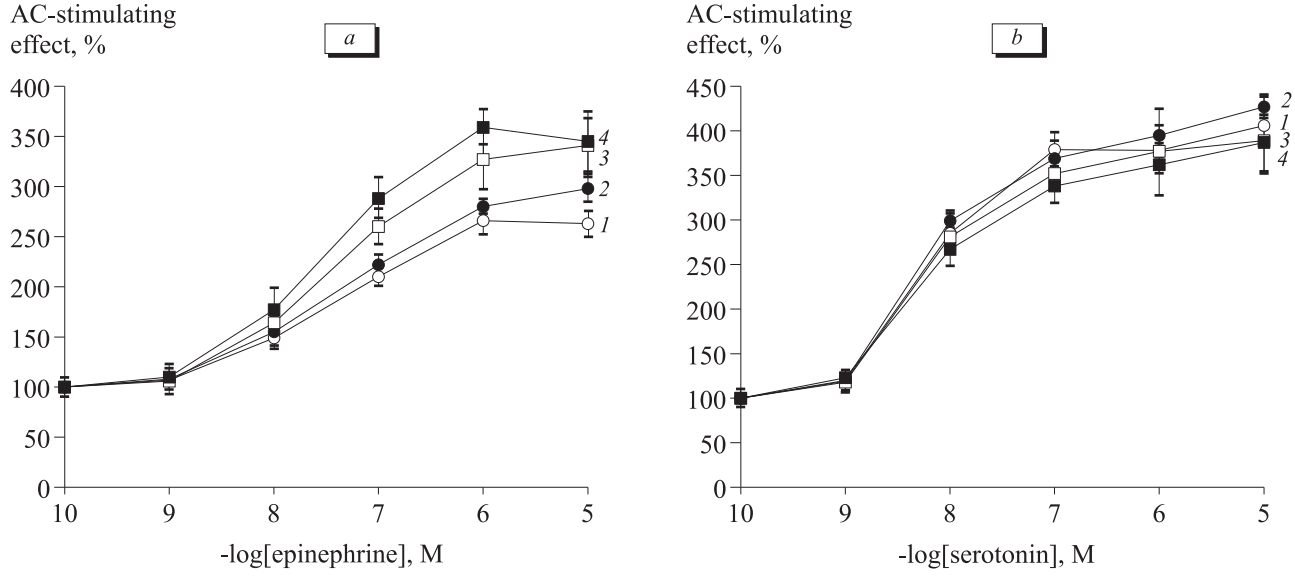

Fig 3. Stimulatory effects of epinephrine (*a*) and serotonin (*b*) on testicular AC in control and diabetic rats.

same stimulatory effect on AC during diabetes. The increase in the effect of epinephrine on AC is probably related to dysfunction of G_i protein-coupled signaling pathways in the testes of diabetic animals. Biogenic amines differ from hCG and PACAP in this property.

The reduced response of AC to spermatogenesis-regulating gonadotropins (hCG) and PACAP probably determines the significant decrease in the number and mobility of spermatozoa in the seminal fluid, increase in the ratio of immature and defective spermatozoa, decrease in the concentration of androgens, and other dysfunctions. These disorders are associated with impairment of testicular function in patients with type 2 diabetes mellitus [14]. Changes in hormone sensitivity of the AC system contribute to the development of hypogonadism, infertility, and other reproductive diseases (main complications of diabetes mellitus). Identification and study of functional activity of the AC system during diabetes hold much promise for the diagnostics and therapy of these diseases.

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