Hindawi Publishing Corporation
Dataset Papers in Pharmacology
Volume 2013, Article ID 698435, 14 pages
http://dx.doi.org/10.7167/2013/698435

Dataset Paper

Alterations of Hormone-Sensitive Adenylyl Cyclase System in the Tissues of Rats with Long-Term Streptozotocin Diabetes and the Influence of Intranasal Insulin

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Received 31 May 2012; Accepted 19 July 2012

Academic Editors: T. H.-W. Huang, Y. Huang, W.-L. Lu, and Y. Uezono

One of the causes of complications in type 1 diabetes mellitus (T1DM) is the changes in adenylyl cyclase (AC) signaling system, identified on the early stages of the disease. However, the most significant disturbances in this system occur on the later stages of T1DM, which ultimately leads to severe complications, but functional state of the AC system in late T1DM is poorly understood. The aim of this work was to study alterations in AC system sensitive to biogenic amines and polypeptide hormones in the heart, brain, and testes of male rats with long-term, 7-month, streptozotocin T1DM and to assess the influence on them of 135-day therapy with intranasal insulin. It was shown that AC effects of β-adrenergic agonists in the heart, serotonin receptor agonists and PACAP-38 in the brain, chorionic gonadotropin and PACAP-38 in the testes, and somatostatin in all investigated tissues in long-term T1DM were drastically decreased. The treatment with intranasal insulin (0.48 IU/day) significantly restored these effects. The results were obtained suggesting that long-term T1DM induces significant alterations in hormone-sensitive AC system in the heart, brain, and testes that are much more pronounced, compared with short-term T1DM, and include a large number of hormonal regulations.

1. Introduction

The type 1 diabetes mellitus (T1DM), one of the most severe metabolic disorders in humans, characterized by hyperglycemia due to a relative or an absolute lack of insulin, leads to many complications, such as coronary heart diseases, hypertension, atherosclerosis, neurodegenerative diseases, cognitive deficit, and dysfunctions of the reproductive system [1, 2]. One of the causes of these complications is the alterations in hormone-sensitive signaling systems, the adenylyl cyclase (AC) system in particular [3, 4]. It was shown that changes in the functional activity of AC system in the heart, brain, and reproductive tissues in experimental T1DM were tissue and hormone specific [5–10]. Generally, the effects of hormones acting on AC via G proteins of the inhibitory type (G_i) were changed to a greater degree compared with those realized via G proteins of the stimulating type (G_s), likely due to a decrease of G_i proteins expression and a reduction of their functional activity and coupling with upstream and downstream signal proteins.

The degree of alterations and abnormalities in hormonal signaling systems is well correlated with the severity of T1DM and its complications [11, 12]. As a result, at the later stages of the disease characterized by pronounced clinical symptoms, there are all reasons to expect the changes in these systems to be much expressed. However, the functional state of hormonal signaling systems in late T1DM has been studied rather poorly yet, which greatly complicates the treatment of this disease and the monitoring of diabetic patients. The aim of this work was to identify and study the alterations in AC system sensitive to biogenic amines and polypeptide hormones in the heart, brain, and testes of male rats with long-term, 7-month, streptozotocin (STZ) T1DM and to reveal the influence of 135-day therapy with intranasal insulin (I-I) on the functioning and sensitivity of this system to hormones. The intranasal route of insulin

delivery was chosen proceeding from the fact that I-I has no significant influence on the level of peripheral glucose and, therefore, does not provoke hypoglycemic episodes [13, 14]. I-I normalizes metabolic processes and improves cognitive functions, but the molecular mechanisms and targets of its action have not been well defined [15, 16]. It should be noted also that the information concerning the influence of I-I on human and experimental T1DM is scarce [17, 18].

2. Methodology

The model of long-term streptozotocin T1DM in adult male rats and the intranasal insulin treatment were as follows. For experiments, adult male Wistar rats housed in plastic sawdust-covered cages with a normal light-dark cycle and free access to food and water were obtained. The experiments were carried out under the Bioethics Committee of Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia (Institutional Guidelines, December 23, 2010) and "*Guidelines for the treatment of animals in behavior research and teaching*" [19]. All efforts were made to minimize animal suffering and reduce the number of animals used.

After a one-week adaptation period, rats aged 17-18 weeks were randomly divided into diabetic and control groups. Experimental diabetes mellitus of the type 1 (T1DM) was induced by a three consecutive intraperitoneal injection of freshly prepared STZ (Sigma, St. Louis, Mo, USA) in 0.1 M citrate buffer (pH 4.5) on the first, tenth, and eightieth days of experiments at doses 40, 35, and 30 mg/kg of body weight, respectively. Daily serum glucose level was assessed using test strips One Touch Ultra (USA) and a glucometer (LifeScan Johnson & Johnson, Denmark) via puncture of the tail. Nine days after each STZ administration, animals with fasting blood glucose level over 12 mM were considered diabetic and were used in further experiments. Diabetic rats had, in addition, a very pronounced glucosuria. The monitoring of glucose concentration in the urine was carried out using test strips (Combi-Screen Analyticon, Germany). The insulin concentration in rat serum was determined using Rat Insulin ELISA (Mercodia AB, Sweden).

At the seventy-fifth day of experiment, 5 days before the last injection of STZ, diabetic rats were divided into two equal groups. One group of diabetic rats (Group DI) was treated with I-I and the other with citrate buffer without insulin (Group D). Control animals were also divided into two equal groups: one received I-I (Group CI) and the other buffer without it (Group C). Intranasal delivery of insulin to the rat brain was performed as described previously by Thorne and coworkers [20]. Crystalline insulin at concentration 24 IU/mL was dissolved in 0.1 M citrate buffer, pH 4.5, and administered intranasally to both diabetic (Group DI) and control (Group CI) rats once a day. Each rat was placed in a supine position and then an average of 20 μL of insulin solution (0.48 IU) was administered by Eppendorf pipette as 5 μL drops in each nostril, in turn, every 1-2 min. Control animals were given the equal volume of saline, pH 4.5. The I-I treatment of rats from Groups DI and CI was carried out during 135 days. Twenty-four hours after the last intranasal administration of insulin or saline (on the 210th day of the experiment), the animals were decapitated under anesthetics, and their heart, brain tissues (cerebral cortex, striatum, hypothalamus, and olfactory bulbs), and testes were rapidly dissected and frozen on dry ice. Finally, four groups of rats were investigated: control animals (Group C, $n = 8$), control animals treated with I-I (Group CI, $n = 7$), diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM) (Group D, $n = 8$), and diabetic animals treated with I-I (Group DI, $n = 7$).

The chemicals used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Calbiochem (San Diego, CA, USA). STZ, β,γ-imidoguanosine-5′-triphosphate (GppNHp), forskolin, human chorionic gonadotropin (hCG), somatostatin-14, pituitary adenylyl cyclase-activating peptide-38 (PACAP-38), serotonin, noradrenaline, isoproterenol, dopamine, and bromocriptine were purchased from Sigma-Aldrich (St. Louis, MO, USA). 5-nonyloxytryptamine and 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole (EMD-386088) were purchased from Tocris Cookson Ltd. (UK). [α-^{32}P]-ATP (4 Ci/mmol) was purchased from Isotope Company (St. Petersburg, Russia).

To study the functional activity of hormone-sensitive AC system in long-term T1DM, we chose the hormones that regulate AC activity via G_s-coupled (stimulation) and G_i-coupled receptors (inhibition) and play a key role in the functioning of the cardiovascular, nervous, and reproductive systems. The brain serotonin-regulated AC system was examined using 5-nonyloxytryptamine, a selective agonist of G_i-coupled 5-hydroxytryptamine receptors (5-HTRs) of the subtype 1B (5-HT_{1B}R); EMD-386088, a selective agonist of G_s-coupled 5-HT_6R; and the brain dopamine-regulated AC system using nonselective dopamine and bromocriptine, a selective agonist of D_2-dopamine receptors (D_2-DARs). In all investigated tissues, the AC inhibiting the effect of somatostatin realized via G_i-coupled somatostatin receptors was studied. The myocardial adrenergic signaling was studied using noradrenaline, an agonist of β_1- and α_2-adrenergic receptors (β_1-AR and α_2-AR), and isoproterenol, a nonselective β-AR agonist, and in the testes using hCG, a glycoprotein hormone produced during pregnancy, and PACAP-38 that stimulate AC via G_s-coupled luteinizing hormone/hCG and PAC1 receptors, respectively.

The preparation of cardiac membranes from the rat heart was performed according to Baker and Potter [21], with some modifications. The dissected hearts were placed in ice-cold 0.9% NaCl, and atria, fat, and valves were removed. The tissues were cut into small pieces; homogenized with a Polytron in 20 volumes of ice-cold 40 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$, 320 mM sucrose, and a cocktail of protease inhibitors 500 μM *O*-fenantrolin, 2 μM pepstatin, and 1 mM phenylmethylsulfonyl fluoride (Buffer A); and centrifuged at 480 ×g for 10 min at 4°C. The pellet was discarded, and the supernatant was centrifuged at 27 500 ×g for 20 min at 4°C. The pellet was resuspended in Buffer A (without sucrose) and then centrifuged at 27 500 ×g for 20 min. The preparation of synaptosomal membranes from the rat brain was performed as described earlier [22]. The

brain tissues were dissected on ice and homogenized with a Polytron in 30 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 2 mM EGTA, 10% (w/v) sucrose, and a cocktail of protease inhibitors (Buffer B). The obtained material underwent centrifuge procedures, each performed at 4°C. The crude homogenate was centrifuged at 1000 ×g for 10 min; the resulting pellet was discarded and the supernatant was centrifuged at 9000 ×g for 20 min. The pellet was resuspended in Buffer B (without sucrose) and centrifuged at 35 000 ×g for 10 min. The isolation of plasma membranes from testes was carried out as described earlier [23]. The testes were placed in ice-cold Buffer A and homogenized with a Polytron. The homogenate was centrifuged at 1500 ×g for 10 min at 4°C. The supernatant was centrifuged at 20 000 ×g for 30 min at 4°C. The resulting pellet was washed in 10 volumes of Buffer A (without sucrose) and centrifuged again at 20 000 ×g for 30 min. The final pellet was resuspended in the 50 mM Tris-HCl buffer (pH 7.4) to produce the membrane fraction with a protein concentration range 1–3 mg/mL and stored at −70°C. The protein concentration of each membrane preparation in all experiments was measured by the method of Lowry and colleagues using BSA as a standard.

The adenylyl cyclase (AC, EC 4.6.1.1) activity was measured as described previously [22]. The reaction mixture (final volume 50 μL) contained 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM ATP, 1 μCi [α-^{32}P]-ATP, 0.1 mM cAMP, 20 mM creatine phosphate, 0.2 mg/mL creatine phosphokinase, and 15–45 μg of membrane protein. Incubation was carried out at 37°C for 10 min. The reaction was initiated by the addition of membrane protein and terminated by the addition of 100 μL of 0.5 M HCl, followed by immersing the tubes with mixture in a boiling water bath for 6 min; 100 μL of 1.5 M imidazole was added to each tube. In these conditions, the AC activity was linear. [^{32}P]-cAMP formed as a result of the enzyme reactions was separated using alumina for column chromatography. The samples were placed on neutral alumina columns, and cAMP was eluted with 10 mL of 10 mM imidazole-HCl buffer (pH 7.4). The eluates were collected in scintillation vials and counted using an LS 6500 scintillation counter (Beckman Instruments Inc., USA). Each assay was carried out in triplicate at least three times, and the results were expressed as picomole (pmol) cAMP/min per milligram (mg) of membrane protein. The basal activity was measured in the absence of hormones and forskolin. To measure AC inhibition by hormones, the enzyme was activated by forskolin (10^{-5} M).

The data are presented as the weighted mean ± weighted standard deviation. The difference in the weight and in glucose and insulin plasma levels of control and diabetic animals and in the basal activity of AC in the tissue membrane fractions of control and diabetic animals as well as the difference in the AC activity in the membrane fractions treated by hormones and nonhormonal AC regulators (GppNHp and forskolin) in each case was statistically assessed using the one-way analysis of variance (ANOVA, t-test) and was considered significant at $P < 0.05$. The assumption of normality was assessed using Shapiro-Wilk test. The results of the test confirmed that the obtained data are a sample from a normal distribution at $\alpha = 0.05$.

3. Dataset Description

The dataset associated with this Dataset Paper consists of 8 items, which are described as follows.

Dataset Item 1 (Table). Body weight, plasma glucose, and plasma insulin levels in control rats without (Group C) and with (Group CI) I-I treatment and in rats with long-term, 7-month, STZ T1DM without (Group D) and with (Group DI) I-I treatment. The plasma glucose level in diabetic rats was considerably increased during the whole period after the first treatment with STZ due to insulin deficiency, and their body weight was significantly lower as compared with control. In rats with 7-month STZ T1DM, glucose levels were approximately three times higher and the weight was reduced by 14% compared with the respective control (Table 1). In diabetic rats, the therapy with I-I led to an increase of the body weight and to a decrease of the elevated glucose level but had no effect on insulin deficit. In control animals, I-I changed neither body weight nor glucose level and decreased a little insulin level.

Column 1: Group Name

Column 2: Body Weight (g)

Column 3: Plasma Glucose (mM)

Column 4: Plasma Insulin (ng mL^{-1})

Dataset Item 2 (Table). The basal AC activity in plasma membrane fractions isolated from the heart, brain, and testes of insulin-treated and -untreated diabetic and control rats (in picomole (pmol) cAMP/min per milligram (mg) of membrane protein). Group C is the control animals ($n = 8$); CI, the control animals treated with intranasal insulin (I-I) ($n = 7$); D, the diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM) ($n = 8$); DI, the diabetic animals treated with I-I ($n = 7$). The basal AC activity in the heart and testes of diabetic rats, compared with control, was significantly decreased (Table 2). The treatment with I-I did not influence on the basal AC activity in the heart and testes of control animals but restored it in diabetic rats. In the brain of diabetic rats, the basal AC activity did not change considerably compared with control, and I-I had little effect on it in both control and diabetic animals. This indicates weakening of catalytic function of AC in the diabetic heart and testes, but not in the brain.

Column 1: Group Name

Column 2: Series

Column 3: Heart

Column 4: Brain

Column 5: Testes

Dataset Item 3 (Table). AC stimulating effects of GppNHp activating G_s proteins directly interacting with catalytic site

Table 1: Body weight, plasma glucose, and plasma insulin levels in control rats without (Group C) and with (Group CI) I-I treatment and in rats with long-term, 7-month, STZ T1DM without (Group D) and with (Group DI) I-I treatment.

Groups of Rats	Body Weight (g)	Plasma Glucose (mM)	Plasma Insulin (ng mL^{-1})
Group C ($n = 8$)	412 ± 27 (C-D, $P = 0.0018$; C-CI, $P = 0.3835$)	4.9 ± 0.5 (C-D, $P = 0.0003$; C-CI, $P = 0.9913$)	2.2 ± 0.3 (C-D, $P = 10^{-9}$; C-CI, $P = 0.0017$)
Group CI ($n = 7$)	428 ± 39 (CI-DI, $P = 0.0234$)	4.9 ± 0.7 (CI-DI, $P = 0.0164$)	1.5 ± 0.4 (CI-DI, $P = 0.00008$)
Group D ($n = 8$)	354 ± 32 (D-DI, $P = 0.1497$)	16.7 ± 5.0 (D-DI, $P = 0.0458$)	0.3 ± 0.2 (D-DI, $P = 0.8523$)
Group DI ($n = 7$)	379 ± 30	11.0 ± 4.9	0.4 ± 0.2

Values are expressed as the mean ± standard deviation.

Table 2: The basal AC activity in plasma membrane fractions isolated from the heart, brain, and testes of insulin-treated and -untreated diabetic and control rats (in pmol cAMP/min per mg of membrane protein). C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I.

Groups of Rats	Heart	Brain	Testes
Group C ($n = 8$)	30.4 ± 0.3 (C-D, $P = 0.00000147$; C-CI, $P = 0.1433$)	26.8 ± 0.5 (C-D, $P = 0.0056$; C-CI, $P = 0.3619$)	20.0 ± 0.2 (C-D, $P = 10^{-13}$; C-CI, $P = 0.1685$)
Average of the first series	30.5	29.0	18.1
Average of the second series	26.6	28.8	18.8
Average of the third series	27.3	25.6	21.0
Average of the fourth series	31.0	25.3	18.2
Group CI ($n = 7$)	28.9 ± 0.3 (CI-DI, $P = 0.0003$)	23.8 ± 0.7 (CI-DI, $P = 0.7917$)	18.2 ± 0.2 (CI-DI, $P = 10^{-7}$)
Average of the first series	31.1	25.7	16.0
Average of the second series	32.0	23.4	19.4
Average of the third series	26.3	22.1	19.5
Average of the fourth series	32.2	32.2	17.8
Group D ($n = 8$)	23.9 ± 0.5 (D-DI, $P = 0.0012$)	25.1 ± 0.2 (D-DI, $P = 0.2915$)	11.1 ± 0.3 (D-DI, $P = 0.0001$)
Average of the first series	21.5	25.8	10.1
Average of the second series	24.8	25.5	11.4
Average of the third series	24.2	24.7	10.6
Average of the fourth series	21.6	24.6	12.4
Group DI ($n = 7$)	26.8 ± 0.2	25.5 ± 1.3	12.4 ± 0.2
Average of the first series	28.9	23.9	11.5
Average of the second series	25.3	29.2	13.7
Average of the third series	24.4	25.1	13.9
Average of the fourth series	26.0	27.0	14.6

Values are expressed as the weighted mean ± weighted standard deviation of four individual experiments.

of AC in the tissues of diabetic and nondiabetic rats. Group C is the control animals ($n = 8$); CI, the control animals treated with intranasal insulin (I-I) ($n = 7$); D, the diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM) ($n = 8$); DI, the diabetic animals treated with I-I ($n = 7$). In the heart, brain, and testes of control rats, AC stimulating effects of GppNHp (10^{-5} M), nonhydrolysable analog of GTP, were 229, 204, and 105% over the basal activity of the enzyme, respectively. It is a common knowledge that GppNHp activates G_s proteins and thus stimulates the basal AC activity. In the diabetic heart and testes, AC effects of GppNHp were decreased, most significantly in the testes, and I-I restored the effects of GppNHp in the heart but did not in the testes (Figure 1(a) and Table 3(a)). In the diabetic brain, the AC effect of GppNHp decreased insignificantly. These data indicate the attenuation of catalytic function of the membrane-bound AC in the heart and testes of rats with long-term T1DM, whereas in the diabetic brain the enzyme catalytic properties were not altered. Note that in the short-term T1DM, we found no significant changes in the basal and

Table 3: The stimulating effects of GppNHp (a) and forskolin (b) on the basal AC activity in the heart, brain, and testes in diabetic and control rats treated with I-I or saline. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I. GppNHp and forskolin were taken at 10^{-5} M. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate.

(a)

Groups of Rats	Heart	Brain	Testes
Group C ($n = 8$)	69.6 ± 1.7 (C-D, $P = 10^{-9}$; C-CI, $P = 0.3460$)	54.6 ± 0.6 (C-D, $P = 0.0322$; C-CI, $P = 0.2613$)	20.9 ± 0.3 (C-D, $P = 10^{-6}$; C-CI, $P = 0.3268$)
Average of the first series	68.0	63.5	20.1
Average of the second series	67.8	54.3	26.4
Average of the third series	74.7	52.6	23.8
Group CI ($n = 7$)	66.8 ± 1.0 (CI-DI, $P = 0.0040$)	54.0 ± 2.4 (CI-DI, $P = 0.2172$)	24.4 ± 1.0 (CI-DI, $P = 10^{-10}$)
Average of the first series	75.0	59.8	23.8
Average of the second series	63.1	45.4	24.9
Average of the third series	64.6	54.2	25.4
Group D ($n = 8$)	43.2 ± 1.1 (D-DI, $P = 10^{-7}$)	50.6 ± 1.5 (D-DI, $P = 0.4860$)	9.0 ± 0.2 (D-DI, $P = 0.3766$)
Average of the first series	42.0	56.0	9.0
Average of the second series	49.1	47.7	8.6
Average of the third series	42.4	48.9	9.8
Group DI ($n = 7$)	59.7 ± 1.2	47.5 ± 2.0	8.5 ± 0.3
Average of the first series	56.7	53.3	8.5
Average of the second series	57.5	41.9	9.6
Average of the third series	62.1	51.9	8.3

(b)

Groups of Rats	Heart	Brain	Testes
Group C ($n = 8$)	201 ± 4 (C-D, $P = 10^{-8}$; C-CI, $P = 0.1781$)	166 ± 4 (C-D, $P = 0.3585$; C-CI, $P = 0.2458$)	41 ± 2 (C-D, $P = 10^{-9}$; C-CI, $P = 0.3261$)
Average of the first series	191	145	40
Average of the second series	204	173	42
Average of the third series	212	156	46
Group CI ($n = 7$)	203 ± 7 (CI-DI, $P = 0.0211$)	164 ± 3 (CI-DI, $P = 0.0033$)	38 ± 2 (CI-DI, $P = 10^{-6}$)
Average of the first series	173	178	44
Average of the second series	189	159	35
Average of the third series	209	158	41
Group D ($n = 8$)	145 ± 3 (D-DI, $P = 0.0004$)	152 ± 7 (D-DI, $P = 0.5864$)	18 ± 1 (D-DI, $P = 0.2506$)
Average of the first series	131	175	14
Average of the second series	155	138	23
Average of the third series	153	136	21
Group DI ($n = 7$)	166 ± 2	153 ± 3	23 ± 2
Average of the first series	171	161	27
Average of the second series	176	139	21
Average of the third series	162	135	18

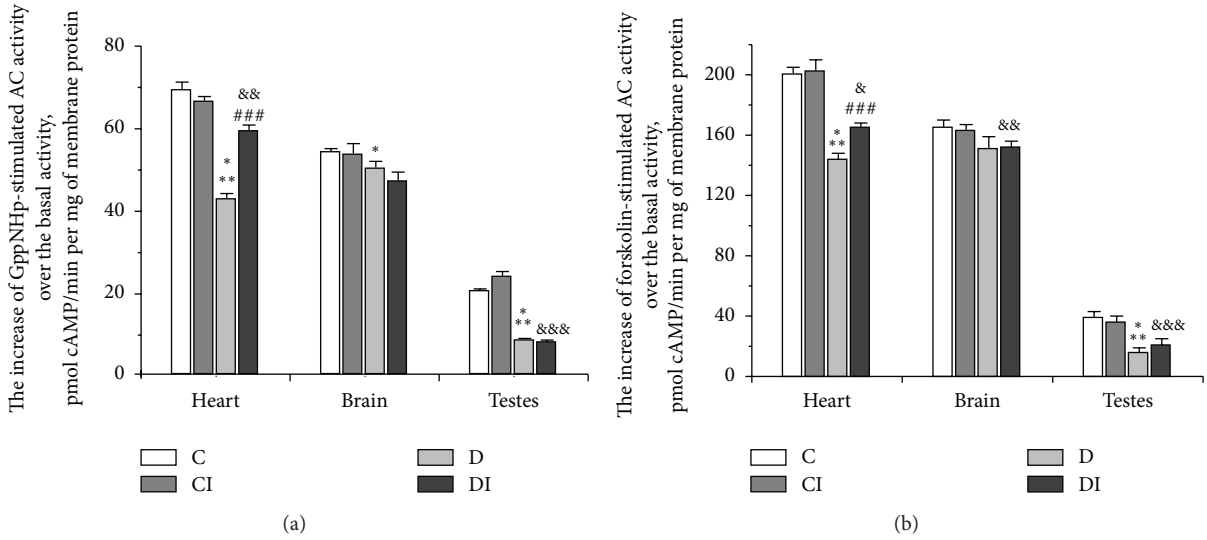

Figure 1: The stimulating effects of (a) GppNHp and (b) forskolin on the basal AC activity in the heart, brain, and testes in diabetic and control rats treated with I-I or saline. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I. GppNHp and forskolin were taken at 10^{-5} M. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate. ∗ and ∗∗∗ denote the statistically significant difference between Groups C and D at $P < 0.05$ and 0.001; ###, the same between Groups D and DI at $P < 0.001$; &, &&, and &&&, the same between Groups CI and DI at $P < 0.05$, 0.01, and 0.001, respectively.

GppNHp-stimulated AC activities in the heart and a small decrease of these activities in the testes [10, 25].

Column 1: Group Name

Column 2: Series

Column 3: Heart

Column 4: Brain

Column 5: Testes

Dataset Item 4 (Table). AC stimulating effects of forskolin directly interacting with catalytic site of AC in the tissues of diabetic and nondiabetic rats. Group C is the control animals ($n = 8$); CI, the control animals treated with intranasal insulin (I-I) ($n = 7$); D, the diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM) ($n = 8$); DI, the diabetic animals treated with I-I ($n = 7$). In the heart, brain, and testes of control rats, AC stimulating effects of diterpene forskolin (10^{-5} M) were 661, 619, and 205%, respectively. It is a common knowledge that forskolin directly interacts with catalytic site of the enzyme. In the diabetic heart and testes, AC effects of forskolin were decreased, most significantly in the testes, and I-I restored the effects of forskolin in the heart but did not in the testes (Figure 1(b) and Table 3(b)). In the diabetic brain, the AC effect of forskolin did not change. These data indicate the attenuation of catalytic function of the membrane-bound AC in the heart and testes of rats with long-term T1DM, whereas in the diabetic brain, the enzyme catalytic properties were not altered. Note that in the short-term T1DM, we found no significant changes in the basal and forskolin-stimulated AC activities in the heart and a small decrease of these activities in the testes [10, 25].

Column 1: Group Name

Column 2: Series

Column 3: Heart

Column 4: Brain

Column 5: Testes

Dataset Item 5 (Table). The AC effects of hormones activating the enzyme via G_s protein-coupled receptors in the tissues of diabetic rats and under the influence of I-I. In the diabetic heart, the AC stimulating effects of isoproterenol and noradrenaline, the agonists of β-adrenergic receptors (β-AR), were decreased and partially restored by I-I. A most significant decrease was observed with noradrenaline acting preferably via β_1-AR undergoing considerable down-regulation in T1DM [26], whereas AC effect of isoproterenol, nonselective β-AR agonist, was reduced to a lesser extent (Figure 2(a) and Table 4(a)). In our view, this may be due to the fact that the signaling pathways mediated via β_2-AR, the main target of isoproterenol, in the heart of rats with T1DM, even short-term, were preserved or enhanced, this being a compensatory mechanism triggered by reducing the functional activity of β_1-AR [11, 27]. Thus, in the heart of 14-week STZ rats, the content of β_1-AR protein as well as β_1-AR mRNA was decreased to 35 and 45%, respectively, and the decrease of maximal chronotropic response to selective β_1-AR agonists was 70% of that in control, whereas the number

Table 4: The stimulating effects of hormones on AC activity in (a) the heart, (b) brain, and (c) testes of diabetic and control rats with and without therapy by I-I. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I. Isoproterenol, noradrenaline, serotonin, EMD-386088, and dopamine were taken at 10^{-5} M, PACAP-38 at 10^{-7} M, and hCG at 10^{-8} M. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate.

(a) In picomole (pmol) of cAMP/min per milligram (mg) of membrane protein

Groups of Rats	Isoproterenol	Noradrenaline
Group C ($n = 8$)	65.0 ± 2.7 (C-D, $P = 0.0004$; C-CI, $P = 0.1348$)	47.2 ± 1.1 (C-D, $P = 10^{-8}$; C-CI, $P = 0.4434$)
Average of the first series	73.7	53.6
Average of the second series	54.9	48.1
Average of the third series	66.6	46.0
Group CI ($n = 7$)	60.3 ± 0.9 (CI-DI, $P = 0.2191$)	52.7 ± 1.9 (CI-DI, $P = 0.1188$)
Average of the first series	61.6	42.6
Average of the second series	61.1	56.7
Average of the third series	57.3	55.0
Group D ($n = 8$)	47.0 ± 1.8 (D-DI, $P = 0.0032$)	29.5 ± 1.6 (D-DI, $P = 0.0025$)
Average of the first series	43.9	30.3
Average of the second series	44.4	32.3
Average of the third series	55.2	25.8
Group DI ($n = 7$)	56.2 ± 1.7	37.9 ± 2.3
Average of the first series	53.8	31.9
Average of the second series	56.1	51.4
Average of the third series	62.0	49.8

(b) In picomole (pmol) of cAMP/min per milligram (mg) of membrane protein

Groups of Rats	Isoproterenol	Serotonin	EMD-386088	Dopamine	PACAP-38
Group C ($n = 8$)	42.6 ± 1.1 (C-D, $P = 10^{-9}$; C-CI, $P = 0.0374$)	81.3 ± 2.2 (C-D, $P = 0.0066$; C-CI, $P = 0.7467$)	28.3 ± 1.8 (C-D, $P = 0.0004$; C-CI, $P = 0.3930$)	52.2 ± 1.0 (C-D, $P = 0.1771$; C-CI, $P = 0.0050$)	34.1 ± 0.3 (C-D, $P = 10^{-8}$; C-CI, $P = 0.0382$)
Average of the first series	41.6	90.0	29.5	51.8	33.7
Average of the second series	42.6	81.1	27.3	50.0	36.3
Average of the third series	45.8	75.9	33.5	55.1	36.1
Group CI ($n = 7$)	40.3 ± 1.2 (CI-DI, $P = 0.0226$)	81.8 ± 2.1 (CI-DI, $P = 0.0107$)	34.2 ± 2.0 (CI-DI, $P = 0.2647$)	47.7 ± 1.2 (CI-DI, $P = 0.4680$)	37.8 ± 1.2 (CI-DI, $P = 0.2633$)
Average of the first series	40.6	83.2	30.0	46.6	40.6
Average of the second series	42.8	92.2	28.1	45.1	35.3
Average of the third series	37.3	75.1	39.0	51.2	38.7
Group D ($n = 8$)	28.0 ± 0.8 (D-DI, $P = 0.0031$)	67,2 ± 4.0 (D-DI, $P = 0.1032$)	21.4 ± 1.2 (D-DI, $P = 0.00065$)	44.4 ± 5.1 (D-DI, $P = 0.8898$)	22.3 ± 0.7 (D-DI, $P = 10^{-5}$)
Average of the first series	26.8	51.5	20.5	56.8	22.2
Average of the second series	29.4	72.6	19.4	32.7	25.0
Average of the third series	30.0	75.3	23.4	47.6	19.2
Group DI ($n = 7$)	33.9 ± 1.7	74.8 ± 1.3	29.5 ± 2.0	46.2 ± 1.7	37.9 ± 2.0
Average of the first series	33.9	75.9	25.4	50.2	34.7
Average of the second series	40.5	76.7	29.6	43.3	32.8
Average of the third series	31.7	70.8	33.2	45.6	40.2

(c) In picomole (pmol) of cAMP/min per milligram (mg) of membrane protein

Groups of Rats	hCG	PACAP-38
Group C ($n = 8$)	105 ± 4 (C-D, $P = 10^{-7}$; C-CI, $P = 0.0270$)	53 ± 3 (C-D, $P = 10^{-5}$; C-CI, $P = 0.4720$)
Average of the first series	127	44
Average of the second series	105	52
Average of the third series	95	58
Group CI ($n = 7$)	89 ± 5 (CI-DI, $P = 0.0039$)	50 ± 2 (CI-DI, $P = 0.0007$)
Average of the first series	110	50
Average of the second series	76	45
Average of the third series	84	69
Group D ($n = 8$)	40 ± 3 (D-DI, $P = 10^{-7}$)	29 ± 2 (D-DI, $P = 0.1024$)
Average of the first series	40	25
Average of the second series	47	35
Average of the third series	36	30
Group DI ($n = 7$)	67 ± 2	34 ± 2
Average of the first series	66	29
Average of the second series	63	36
Average of the third series	71	40

of β_2-AR and the chronotropic response to selective β_2-AR agonist fenoterol did not change [27].

Column 1: Group Name

Column 2: Series

Column 3: Isoproterenol

Column 4: Noradrenaline

Dataset Item 6 (Table). In the diabetic brain, the AC effects of isoproterenol, serotonin, selective 5-HT_6R agonist EMD-386088, and PACAP-38 were significantly decreased, but the corresponding effect of dopamine did not change (Figure 2(b) and Table 4(b)). The decreased AC effects of hormones were completely (PACAP-38, EMD-386088) or partially (isoproterenol, serotonin) restored by I-I treatment. A decrease of the AC effect of EMD-386088 indicates the impairment of 5-HT_6R-mediated pathways in the diabetic brain but does not exclude attenuation of the functional activity of the other types of 5-HTR that are also coupled with AC via G_s proteins. As we showed earlier, in the model of short-term T1DM and the neonatal model of type 2 diabetes mellitus (T2DM), although the stimulating effects of serotonin on AC activity and GTP binding of G_s proteins in the diabetic brain were reduced, the corresponding effects of EMD-386088 and, as a result, 5-HT_6R-mediated AC signaling did not change [16, 25]. There was a decrease in the number of 5-HT_6R and a reduced AC response to selective 5-HT_6R agonists in the brain of patients with prolonged Alzheimer's disease due to the neurodegenerative alterations in neuronal and glial cells [28]. The decrease in the sensitivity of AC to β-AR-agonist isoproterenol points to a weakening of β-AR signaling in the diabetic brain, which negatively influences the synaptic transmission provoked by impairment of the mechanism involving increase of intracellular cAMP concentration and protein synthesis [29] and induces disturbances in the cerebral microvessels, whose functions are controlled via different signaling systems, the adrenergic in particular [30]. A significant reduction of AC effect of PACAP-38 having an important role in protection of neuronal cells from damage and neurodegenerative changes [31] speaks in favor of abnormalities in PACAP-mediated neuroprotection in the case of long-term T1DM. It has been noticed that short-term T1DM and neonatal T2DM had no effect on the regulation of brain AC activity and G_s protein binding by PACAP-38 [16, 25]. We showed that the 135-day therapy with I-I prevents the decline of AC stimulation induced by PACAP-38 in the diabetic brain. Thus, the ability of I-I to restore AC signaling pathways regulated by PACAP-38 and some biogenic amines may be one of the main mechanisms of positive influence of I-I on the impaired CNS functions in human and experimental DM [16, 24, 32]. The AC effect of dopamine, contrary to the other investigated hormones, did not change in the diabetic brain. Earlier it was shown that dopamine-stimulated cAMP production in the brain of rats with 14-week alloxan DM or neonatal T2DM was markedly increased [16, 33]. This may be due to a compensatory increase in the expression of G_s protein-coupled D_1-DAR, as in the case of short-term STZ T1DM [34].

Column 1: Group Name

Column 2: Series

Column 3: Isoproterenol

Column 4: Serotonin

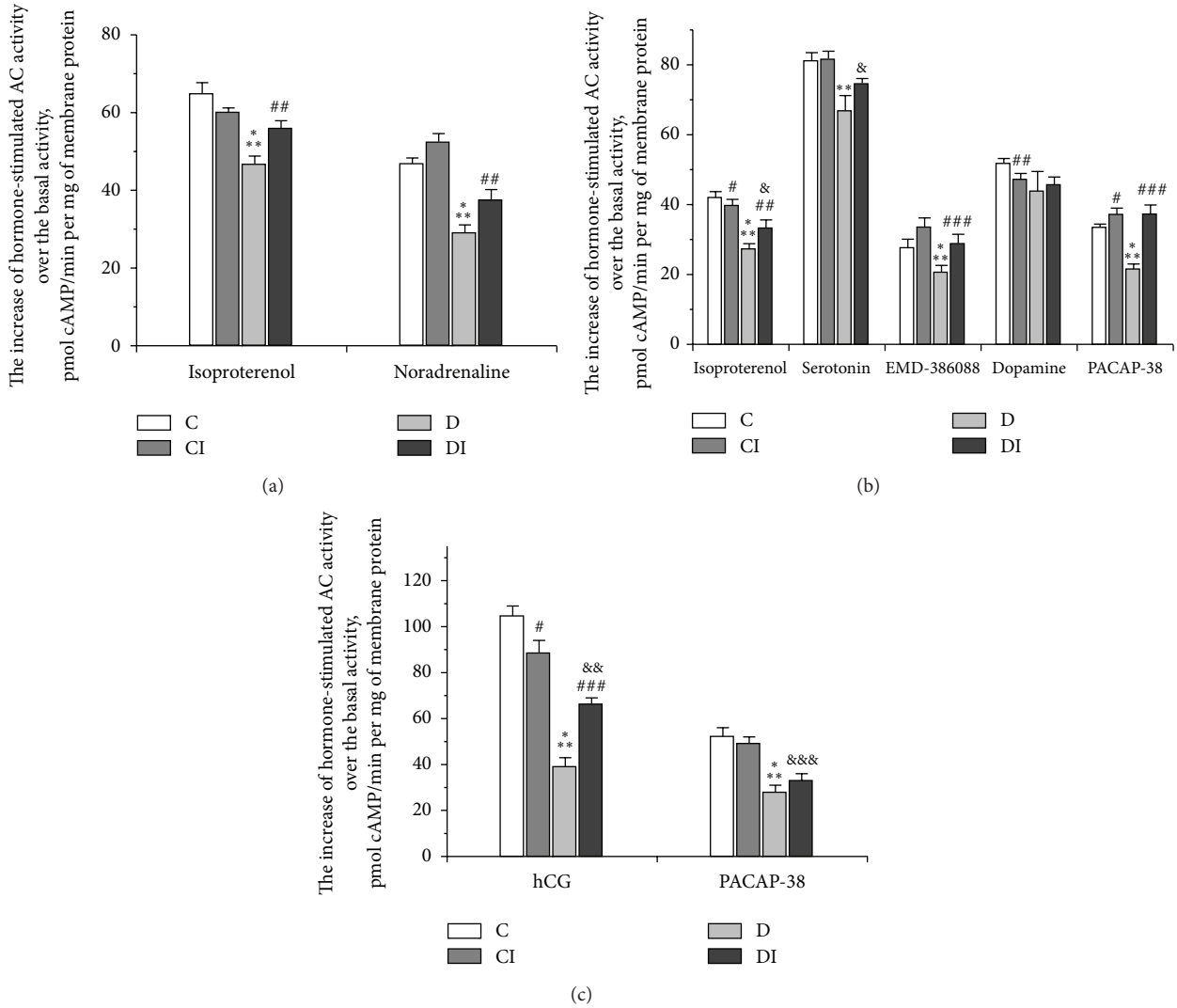

Figure 2: The stimulating effects of hormones on AC activity in (a) the heart, (b) brain, and (c) testes of diabetic and control rats with and without therapy by I-I. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I. Isoproterenol, noradrenaline, serotonin, EMD-386088, and dopamine were taken at 10^{-5} M, PACAP-38 at 10^{-7} M, and hCG at 10^{-8} M. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate. ∗∗ and ∗∗∗ denote the statistically significant difference between Groups C and D at $P < 0.01$ and 0.001; #, ##, and ###, the same between Groups D and DI at $P < 0.05$, 0.01, and 0.001; &, &&, and &&&, the same between Groups CI and DI at $P < 0.05$, 0.01, and 0.001, respectively.

Column 5: EMD-386088

Column 6: Dopamine

Column 7: PACAP-38

Dataset Item 7 (Table). In the diabetic testes, the AC effects of hCG, structural and functional homologue of luteinizing hormone, and PACAP-38 were decreased significantly, and the effect of hCG was partially restored by I-I treatment (Figure 2(c) and Table 4(c)). The reduction in hCG- and PACAP-induced AC stimulation in the rat testes in long-term T1DM was more pronounced compared with that in short-term T1DM [10]. These results indicate that the abnormalities in testicular function in human and experimental T1DM can be ascribed to the changes in sensitivity of AC to gonadotropins and peptide hormones belonging to the vasoactive intestinal peptide/PACAP family. It should be mentioned that AC stimulation induced by vasoactive intestinal peptide in the seminal vesicle of rats with STZ T1DM was considerably reduced, which was associated with a decrease of $G\alpha_s$ subunit level [35, 36].

Column 1: Group Name

Column 2: Series

Column 3: hCG

Column 4: PACAP-38

Table 5: The inhibitory effects of hormones on forskolin-stimulated AC activity in the tissues of diabetic and control rats with and without I-I treatment. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I. The stimulating effect of forskolin (10^{-5} M) on the basal AC activity is taken as 100%. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate.

(a) In percentages

	Group C	Group CI	Group D	Group DI
		Heart		
Somatostatin	52 ± 3% (C-D, $P = 10^{-8}$; C-CI, $P = 0.7090$)	55 ± 2% (CI-DI, $P = 10^{-7}$)	88 ± 3% (D-DI, $P = 0.0070$)	78 ± 3%
Average of the first series	51	55	90	77
Average of the second series	59	57	83	83
Average of the third series	50	45	87	73
Noradrenaline	44 ± 2% (C-D, $P = 10^{-6}$; C-CI, $P = 0.0927$)	49 ± 2% (CI-DI, $P = 0.4005$)	73 ± 3% (D-DI, $P = 0.00015$)	49 ± 4%
Average of the first series	44	51	63	52
Average of the second series	43	48	78	56
Average of the third series	42	42	69	43
		Brain		
Somatostatin	50 ± 3% (C-D, $P = 0.0006$; C-CI, $P = 0.1043$)	47 ± 3% (CI-DI, $P = 0.0032$)	66 ± 4% (D-DI, $P = 0.0745$)	58 ± 4%
Average of the first series	47	44	76	50
Average of the second series	52	52	62	67
Average of the third series	53	41	65	60
Noradrenaline	80 ± 3% (C-D, $P = 0.1763$; C-CI, $P = 0.6663$)	87 ± 3% (CI-DI, $P = 0.9149$)	93 ± 3% (D-DI, $P = 0.4016$)	84 ± 3%
Average of the first series	80	79	96	78
Average of the second series	92	86	84	84
Average of the third series	78	91	88	95
Bromocriptine	72 ± 3% (C-D, $P = 0.0276$; C-CI, $P = 0.7451$)	71 ± 4% (CI-DI, $P = 0.1306$)	81 ± 3% (D-DI, $P = 0.4622$)	81 ± 2%
Average of the first series	65	64	92	84
Average of the second series	68	76	74	78
Average of the third series	81	79	79	74
5-Nonyloxytryptamine	67 ± 4% (C-D, $P = 10^{-6}$; C-CI, $P = 0.2520$)	62 ± 5% (CI-DI, $P = 0.0446$)	93 ± 3% (D-DI, $P = 10^{-5}$)	70 ± 3%
Average of the first series	66	59	90	79
Average of the second series	63	66	91	68
Average of the third series	73	64	95	67
		Testes		
Somatostatin	64 ± 3% (C-D, $P = 10^{-7}$; C-CI, $P = 0.3465$)	67 ± 4% (CI-DI, $P = 10^{-6}$)	95 ± 3% (D-DI, $P = 0.7882$)	93 ± 3%
Average of the first series	65	73	104	107
Average of the second series	60	67	91	89
Average of the third series	64	59	96	93

(b) In picomole (pmol) of cAMP/min per milligram (mg) of membrane protein

	Group C	Group CI	Group D	Group DI
		Heart		
Somatostatin	105.2 ± 5.7 (C-D, $P = 0.0039$; C-CI, $P = 0.8304$)	111.8 ± 3.8 (CI-DI, $P = 0.0011$)	127.2 ± 4.1 (D-DI, $P = 0.4273$)	129.0 ± 5.1
Average of the first series	102.5	111.0	130.5	128.4
Average of the second series	119.3	115.0	119.9	137.8
Average of the third series	99.8	91.4	126.6	121.7
Noradrenaline	87.6 ± 4.1 (C-D, $P = 0.0080$; C-CI, $P = 0.0666$)	99.7 ± 4.2 (CI-DI, $P = 0.0596$)	105.4 ± 4.5 (D-DI, $P = 0.0110$)	81.8 ± 6.6
Average of the first series	88.4	104.2	91.8	86.9
Average of the second series	87.1	98.1	112.6	93.0
Average of the third series	84.4	85.9	105.1	70.8
		Brain		
Somatostatin	82.8 ± 4.7 (C-D, $P = 0.0064$; C-CI, $P = 0.0721$)	76.9 ± 5.0 (CI-DI, $P = 0.0203$)	100.4 ± 5.8 (D-DI, $P = 0.0882$)	89.4 ± 5.4
Average of the first series	78.6	71.6	115.5	76.5
Average of the second series	86.3	85.3	94.2	102.5
Average of the third series	87.4	67.8	98.8	92.3
Noradrenaline	132.4 ± 5.3 (C-D, $P = 0.6643$; C-CI, $P = 0.8534$)	142.5 ± 5.5 (CI-DI, $P = 0.1950$)	140.8 ± 4.4 (D-DI, $P = 0.4807$)	128.7 ± 4.7
Average of the first series	133.4	129.6	145.9	119.3
Average of the second series	153.3	141.6	127.7	128.5
Average of the third series	129.5	148.7	133.8	145.9
Bromocriptine	120.3 ± 5.5 (C-D, $P = 0.4123$; C-CI, $P = 0.8973$)	116.1 ± 7.0 (CI-DI, $P = 0.8306$)	122.6 ± 4.0 (D-DI, $P = 0.5505$)	124.6 ± 2.9
Average of the first series	108.5	105.0	139.8	129.0
Average of the second series	112.9	124.1	112.5	119.9
Average of the third series	134.5	129.6	120.6	113.7
5-Nonyloxytryptamine	111.7 ± 6.8 (C-D, $P = 0.000006$; C-CI, $P = 0.1797$)	102.2 ± 8.1 (CI-DI, $P = 0.3600$)	140.9 ± 3.9 (D-DI, $P = 10^{-5}$)	107.7 ± 4.9
Average of the first series	109.6	97.3	136.8	120.9
Average of the second series	105.1	108.2	138.8	104.0
Average of the third series	121.2	104.4	144.4	102.0
		Testes		
Somatostatin	26.3 ± 1.0 (C-D, $P = 10^{-7}$; C-CI, $P = 0.6490$)	25.6 ± 1.5 (CI-DI, $P = 10^{-4}$)	17.1 ± 0.6 (D-DI, $P = 0.0354$)	21.5 ± 0.6
Average of the first series	26.8	27.9	18.8	24.5
Average of the second series	24.6	25.5	16.4	20.4
Average of the third series	26.2	22.4	17.3	21.4

Dataset Item 8 (Table). The AC inhibitory effects of hormones acting via G_i protein-coupled receptors in the tissues of diabetic and nondiabetic rats, which were estimated by the influence of the hormones on forskolin-stimulated AC activity. AC inhibitory effects of somatostatin in all the investigated tissues, noradrenaline in the heart, and 5-$HT_{1B/1D}$R agonist 5-nonyloxytryptamine in the brain were decreased significantly in T1DM compared with control (Figure 3 and Table 5). The corresponding effects of noradrenaline and D_2-DAR agonist bromocriptine in the diabetic brain

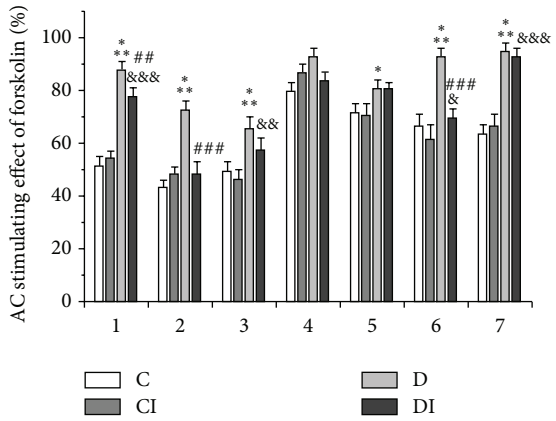

Figure 3: The inhibitory effects of hormones on forskolin-stimulated AC activity in the tissues of diabetic and control rats with and without I-I treatment. C: control animals; CI: control animals treated with intranasal insulin (I-I); D: diabetic animals with 210-day streptozotocin-induced diabetes mellitus of the type 1 (T1DM); DI: diabetic animals treated with I-I; 1, 3, and 7: somatostatin (10^{-6} M) in the heart, brain, and testes, respectively; 2 and 4: noradrenaline (10^{-5} M) in the heart and brain, respectively; 5: bromocriptine (10^{-5} M) in the brain; 6: 5-nonyloxytryptamine (10^{-5} M) in the brain. The stimulating effect of forskolin (10^{-5} M) on the basal AC activity is taken as 100%. Values are the weighted mean ± weighted standard deviation of three individual experiments, each performed in triplicate. ∗ and ∗∗∗ denote the statistically significant difference between Groups C and D at $P < 0.05$ and 0.001; #, ##, and ###, the same between Groups D and DI at $P < 0.05$, 0.01, and 0.001; &, &&, and &&&, the same between Groups CI and DI at $P < 0.05$, 0.01, and 0.001, respectively.

changed, but not very much. The therapy with I-I restored the inhibitory effects of noradrenaline in the heart and of 5-nonyloxytryptamine in the brain but influenced only a little the somatostatin effects in all the studied tissues. The significant decrease of AC effect of 5-nonyloxytryptamine indicates attenuation of 5-HT_1R-mediated AC signaling in the brain in the model of prolonged T1DM. These findings are consistent with the data obtained by the other authors on the weakening of the behavioral response of rats with T1DM to 5-HT_{1A}R agonist 8-hydroxy-2-(dipropylamino) tetralin hydrobromide [37], and with our data concerning a decrease of regulatory effects of 5-nonyloxytryptamine on AC activity and G_i proteins GTP binding in the brain of rats with neonatal T2DM [16]. The abnormalities in 5-HT_1R-mediated signaling pathways are able to induce the impairment of memory and cognitive functions and psychic disorders [38]. The decrease of AC inhibitory effects of noradrenaline in the diabetic heart indicates alterations in cardiac signaling including G_i-coupled α_2-AR, which may be caused by a decrease in the number of α_2-AR and by a reduction of expression and functional activity of G_i proteins in the heart of rats with T1DM [5, 6]. As shown in our previous studies, the changes in α_2-AR signaling can be identified at the early stages of STZ T1DM, being, however, less expressed compared with the later stages [8, 25]. The most dramatic changes were revealed in AC signaling cascades regulated by somatostatin. Somatostatin-induced AC inhibition was significantly reduced in all tissues under study, and this decline surpassed that in short-term STZ T1DM and neonatal T2DM [16, 25]. These data are the evidence for the degree of damage in somatostatin-regulated AC system enhanced with increasing duration and severity of the diabetic state. The reports are available describing the decrease of expression of some types of somatostatin receptors in the hypothalamus and pituitary in rats with T1DM [39]. In the atria of 4-week STZ rats, the suppressive effect of somatostatin on ANP secretion, realized via G_i-coupled sst2-somatostatin receptor, and the level of mRNA and protein content for this receptor was markedly decreased [40]. In the glucagon cells isolated from the pancreatic islets from patients with T2DM, there were found no sst1- and sst4-somatostatin receptors, and in the somatostatin cells, no sst1-, sst2-, sst3-, and sst-4-somatostatin receptors were detected [41]. This gave grounds for a suggestion that the decrease of the level and the function of somatostatin receptors in patients with T2DM and animals with T1DM may be due to the elevated level of circulating somatostatin inducing downregulation of the cognate receptors [42]. Taking into consideration the importance and complexity of the physiological and biochemical effects of somatostatin and its involvement in the pathogenesis of a variety of diseases, it would be right to say that the disturbances in somatostatin-regulated AC system are likely to be responsible for the development of T1DM-associated dysfunctions in the cardiovascular, nervous, and reproductive systems. This indicates the attenuation of G_i protein-coupled signaling cascades, especially somatostatin-regulated, in the heart, brain, and testes of rats with long-term STZ T1DM, and the partial restoration of these cascades with I-I treatment.

Column 1: Group Name

Column 2: Series

Column 3: Hormone

Column 4: Heart

Column 5: Percentage in Heart

Column 6: Brain

Column 7: Percentage in Brain

Column 8: Testes

Column 9: Percentage in Testes

4. Concluding Remarks

The study of the model of long-term T1DM is very important for understanding the molecular mechanisms responsible for the development of complications of this disease and for finding the approaches to their therapy and diagnostics. We showed that in 7-month STZ T1DM the regulatory effects of a large number of hormones including both AC activators, such as ligands of β-AR and 5-HT_6R, PACAP-38, and gonadotropin, and AC inhibitors, such as noradrenaline, somatostatin, and selective agonist of 5-HT1B/1DR, change significantly. The alterations in AC signaling may be regarded as a result of disturbances in the fundamental cellular processes in the tissues and organs of diabetic individuals and also as a mechanism compensating for the impairment of insulin-mediated signaling provoked by prolonged insulin deficiency. The alterations in AC signaling we revealed in long-term T1DM are expressed much better compared with short-term T1DM studied in detail by us and the other authors earlier and cover a wide spectrum of hormonal regulations [5, 8–11]. A prolonged, 135-day, treatment of diabetic rats with I-I resulted in restoration of the sensitivity of the cardiac AC system to nonhormonal regulators (GppNHp, forskolin) and adrenergic agonists of the brain AC system to selective agonists of 5-HTR and PACAP-38 and of the testicular AC system to hCG. Our results give strong evidence for the benefit of I-I in the therapy of diabetic patients with prolonged T1DM aimed at improving functioning of the cardiovascular, nervous, and reproductive systems and preventing their impairments; they indicate that insulin signaling system, the key component of overall hormonal network in the brain, is an important target in the treatment of severe forms of T1DM. The increase in sensitivity of cardiac AC to adrenergic ligands and testicular AC to gonadotropin in diabetic rats receiving I-I suggests that intranasaly adminestered hormone acts on the cardiovascular system and on the testes, a distal component of the hypothalamic-pituitary-gonadal axis, via the central mechanisms due to the impovement of brain signaling impaired in long-term T1DM.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at http://dx.doi.org/10.7167/2013/698435/dataset.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

This work was supported by the Ministry of Education and Science of Russian Federation (Project no. 8486) and by RFBR (Grant nos. 12-04-00434 and 12-04-32034). The authors are grateful to Inga Menina for linguistic assistance.

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