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Hypercorticism during Streptozotocin Diabetes and Mifepristone Administration: The Role of Cyclic Adenosine Monophosphate

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Abstract—The effects of mifepristone on basal and stimulated production of cyclic adenosine monophosphate (cAMP) and corticosteroid hormones (progesterone and corticosterone) have been studied in vitro using adrenal glands from control rats and rats with streptozotocin-induced diabetes. In adrenals of rats with streptozotocin diabetes, both basal and adrenocorticotropic hormone (ACTH) stimulated cAMP production were significantly increased; this was accompanied by an increase in basal and ACTH stimulated production of rat adrenal progesterone and corticosterone in vitro. Repeated administration of mifepristone to control and diabetic rats resulted in a preferential increase of the ACTH-stimulated production of corticosterone, the main glucocorticoid hormone, without additional changes in the level of cAMP production. These results suggest activation of two mechanisms responsible for increased steroidogenesis in experimental animals. In rats with streptozotocin diabetes, the increased formation of the second messenger mediating of ACTH action on adrenocortical cells, cAMP, increases both basal and ACTH-stimulated activity of all stages of steroidogenesis. After long-term administration of mifepristone to both control and diabetic rats activity of the later stages of steroidogenesis increases with a predominant increase in the synthesis of physiologically active hormone corticosterone without additional changes in the level of cAMP production.

Keywords: streptozotocin-induced diabetes, mifepristone, adrenocorticotropic hormone, cyclic adenosine monophosphate, progesterone, corticosterone

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INTRODUCTION

Diabetes mellitus often provokes the long-term activation of the hypothalamus–pituitary–adrenocortical axis (HPA) and patients with type 1 and 2 diabetes have increased blood cortisol; this can deteriorate diabetic hyperglycemia and cause the development of diabetic complications [1–3]. Modeling of diabetes mellitus in experimental animals, particularly by administering streptozotocin (STZ), is accompanied by HPA hyperactivation and increased corticosterone content in serum [4], hairs [5], also by increased urinary excretion of non-metabolized corticosterone [6]. We have previously shown that in rats with STZ diabetes corticosterone production was also increased by adrenals in vitro [7].

Treatment of steroid-induced hyperglycemia (including diabetic hyperglycemia) may include administration of a synthetic steroid drug, mifepristone (MIF); this progesterone/glucocorticoid antagonist exhibits a high affinity for the receptors of these hormones [8]. Due to the properties of the glucocorticoid receptor blocker, MIF has been recommended for the use as an alternative drug therapy for the treatment of clinical and metabolic disorders associated with hypercorticism; it has been introduced into clinical practice as a drug for long-term therapy of Cushing's syndrome, and is also recommended for patients with secondary steroid hyperglycemia [9]. However, in many cases MIF administration is accompanied by an increase in blood levels of ACTH and cortisol, possibly due to the presence of feedback mechanisms between peripheral tissues, adrenals, and the central link of the hypothalamic-pituitary-adrenocortical system [10].

It was previously shown that MIF administration to healthy Wistar rats, or to animals with STZ-diabetes was accompanied by an increase in blood corticosterone and increased urinary excretion of non-metabolized hormone [6, 11]. However, cellular mechanisms of long-term enhancement of steroidogenesis induced by MIF treatment of healthy rats and rats with STZ-diabetes still need better investigation.

It is known that ACTH plays a major role in the regulation of glucocorticoid hormone synthesis in adrenals; ACTH via adenylate cyclase activation, increases formation of cyclic adenosine monophosphate (cAMP) followed by activation of cAMP-dependent protein kinase A (PKA), which is responsible for the phosphorylation of specific transcription factors [12]. The cAMP/PKA signaling system is

involved in the activation of synthesis of the StAR protein, which transfers the cholesterol molecule from the outer to the inner mitochondrial membrane, where it is converted to pregnenolone, an early precursor in the synthesis of corticosteroid hormones. This initial stage is rate-limiting in the biosynthesis of corticosteroid hormones [13]. During chronic exposure to ACTH, adrenal cortical hyperplasia is accompanied by transcription of genes encoding enzymes of the late stages of glucocorticoid hormone synthesis, including 21-hydroxylase and 11β-hydroxylase; these enzymes transform pregnenolone into corticosterone through formation of progesterone in the adrenocortical cells [12, 14]. It is particularly interesting to elucidate both stages of steroidogenesis and mechanisms responsible for increased synthesis of glucocorticoid hormones in diabetes mellitus and during administration of the glucocorticoid receptor antagonist, MIF, and are there any specific features in these mechanisms?

The aim of the study was to investigate the effect of MIF administration to intact animals and animals with STZ diabetes on the basal and ACTH-stimulated production of cAMP and corticosteroid hormones by the adrenal glands in vitro.

MATERIALS AND METHODS

Adult male Wistar rats (n = 48) were used in the study. The animals housed in individual cages with free access to food and water were divided into four groups (n = 12 each): group 1—control, healthy animals treated per os with 0.4 mL water a dispenser for 10 days; group 2—healthy animals treated per os with 0.4 mL aqueous suspension of MIF prepared from the tablet pharmaceutical preparation "Ginestril 0.05" (the dose of the active substance, MIF, was 20 mg/kg); group 3—animals with STZ diabetes, which was induced by intraperitoneal administration of STZ (Sigma, USA; 50 mg/kg in 0.04 M citrate buffer, pH 4.2) to 18 h-starved rats, and on day 8 after STZ administration a 10 day-course of oral administration of 0.4 mL water similarly to group 1 started; group 4—animals with STZ diabetes, which received a 10 day-course of oral administration of MIF suspension similarly to group 2 (the treatment started on day 8 after STZ administration). On the next day after the last administration of water or MIF, rats were removed from the experiment by decapitation.

Adrenals were removed on ice, separated from adipose tissue, weighed and cut into 4 parts. Two parallel samples were prepared from the adrenal glands from two rats of each group. A total of 6 pairs of parallel samples were prepared for each group of animals. Each sample was weighed and incubated twice at 37°C in air with 5% CO_2 (v/v) in 2 mL of standard Krebs–Ringer bicarbonate buffer, pH 7.4, containing glucose. The first incubation lasted 15 min, then the incubation medium was removed and after addition of a fresh portion of the medium (2 mL) and ACTH (Sigma, 25 ng/mL) to one of two samples the incubation was carried out for 2 h.

Immediately after incubation, an aliquot (0.2 mL) of the incubation medium was mixed with the equal volume of 0.2 M HCl and frozen −20°C for subsequent cAMP determination. The cAMP content in the incubation medium was measured using cAMP ELISA kits (Enzo Life Sciences, USA). In accordance with the description of the kit, final concentration of HCl (0.1 M) is used for inhibition of endogenous phosphodiesterases and stabilization of cAMP formed.

For determination of progesterone and corticosterone, two 0.5 mL-aliquots of the incubation medium were immediately frozen after incubation and kept at −20°C.

Determination of progesterone and corticosterone in the incubation medium was carried out using the Progesterone-ELISA kits (XEMA, Russia) and Rat Corticosterone ELISA Kit (Enzo Life Sciences), respectively. Before determining progesterone, it was extracted from the incubation medium with ethyl acetate as described previously [7]. For corticosterone determination, the incubation medium was diluted 200 times with the ELISA buffer for corticosterone determination.

Statistical treatment of the results was carried out using the Statistica 10.0 software package (Statsoft, USA). The Kruskal–Wallis test was used for multiple comparisons; in the case of paired comparisons the Mann–Whitney test was used for independent variables. The results are presented in the text and tables in the form of a sample mean (M) and standard error (SEM). Differences were considered as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In vitro basal production of cAMP by the adrenals (Table 1) was significantly higher in rats with STZ diabetes (groups 3 and 4) as compared with adrenals from healthy rats (groups 1 and 2, respectively). Adrenals from healthy animals and rats with STZ diabetes demonstrated a pronounced response to stimulation of cAMP production by addition of ACTH to the incubation medium.

Administration of MIF did not influence cAMP production by the adrenals of both healthy rats and rats with STZ diabetes STD (Table 1). In the context of cAMP production the adrenal sensitivity in vitro to ACTH did not differ in rats of groups 1, 3 and 4 (the ACTH-induced 10-, 9.4-and 9.1-fold increase in cAMP production, respectively); however, in rats of group 2 (treated with MIF) the stimulation was somewhat lower (6.9-fold).

The basal production of progesterone (Table 2) and corticosterone (Table 3) by adrenals from rats with

Table 1. The effect of ACTH on cAMP production by rat adrenals in vitro

Parameter (number of samples per incubation)	Group of animals (number of animals per group)				*p*
	1—control ($n = 12$)	2—MIF ($n = 12$)	3—STZ diabetes ($n = 12$)	4—STZ diabetes + MIF ($n = 12$)	
Basal cAMP production, pmol/mg of tissue ($n = 6$)	38.5 ± 8.4	58.5 ± 11.1	98.6 ± 15.7	109.1 ± 7.9	1–2 = 0.2298 **1–3 = 0.0358** **2–4 = 0.0131** 3–4 = 0.7842
ACTH stimulated cAMP production, pmol/mg of tissue ($n = 6$)	386.7 ± 32.0	405.8 ± 45.9	931.5 ± 157.5	994.9 ± 71.0	1–2 = 0.9362 **1–3 = 0.0131** **2–4 = 0.0051** 3–4 = 1.0000
p	**0.0051**	**0.0051**	**0.0081**	**0.0051**	

Here and in Tables 2 and 3: STZ diabetes—streptozotocin-induced diabetes mellitus, MIF—mifepriston.

Table 2. The effect of ACTH on progesterone production by rat adrenals in vitro

Parameter (number of samples per incubation)	Group of animals (number of animals per group)				*p*
	1—control ($n = 12$)	2—MIF ($n = 12$)	3—STZ diabetes ($n = 12$)	4—STZ diabetes + MIF ($n = 12$)	
Basal progesterone production, pmol/mg of tissue ($n = 6$)	0.095 ± 0.015	0.108 ± 0.025	0.153 ± 0.015	0.080 ± 0.009	1–2 = 0.5282 **1–3 = 0.0453** 2–4 = 0.2306 **3–4 = 0.0050**
ACTH stimulated progesterone production, pmol/mg of tissue ($n = 6$)	0.137 ± 0.012	0.162 ± 0.015	0.258 ± 0.015	0.156 ± 0.012	1–2 = 0.5751 **1–3 = 0.0050** 2–4 = 0.8101 **3–4 = 0.0202**
p	0.0927	0.5751	**0.0131**	**0.0051**	

Table 3. The effect of ACTH on corticosterone production by rat adrenals in vitro

Parameter (number of samples per incubation)	Group of animals (number of animals per group)				*p*
	1—control ($n = 12$)	2—MIF ($n = 12$)	3—STZ diabetes ($n = 12$)	4—STZ diabetes + MIF ($n = 12$)	
Basal corticosterone production, pmol/mg of tissue ($n = 6$)	58.6 ± 14.1	61.8 ± 15.0	108.2 ± 16.7	151.0 ± 28.6	1–2 = 0.8941 1–3 = 0.1994 2–4 = 0.0606 3–4 = 0.5403
ACTH stimulated corticosterone production, pmol/mg of tissue ($n = 6$)	127.0 ± 26.5	307.4 ± 50.2	465.3 ± 139.1	1526.3 ± 223.3	**1–2 = 0.0252** **1–3 = 0.0453** **2–4 = 0.0142** **3–4 = 0.0202**
p	**0.0358**	**0.0304**	**0.0225**	**0.0122**	

STZ was 1.6- and 1.8-fold higher than the corresponding parameter in rats of the control group, respectively.

Production of progesterone and corticosterone by the adrenal glands of healthy rats and rats with STZ diabetes increased in response to ACTH addition to the incubation medium. Adrenals of healthy rats and rats with STZ-diabetes demonstrated a 1.4- and 1.7-fold increase in progesterone production, respectively, while ACTH-stimulated corticosterone production increased by 2.2- and 4.3-fold, respectively (Tables 2 and 3). Thus, in rats with STZ diabetes, the adrenal sensitivity to ACTH in corticosterone production was almost 2-fold higher than in healthy animals.

It is known that oxidative stress, which causes impairments in the activity of membrane-bound enzymes, is the basis determining metabolic disorders in type 1 and type 2 diabetes mellitus [15]. It has been demonstrated [16] that rats with STZ diabetes are characterized by changes in adenylate cyclase signaling cascades and altered sensitivity to hormones in the brain, myocardium, and testes. Analysis of the literature data in context of our results suggests that in rats with STZ diabetes, adenylate cyclase activity in corticocytes can also change and the increase in cAMP production is associated with an increase in both basal and ACTH-stimulated synthesis of corticosteroid hormones.

To date, adenylate cyclase inhibitors are being studied as therapeutic agents for treatment of chronic pain syndrome, reactive psychosis and other diseases [17, 18]. Our results indicate that these inhibitors can also be promising agents for pathogenetic therapy of hypercorticism, as one of the factors that exacerbate diabetic hyperglycemia.

Phosphodiesterases, which hydrolyze cAMP and thus reduce the level of hormonal signal, as well as a number of protein kinases and regulatory proteins that interrupt the hormonal signal transduction through the hormonal receptor and G-proteins, which are components of the adenylate cyclase signal systems [19] also play an important role in the control of cAMP-dependent cascades in the cell. Thus, in our experiment, cAMP production in vitro by the isolated adrenals reflects the activity of not only the formation but also the degradation of cAMP, thus showing the overall result of changes in the cAMP content in the incubation medium.

Repeated administration of MIF to healthy rats did not cause changes in the basal production of progesterone and corticosterone by the isolated adrenal in vitro (Tables 2 and 3), however, ACTH-stimulated corticosterone production by adrenals of MIF-treated animals was higher than in control animals. In rats with STZ diabetes administration of MIF led to a decrease in basal progesterone production and an increase in basal corticosterone production. In vitro stimulation of adrenals from group 4 rats by ACTH caused a 1.9-fold increase in progesterone production; however the stimulated progesterone production remained lower than that by adrenals group 3 rats under similar conditions (Table 2). At the same time, ACTH-stimulated production of corticosterone by the adrenals of group 4 rats demonstrated a higher (10-fold) increase versus adrenals without ACTH stimulation; this stimulation was more than three times higher than that of adrenals of group 3 rats under similar conditions (Table 3).

The fact that repeated administration of MIF to both healthy rats and rats with STZ diabetes caused an increase in the predominantly ACTH-stimulated production of the main glucocorticoid hormone corticosterone without an additional change in the level of cAMP production, suggests that this increase in the production may be due to increased expression of genes responsible for synthesis of 21-hydroxylase and/or 11β-hydroxylase, the enzymes that convert progesterone through deoxycorticosterone to corticosterone [14]. This suggestion is also supported by the results indicating a decrease in the progesterone production of the adrenal glands of animals with STZ diabetes under the conditions of MIF administration (Table 2). As was shown by us earlier [20], in rats with STZ diabetes at different time-frame of MIF administration, the ACTH-stimulated production of pregnenolone, the early precursor in the synthesis of corticosteroids also decreased. Such decrease in the production of precursors in steroidogenesis (pregnenolone and progesterone) may be associated with redistribution of resources in favor of corticosterone synthesis in adrenals of rats treated with MIF.

The mechanisms responsible for increased synthesis of predominantly glucocorticoid hormone corticosterone by the adrenal cortex induced by repeated administration of MIF to rats may be associated with the presence of glucocorticoid receptors in adrenocortical cells, through which the negative effects of glucocorticoids on steroidogenesis are realized [10]. MIF binding to glucocorticoid receptors with high affinity can prevent realization of these negative effects and stimulate corticosterone synthesis. This may be the reason determining high sensitivity of adrenals from rats with STZ diabetes and MIF to ACTH, because feedback mechanisms play a decisive role in the regulation of endocrine gland activity.

CONCLUSIONS

The results obtained in this study indicate activation of various mechanisms responsible for increased adrenal steroidogenesis in rats with STZ diabetes and treated with repeated administration of MIF.

In adrenals from rats with STZ-diabetes both basal and ACTH-stimulated activity of all stages of steroidogenesis increased; this was mediated by the increased formation of cAMP, the second messenger mediating ACTH action on adrenocortical cells.

Long-term administration of MIF to intact rats and rats with STZ diabetes activated the late stages of steroidogenesis followed by increased synthesis of the physiologically active hormone, corticosterone.

COMPLIANCE WITH ETHICAL STANDARDS

The experiments were carried out in accordance with the Rules of the Laboratory Practice Using Experimental Animals, approved by order of the Ministry of Public Health of the Russian Federation (no. 267 of June 19, 2003), and ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (1986).

The authors declare that they have no conflict of interest.

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