Dysregulation of Corticosterone Secretion in Streptozotocin-Diabetic Rats: Modulatory Role of the Adrenocortical Nitrergic System


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An increased activity of the hypothalamo-pituitary-adrenal axis resulting in exaggerated glucocorticoid secretion has been repeatedly described in patients with diabetes mellitus and in animal models of this disease. However, it has been pointed out that experimental diabetes is accompanied by a decreased glucocorticoid response to ACTH stimulation. Because previous studies from our laboratory demonstrate the involvement of nitric oxide (NO) in the modulation of corticosterone production, present investigations were designed to evaluate 1) the impact of streptozotocin (STZ)-induced diabetes on the adrenocortical nitrergic system and 2) the role of NO in the modulation of adrenal steroidogenesis in STZ-diabetic rats. Four weeks after STZ injection, increased activity and expression levels of proteins involved in L-arginine transport and in NO synthesis were detected, and increased levels of thiobarbituric acid reactive species, carbonyl adducts, and nitrotyrosine-modified proteins were measured in the adrenocortical tissue of hyperglycemic rats. An impaired corticosterone response to ACTH was evident both in vivo and in adrenocortical cells isolated from STZ-treated animals. Inhibition of NO synthase activity resulted in higher corticosterone generation in adrenal tissue from STZ-treated rats. Moreover, a stronger inhibition of steroid output from adrenal cells by a NO donor was observed in adrenocortical Y1 cells previously subjected to high glucose (30 mM) treatment. In summary, results presented herein indicate an inhibitory effect of endogenously generated NO on steroid production, probably potentiated by hyperglycemia-induced oxidative stress, in the adrenal cortex of STZ-treated rats. (Endocrinology 151: 203–210, 2010)

Patients with poorly controlled diabetes mellitus exhibit an altered endocrine function, reflected e.g. by changes in the levels of thyroid hormones, GH/IGF-I and LH/FSH (1–4). In particular, an up-regulated hypothalamo-pituitary-adrenal (HPA) axis (5, 6) and increasing circulating glucocorticoid levels (7) have also been shown, both in humans and in animal models of this disease (8, 9). Although these changes have been mainly attributed to effects exerted at higher levels of the HPA axis, several authors have stressed the contribution to this phenomenon of the consequences of the disease at an adrenal level (8, 9).

ACTH is the major regulator of glucocorticoid production in the adrenal cortex, but several studies suggest that local factors may play roles as paracrine or autocrine modulators of adrenal steroidogenesis. Growth factors, cytokines, and peptides, among other molecules produced locally, synergize or antagonize the action of ACTH (10–12). Previous studies from our laboratory have demonstrated that endogenously generated nitric oxide (NO) is involved in the modulation of corticosterone production in rat adrenal zona fasciculata (ZF) cells (13) and that adrenal NO synthase (NOS) activity is dependent on extracellular...
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TABLE 1. Whole body weight, adrenal weight, and nonfasting serum glucose levels of the control, diabetic, and insulin-treated diabetic groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ</th>
<th>STZ + INS</th>
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<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>405.7 ± 7.0</td>
<td>304.2 ± 12.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>516.6 ± 8.8&lt;sup&gt;a,c&lt;/sup&gt;</td>
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<tr>
<td>Adrenal weight (mg)</td>
<td>25.3 ± 1.2</td>
<td>32.1 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.0 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative adrenal weight (mg/kg body weight)</td>
<td>61.8 ± 3.2</td>
<td>107.5 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.0 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>121.3 ± 4.1</td>
<td>702.0 ± 48.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.9 ± 24.7&lt;sup&gt;c&lt;/sup&gt;</td>
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Rats were rendered diabetic by STZ injections [40 mg/kg ip in sterile 0.1 M citrate buffer (pH 4.2) for 3 successive days]. Three days after the induction of diabetes, a group of STZ-treated animals was inserted with a slow-release insulin implant (STZ + INS group: two Linplants per rat releasing ~2.5 U insulin/d). Measurements were carried out 4 wk thereafter. Values are presented as means ± SEM; n indicates the number of rats used in each group.

<sup>a</sup> P < 0.001; <sup>b</sup> P < 0.01 vs. control; <sup>c</sup> P < 0.001 vs. STZ (ANOVA followed by Tukey’s test).

L-arginine levels and on the activity of its uptake system (13–15).

Although several reports have addressed the participation of NO and nitrosative stress in the development and progression of diabetic complications in different tissues (e.g., renal, vascular, and neural) (16, 17), the effect of diabetes on adrenal NOS activity has not been characterized. Accordingly, the present investigations were designed first to evaluate the impact of streptozotocin (STZ)-induced diabetes on the adrenocortical nitrergic system and then, considering the modulatory role described for NO on corticosterone secretion, to study the involvement of NOS activity on the modulation of adrenal steroidogenesis in STZ-diabetic rats.

Materials and Methods

Chemicals

ACTH was obtained from Laboratorios ELEA (ACTHelea; Buenos Aires, Argentina). Antibodies raised against NOS-1, NOS-2, and NOS-3 and those against actin were from Santa Cruz Biotechnology (Santa Cruz, CA); peroxidase-conjugated goat antirabbit IgG was from Bio-Rad (Hercules, CA). Moloney murine leukemia virus reverse transcriptase was from Promega (Madison, WI). Corticosterone and progesterone antisera were kindly provided by Dr. A. Bélanger (Laval University, Quebec, Canada). STZ, diethylenetriamine (DETA)/NO adduct, kindly provided by Dr. A. Be´langer (Laval University, Quebec, Canada). Antibodies raised against NOS-1, L-arginine methyl ester hydrochloride (L-NAME), 8-bromo-L-arginine monohydrochloride (8Br-cAMP), BSA, and anti-nitrotyrosine antibodies were from Sigma (Buenos Aires, Argentina). L-[1,2,6,7-3H]arginine monohydrochloride (0.25 mCi, 58 Ci/mmol), [1,2,6,7-3H(N)]progesterone (0.25 mCi, 102.1 Ci/mmol), and [1,2,6,7-3H(N)]corticosterone (0.25 mCi, 76.5 Ci/mmol) were from PerkinElmer (Boston, MA). Fetal calf serum, Taq polymerase, penicillin, and streptomycin were from Invitrogen (Life Technologies, Buenos Aires, Argentina). Pierce ECL Western blotting substrate was from Thermo Fisher Scientific (Rockford, IL). The enzymatic-colorimetric kit (GOD/POD reagent) for the determination of serum glucose levels was from Laboratorios Wiener (Rosario, Argentina).

Animals and experimental procedures

Adult male Wistar rats (300–350 g body weight) were injected with STZ [40 mg/kg ip in sterile 0.1 M citrate buffer (pH 4.2), for 3 successive days] or vehicle (control group). Three days after the induction of diabetes, a group of STZ-treated animals was inserted under light ketamine/xylazine anesthesia with a slow-release insulin implant (STZ + INS group: two Linplants per rat releasing ~2.5 U insulin/d; Linshin Canada Inc., Scarborough, Ontario, Canada). The animals were kept in cages under controlled conditions (23 ± 2 °C, lights on 0700–1900 h) with free access to water and laboratory chow. Four weeks after the initiation of hyperglycemia and during the morning hours (between 0900 and 1000 h), animals were killed by decapitation according to protocols approved by the animal care and use committee from the University of Buenos Aires.

Only those animals presenting serum glucose levels over 300 mg/dl were used throughout the experiments. Table 1 summarizes different parameters determined at the end of the study in the animals used throughout.

In vivo experiments

Each group of animals (control, STZ, and STZ + INS) was either injected with ACTH (10 IU/kg ip) or with pyrogen-free saline solution and kept 60 min thereafter. Blood was collected, and serum was obtained.

ZF tissue preparation

Adrenal glands were rapidly dissected and placed on a chilled plate. ZF adrenal tissue was homogenized in 0.2 ml/gland of 10 mM HEPES (pH 7.4), 320 mM sucrose, 0.1 mM EDTA, 0.1 mM dithiothreitol, and the following protease inhibitors: 2 μM pepstatin, 10 μM Leupeptin, 25 μM apronitin, and 2 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 9000 g for 20 min. The obtained supernatant was further centrifuged at 9000 × g for 20 min, and this supernatant (postmitochondrial fraction) was used in the following determinations.

NOS activity

NOS activity was determined in postmitochondrial fractions by monitoring the conversion of L-[3H]arginine to L-[3H]citrulline as described elsewhere (15). Briefly, protein fractions were brought to 100 μl in a reaction mixture that contained final concentrations of 10 mM HEPES (pH 7.4), 20 μM L-arginine, 0.5 mM dithiothreitol, 1 mM NADPH, 187 nM L-[3H]arginine, and 1.25 mM CaCl₂ and incubated at 37°C for 15 min. The reaction was terminated by the addition of 800 μl ice-cold stop buffer (10 mM EGTA, 10 mM EDTA, and 50 mM HEPES, pH 5). L-[3H]Citrulline was separated by anion exchange chromatog-
raphy and quantified by liquid scintillation spectroscopy. NOS enzyme activity is indicated as picomoles of \(\text{L-}^{[3\text{H}]}\text{citrulline}\) formed per milligram protein per minute.

**Measurement of carbonyl content and of lipid peroxides [thiobarbituric acid reactive species (TBARS)]**

TBARS were determined as previously described (18). Determination of protein carbonyl content was carried out according to Fields and Dixon (19).

**RNA isolation and RT-PCR**

Total RNA was obtained from rat adrenal ZF using Trizol reagent according to the manufacturer’s instructions. RNA (1 \(\mu\)g) was pretreated with ribonuclease-free deoxyribonuclease I to eliminate any possible DNA contamination and then incubated in a mixture containing 0.5 mM dNTPs mix, 25 ng/\(\mu\)l (8 \(\mu\)M) random primers, 1 \(\times\) first-strand buffer, 25 U ribonuclease inhibitor, and 200 U Moloney murine leukemia virus reverse transcriptase in a final volume of 25 \(\mu\)l for 1 h at 42 C. The reaction was stopped by heating at 90 C for 5 min. The reaction mixture was then brought to 100 \(\mu\)l with diethylpyrocarbonate-treated water and stored at −70 C. Reverse transcriptase was omitted in selected tubes as a control of amplification from contaminating genomic DNA.

PCR were carried out in a Tpersonal thermocycler (Biorad, California) and were performed using 2 \(\mu\)l cDNA in a final volume of 20 \(\mu\)l of the following reaction mixture: 1 \(\times\) PCR buffer, 1.5 mM MgCl\(_2\), 0.2 mM of each dNTP, 500 nm of each specific oligonucleotide primer, and 0.625 U Taq polymerase. The sequence for the oligonucleotide primers used is shown in supplemental Table 1 (published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endo.org).

**Immunoblot analysis**

Samples were boiled for 5 min in SDS-PAGE loading buffer and electrophoresed on 7.5 or 12% polyacrylamide gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes for 1 h at 15 \(\times\) V in a Trans-Blot SD system (Bio-Rad) in 48 mM Tris-HCl (pH 9.2), 39 mM glycine, and 1.3 mM SDS. Polyvinylidene difluoride membranes were blocked in 20 mM Tris-HCl (pH 7.4), 137 mM NaCl, 0.1% Tween 20, and 5% nonfat milk for 60 min at room temperature and then incubated overnight with a 1:500 dilution of the respective NOS, steroidogenic acute regulatory protein (StAR) (1:1000), nitrotyrosine (1:2000), or actin (1:1000) antisera at 4 C. Membranes were then incubated with a 1:20,000 dilution of a goat antirabbit IgG antibody horseradish peroxidase conjugate, and the bands were visualized by chemiluminescence.

**ZF cell preparation and L-arginine influx measurement**

ZF cells from control and STZ-treated rats were isolated using published procedures (20). L-Arginine uptake was assessed by the method of Rao and Butterworth (21) with brief modifications. Briefly, the cells were suspended in 6.5 ml Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose and 0.5% BSA, aliquoted (500 \(\mu\)l/tube), and preincubated for 15 min at 37 C. Uptake was initiated by the addition of \(\text{L-}^{[3\text{H}]}\text{arginine}\) (50 \(\mu\)M, 5–7 \(\times\) 10\(^5\) dpm) and finished 2 min later by adding 4 ml ice-cold PBS and rapidly filtering the samples under vacuum through Whatman GF-C filters soaked in 0.3% polyethylenimine to reduce nonspecific binding of \(\text{L-}^{[3\text{H}]}\text{arginine}\). The filters were washed twice with 4 ml fresh buffer, and radioactivity was counted in a liquid scintillation spectrometer. Nonspecific uptake of \(\text{L-}^{[3\text{H}]}\text{arginine}\) was assessed in the presence of 10 \(\mu\)M \(\text{L-arginine}\) and was subtracted from all values.

**Measurement of nitrite levels**

Nitrite levels were determined in the incubation media of isolated adrenal cells. For this purpose, the cells were incubated for 2 h at 37 C in Ham’s F10 medium, and then 80-\(\mu\)l aliquots of the incubation media were mixed with the Griess reagent and incubated at room temperature for 10 min. Absorbance was determined at 570 nm, and sodium nitrite was used as standard.

**In vitro studies with L-NAME**

Control and STZ-treated rats were killed, and their adrenal glands were excised and dissected in quarts. Two adrenal quarters per tube were incubated in Ham’s F10 with or without 5 \(\mu\)M L-NAME for 15 min and further incubated with or without 200 \(\mu\)M 8Br-cAMP for 45 min.

**Experiments with Y1 cells**

Murine Y1 adrenocortical tumor cells were generously provided by Dr. B. Schimmer (University of Toronto, Toronto, Canada). Cells were maintained at 37 C in growth medium (Ham’s F10) containing fetal bovine (2.5%) and horse (12.5%) sera. In our experimental setting, cells were incubated with no additions (normal glucose concentration), or 25 mM d-glucose was added to the incubation media (high glucose) in selected wells after every medium change, and incubation was continued for 7 d. The cells were further incubated in the absence or presence of 250 \(\mu\)M DETA/NO (NO donor, half-life 20 h) for 24 h with or without the addition of 50 \(\mu\)M 8Br-cAMP for the last 3 h. The media were collected, and the cells were lysed with 200 \(\mu\)l 20 mM Tris-HCl (pH 4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 5 \(\mu\)g/ml E64, 1.25 \(\mu\)M orthovanadate, and 1 mg/ml leupeptin.

Cell viability was assessed by the trypan blue dye exclusion test after the treatments. No significant difference was observed for any of the treatments.

**Steroid determinations**

Corticosterone levels in serum and incubation media were determined by RIA as previously described (14). For serum determinations, corticosterone was extracted with dichloromethane. Progesterone concentration was determined in the incubation media by RIA. Results are expressed as percentage of inhibition of progesterone release by the NO donor as compared with its respective control (without DETA/NO).

**Statistical analysis**

Data were tested for normality using the Kolmogorov-Smirnoff test. All values are expressed as mean ± SEM of n experiments. Differences between groups were analyzed by unpaired \(t\) test or by factorial one-way ANOVA, as appropriate. When the ANOVA yielded significant differences (\(P < 0.05\), post hoc comparisons (Tukey’s test) were made to determine the statistical levels of difference between groups. All calculations
were performed using GraphPad InStat version 3.06 for Windows (GraphPad Software, San Diego, CA).

Results

Four weeks after injection, STZ-diabetic rats exhibited clearly elevated glycemic levels, lower body weights, and higher relative adrenal weights (Table 1). These alterations were corrected by insulin treatment.

Adrenocortical NOS activity was significantly increased in STZ-diabetic rats, and this effect was prevented by insulin (Fig. 1A). Both NOS-1 and NOS-3 mRNA levels were elevated in STZ-treated animals (Fig. 1B). NOS-2 was not detected in our experimental setting (data not shown).

**FIG. 1.** NOS activity in adrenal cortex of STZ-treated rats after 4 wk diabetes. Panel A, NOS activity in postmitochondrial fractions was determined by radiochemical conversion of L-[3H]arginine to [3H]citrulline as described in Materials and Methods. Each bar represents mean ± SEM; n = 6. ***, P < 0.001 vs. control; †††, P < 0.001 vs. STZ (ANOVA followed by Tukey’s test). Panel B, Representative semiquantitative RT-PCR experiment showing the mRNA levels of both NOS-1 and NOS-3 isoforms and densitometric analysis of three independent RT-PCR experiments (normalized to 18S rRNA). Each bar, corresponding to NOS-1 ( ■) and NOS-3 ( ■) mRNA concentrations, represents mean ± SEM; n = 3. ***, P < 0.001; *, P < 0.05 vs. respective control; †††, P < 0.001; †, P < 0.05 vs. respective STZ treatment (ANOVA followed by Tukey’s test). Panel C, Western blot analysis of nitrotyrosine-modified proteins in adrenal tissue of control and STZ-treated rats. C, control; INS, insulin.

**FIG. 2.** L-Arginine transport in adrenocortical cells obtained from STZ-treated rats after 4 wk diabetes. Panel A, Influx of L-[3H]arginine, expressed as picomoles per minute per milligram protein ± SEM (n = 4) in isolated adrenocortical cells. *, P < 0.05 by unpaired t test. Panel B, Representative semiquantitative RT-PCR showing mRNA levels of cationic amino acid transporters CAT-1 ( ■), CAT-2 ( ■), CD-98 ( ■), and γ′LAT-2 ( ■) and densitometric analysis of three independent RT-PCR experiments (normalized to 18S rRNA). ***, P < 0.001; **, P < 0.01; *, P < 0.05 vs. control; †††, P < 0.01; †, P < 0.05 vs. respective STZ (ANOVA followed by Tukey’s test). Panel C, Nitrite levels were determined by the Griess reaction in the incubation medium. Results are expressed as means ± SEM (n = 3). **, P < 0.01 by unpaired t test. C, control; INS, insulin.
shown). Increased levels of nitrotyrosine-modified proteins (Fig. 1C), TBARS, and carbonyl content were also evident in the adrenal cortex of hyperglycemic diabetic animals (TBARS in μm malondialdehyde/mg protein: controls, 2.82 ± 0.02; STZ, 6.45 ± 0.55; STZ + Ins, 2.89 ± 0.23; n = 3; STZ vs. controls, P < 0.05) (carbonyl content in mM/mg proteins: controls, 42.7 ± 6.5; STZ, 74.1 ± 1.9; STZ + Ins, 51.5 ± 3.4; n = 4; STZ vs. controls, P < 0.01).

In vivo NO generation is highly dependent on the activity of the l-arginine uptake systems. Dispersed adrenocortical cells from STZ-treated rats showed a clear increase in l-arginine uptake compared with control cells (Fig. 2A). Accordingly, STZ-diabetic rats showed higher mRNA levels of the l-arginine transporters CAT-2, CD-98, (Fig. 2A). Furthermore, nitrite levels measured in the incubation media of cells obtained from STZ-treated animals were significantly increased (Fig. 2C).

After 4 wk STZ injection, diabetic rats exhibited higher basal corticosterone levels compared with control animals. However, upon acute ACTH stimulation, STZ-treated rats exhibited a significantly lower corticosterone response than control animals. These effects were prevented by insulin treatment (Fig. 3A). Adrenocortical cells isolated from STZ-treated rats also showed an impaired response to ACTH stimulation (Fig. 3B). Higher levels of StAR mRNA and protein were detected in the adrenal cortex of diabetic animals (Fig. 3C).

NOS inhibition by means of ε-NAME resulted in higher basal and 8Br-cAMP-stimulated corticosterone output from adrenal quarters obtained from STZ-treated rats (Fig. 4).

Finally, Fig. 5 shows that pretreatment of adrenocortical cells with 30 mM D-glucose concentrations (high glucose) for 7 d resulted in a higher NO-induced inhibition of basal and 8Br-cAMP-stimulated steroiogenesis compared with cells incubated in euglycemic conditions (5.5 mM D-glucose, normal glucose concentration).

Discussion

According to the present results, uncontrolled STZ-diabetic rats show an increase in the expression levels of both constitutive NOS isoforms and in NOS activity in the adrenal cortex. As we have previously demonstrated, this enzymatic activity depends on the extracellular concentration of l-arginine and on the activity of its uptake system (22). Thus, nitrite generation and, especially, the augmentation in l-arginine transport (23, 24) further reflect the activation of the adrenocortical nitrergic system in animals with experimentally induced diabetes mellitus. Accordingly, different authors demonstrated increases in NOS activity related to higher l-arginine uptake levels in different tissues of STZ-treated rats (25, 26). Moreover, our results are in agreement with previous observations on positive NADPH diaphorase staining and increased NOS activity in the rat adrenal cortex after 8 wk STZ treatment (27).
NOS activity and in the expression profile of the different NOS isoforms have been reported in different stages of the disease (28–32), suggesting a tissue- and progression of the disease-dependent regulation of NOS expression levels and activity in STZ-treated rats.

Very little is known about the mechanisms underlying the up-regulation of NOS-3 and NOS-1 expression levels in uncontrolled diabetes in the adrenal cortex of the STZ-treated rat. One possible mechanism was suggested by Cosentino et al. (33) who demonstrated the involvement of protein kinase C activity in the up-regulation of NOS-3 expression levels in endothelial cells cultured in high-glucose medium. In addition, the activity of NOS-3 could be modulated by a plethora of regulatory mechanisms including, among others, its phosphorylation and subcellular localization (see Ref. 34 for a review). Information on the regulation of NOS-1 activity in diabetic animals is also very scarce (35). Summarizing, it is clear that the mechanisms involved in the observed increases in NOS activity, and expression levels in the adrenal cortex of STZ-treated rats deserve further investigation.

Several studies have addressed the effects of untreated or poorly controlled type 1 diabetes on the activity of the HPA axis, both in humans and in animal models of the disease (8, 9, 36). In accordance with previous reports (9), our data show that, after 4 wk diabetes, STZ-treated rats exhibited higher basal serum corticosterone levels and a lower response to ACTH than their control counterparts. In this sense, Chan et al. (8, 9) suggested that in STZ-diabetic rats, long-term effects of the disease at the adrenal level are also involved in the impaired response of the HPA axis. The prevention of these and other defects by insulin replacement observed in our experiments suggests that these alterations arise as a consequence of mechanisms elicited by insulin deficiency.

Cholesterol transport to the inner mitochondrial membrane, the limiting step in steroid biosynthesis, involves the activity of several proteins (e.g. StAR and 18-kDa translocator protein) (37, 38). In particular, both StAR phosphorylation and synthesis of new StAR protein have been involved in the regulation of substrate availability to cytochrome P450 (CYP)11A1 (39). Our results show higher StAR protein and mRNA levels in the adrenal cortex of STZ-treated rats. Lehoux et al. (40) described similar findings in rats chronically stimulated with ACTH. Thus, increased StAR expression levels detected in our animals could arise as a consequence of protracted exposure to elevated ACTH levels present in diabetic rats (9). In turn, increased levels of StAR protein could be involved in the higher basal corticosterone production observed in our animals.

Increased adrenal weight detected in our diabetic rats would also reflect a prolonged exposure to augmented ACTH concentrations. According to Rebuffat et al. (41), the presence of an intact HPA axis is necessary for the rise in plasma corticosterone levels and the development of adrenocortical hypertrophy in STZ-diabetic rats. Both the decreased response to exogenous ACTH detected in vivo and the blunted stimulation by this trophic hormone of isolated adrenal cells from STZ-treated rats observed in our experiments strongly suggest that the diabetic state can negatively affect steroid production at the adrenal.
level. Because acute stimulation of corticosterone release by both ACTH and 8Br-cAMP is decreased, the negative effect of NO seems to be exerted beyond the receptor and the cAMP-generation system level.

In previous papers, we demonstrated that locally produced NO exerts a regulatory role on steroidogenesis in the rat adrenal cortex (13–15). To analyze the effects of augmented NOS activity on steroid production in the adrenal cortex of STZ-diabetic rats, we assessed the in vitro effect of a NOS inhibitor on corticosterone secretion. NOS blockade induced a clear-cut increase in steroid release in adrenal quarters obtained from STZ-treated rats. Hence, the up-regulation of the adrenocortical nitrergic system detected in diabetic rats would exert an inhibitory effect on corticosterone production. This effect could be attributed to the interaction of NO with the heme moiety of CYP11A1 (14) or to a decrease in StAR expression levels, as previously demonstrated (15). Because ACTH is the principal postnatal trophic drive for the adrenal gland, as well as for glucocorticoid synthesis and release, we hypothesize that the elevated ACTH stimulatory tone previously described in STZ-treated animals (as well as in patients with type 1 and type 2 diabetes mellitus) (5–9) is able to override the modulatory negative effect of NO on adrenal steroidogenesis. Thus, prolonged exposure to increased ACTH would result in adrenal hypertrophy and increased StAR protein levels as stated before. Accordingly, a higher basal corticosterone production detected in vivo in diabetic animals previously (9, 41), and in the present experiments, should also be expected.

Many studies have addressed the long-term consequences of high glucose levels on the morphology and function of different cell types (41–43). In particular, adrenal cells subjected to long-term high-glucose treatment exhibit altered oxidative stress parameters including increased reactive oxygen species generation (44). Our results, obtained in Y1 cells exposed for 7 days to high glucose and treated with a NO donor, suggest that cellular changes induced by hyperglycemia may potentiate the inhibition exerted by NO on steroid synthesis. Our results showing higher NO levels and augmented oxidative stress lead us to propose that peroxynitrite could be generated in the adrenal cortex of STZ-treated rats, a fact that is further evidenced by the increase in nitrotyrosine-modified proteins. We hypothesize that an impairment in the activity of steroidogenic CYP enzymes, as it was described for other CYP enzymes (45, 46) or else a modification in the activity of different autocrine or paracrine steroidogenic modulatory systems (e.g., prostaglandin synthesis) are among the possible consequences of peroxynitrite production. The mechanisms involved in this detrimental effect of STZ-induced diabetes at the adrenal level deserve further investigation.

Based on our results obtained in vivo, a contribution of NO-associated vascular (47) or neuronal mechanisms (48) to changes in corticosterone secretion observed in STZ-diabetic animals cannot be ruled out. However, results arising from the in vitro experiments strongly suggest that steroidogenic cells are directly targeted by increased NO production.

In summary, our results indicate that NOS activity is up-regulated in the adrenal cortex of STZ-treated rats resulting in an inhibitory tone on steroid production. Moreover, the combination of hyperglycemia with higher NO levels further potentiates this effect. The effects described herein could be involved in the impaired adrenal response to exogenous ACTH described in type 1 diabetes.

Acknowledgments

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