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Adrenal Gland Involvement in the Regulation of Renal 11β-Hydroxysteroid Dehydrogenase 2

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Abstract Renal 11β-hydroxysteroid dehydrogenase 2 (HSD2) catalyzes the conversion of active glucocorticoids to inert 11β-keto compounds, thereby preventing the illicit binding of these hormones to mineralocorticoid receptors (MRs) and, thus, conferring aldosterone specificity. Absence or inhibition of HSD2 activity, originates a hypertensive syndrome with sodium retention and increased potassium elimination. Recent studies from our laboratory reported an increment of HSD2 activity in intact-stressed rats. To evaluate the adrenal involvement in this increase, we analyzed HSD2 activity and protein abundance in Intact, Sham-operated, and adrenalectomized rats under stress situations (gavage with an overload of 200 mM HCl (10 ml) and simulated gavage) or with corticosterone replacement. HSD2 activity was assessed in renal microsomal preparations obtained from different groups of animals. HSD2 protein abundance was measured by Western-blot. Circulating corticosterone was determined by radioimmunoassay. Sham-operated animals showed an increase in HSD2 activity and abundance compared to Intact and adrenalectomized rats suggesting the involvement of stress-related adrenal factors in HSD2 regulation. In the case of acidotic adrenalectomized animals, there was an increase in renal HSD2 activity when, along with the HCl overload, the rats were injected with corticosterone. This increment occurred without an increase in enzyme abundance. These results suggest the importance of circulating levels of glucocorticoids to respond to a metabolic acidosis, through regulation of HSD2 stimulation. The group subjected to a simulated gavage showed an increase in enzyme activity and protein abundance, thus demonstrating the need for both adrenal and extra-factors in the modulation of renal HSD2. The adrenalectomized animals injected with different doses of corticosterone, produced a progressive increase in enzyme activity and abundance, being significant for the dose of 68 μg corticosterone/100 g body weight. The highest dose (308 μg/100 g body weight) did not show any variation in activity and abundance compared to the control group. This biphasic effect of glucocorticoids could be explained taking into account their permissive and suppressive actions, depending on their blood levels. Knowing that stress induces multifactorial responses, it should not be suprising to observe a differential regulation in renal HSD2, confirming that different stressors act through different factors of both, adrenal and extra-adrenal origin. J. Cell. Biochem. 92: 591–602, 2004.

Key words: acidosis; corticosterone; HSD2; kidney; stress

Aldosterone acts through the mineralocorticoid receptor (MR) to promote the vectorial transport of sodium, potassium, and hydrogen ions in kidney and colon epithelia. The affinity of the isolated or cloned MR is similar for aldosterone, corticosterone (B), cortisol, or deoxycorticosterone regardless of the tissue [Krozowski and Funder, 1983; Arriza et al., 1987]. Corticosterone and cortisol circulate at much higher concentrations than aldosterone (100–1,000 fold), without any mineralocorticoid effects [Krozowski and Funder, 1983; Monder and Shackleton, 1984; Mantero et al., 1996]. In mineralocorticoid target cells, the binding of aldosterone to its specific receptor is preserved by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2), a microsomal enzyme, NAD^+-dependent, that converts corticosterone

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and cortisol to the inactive metabolites 11-dehydrocorticosterone and cortisone [Edwards et al., 1988; Funder et al., 1988; Bonvalet et al., 1990; Alfaidy et al., 1995; Mantero et al., 1996; Farman and Bocchi, 2000; Hirasawa et al., 2000]. According to previous reports, HSD2 could be present in different tissues as an inactive dimer or as an active monomer. The dimer formation, as a latent form of the enzyme, is a feature not shared by many of the short-chain alcohol dehydrogenases which exist as active dimers or tetramers [Krozowski, 1994; Farman and Bocchi, 2000; Gomez-Sanchez et al., 2001].

In patients exhibiting lethal mutations in the gene encoding HSD2, i.e., individuals suffering from the syndrome of apparent mineralocorticoid excess (AME) [Obeyesekere et al., 1995; Ferrari et al., 1996; Mantero et al., 1996; White et al., 1997; Stewart and Krozowski, 1999, Ferrari and Krozowski, 2000], or in mice lacking HSD2 [Kotelevtsev et al., 1999], 11β-hydroxy-glucocorticoids bind to the MR, leading to sodium retention, hypokalemia, and severe hypertension. A similar type of hypertension is induced by ingestion of licorice, which contains glycyrrhetinic acid, a potent inhibitor of HSD2 [Monder et al., 1989; Souness and Morris, 1989; Gomez-Sanchez and Gomez-Sanchez, 1992; Mantero et al., 1996; Ferrari and Krozowski, 2000].

Very little is known about the regulation of HSD2 by stress, a situation leading to stimulation of the adrenomedullar system, as well as to the activation of the hypothalamic-pituitary-adrenal (HPA) axis [Sapolsky et al., 2000; Pacak and Palkovits, 2001], and the information seems contradictory. For example, Alfaidy et al. [1995] did not observe modifications on enzyme activity in adrenalectomized (ADX) animals injected with aldosterone, corticosterone, or dexamethasone. On the other hand, Li et al. [1996], found an increase in renal HSD2 activity in rats injected with dexamethasone for 7 days, along with a decrease in the levels of its mRNA, while Diederich et al. [1996], observed a glucocorticoid-mediated inhibition of enzyme activity, in human kidney slices. Recently, Suzuki et al. [2003] found a concentration-dependent increase in HSD2 activity, mRNA and protein expression in bronchial epithelial cells exposed to dexamethasone for 3 days. This stimulation could probably be mediated by glucocorticoid receptors as RU38486, a potent glucocorticoid receptor antagonist, completely blocked dexamethasone effects. However, a glucocorticoid-responsive element has not yet been described in the HSD2 promoter region [Nawrocki et al., 2002].

A marked increase in HSD2 activity and protein expression has also been observed by our group in rats subjected to different stressful situations [Igarreta et al., 1999 and data not shown].

This study aims first to elucidate, if adrenal glands are involved in the HSD2 stimulation observed by our group [Igarreta et al., 1999] in response to stress, and if those changes in activity were associated to changes in protein abundance. Second, if HSD2 activity and protein abundance are modified by glucocorticoids under basal conditions (unstressed situation). For this purpose, we carried out a series of comparative experiments on ADX animals subjected to different stress situations or injected with increasing doses of corticosterone. The results showed that both adrenal and extra-adrenal factors were required for HSD2 activation under stress. Considering that norepinephrine is present in the kidney [Jacobson and Kokko, 1985; Kurokawa, 1985; Tanioka et al., 2002; Ye et al., 2002], one possible explanation for enzyme activation in the absence of adrenals could be catecholamine-mediated mechanisms. On the other hand, glucocorticoids produced a weak up-regulation of HSD2 activity and abundance, thus constituting one of the possible adrenal factors involved in such activation.

MATERIALS AND METHODS

Chemicals

Tritiated corticosterone ([$^3H_{1,2,6,7}$corticosterone, specific activity = 70 μCi/μmol) and chemiluminescence reagent were purchased from New England Nuclear, Life Sciences Products, Boston, MA. Autoradiography films were purchased from Dupont, Wilmington, DE. The sheep anti-rat HSD2 antibody was a generous gift of Dr Gomez-Sanchez [Gomez-Sanchez et al., 2001]. The second antibody (peroxidase-labeled rabbit antisheep IgG) was purchased from Rockland, Gilbertsville, PA. The other reagents were obtained from Merck-Darmstadt (Darmstadt, Federal Republic of Germany), Sigma (St. Louis, MO), or Aldrich (Milwaukee, WI), unless otherwise specified.

Animals

The animals used for these experiments were treated in accordance with the principles and guidelines of the Guide for the Care and Use of Laboratory Animals, National Research Council (National Academy Press, Washington, DC 1996). We used male Sprague–Dawley rats weighting 300–350 g, kept on rat chow and water ad libitum (this strain of rats does not develop accessory adrenals after adrenalectomy).

Rats were bilaterally adrenalectomized or sham-operated through dorsal incisions under halothane anesthesia, 48 h before the different treatments. This allows for almost complete depletion of circulating glucocorticoids [Damasco and Coronel de Pedazzini, 1985]. Rat chow and 0.9% NaCl solution were given to ADX rats ad libitum. Two hours after each treatment, kidneys were removed for experimental studies. All in vivo experiments were carried out between 9:00 am and 11:00 am.

Animal treatment for stress studies. Food was withdrawn 12 h before treatment. Animals were divided into the following groups:

- Sham-operated: This group was subjected to simulated adrenalectomy 48 h prior to kidneys removal.
- ADX: The animals were adrenalectomized 48 h prior to kidneys removal.
- ADX + acidosis: Animals were adrenalectomized 48 h prior to stress experiments.

Acidosis was achieved by gavage (oropharyngeal gastric intubation), with an overload of 10 ml of 200 mM HCl, 2 h prior to removal of the kidneys. This is a standard procedure used by the National Toxicology Program and the Environmental Protection Agency (see official websites) in chronic treatment of test animals for carcinogens, and by other groups to test ascorbate [Chen et al., 2000], chlorine [Meier et al., 1985; Carlton et al., 1986], and chloramines [Carlton et al., 1986]. This treatment resulted in a metabolic acidosis within 2 h (acute acidosis), as confirmed by blood parameters determination using a MiniQ acid/base analyzer. We observed a significant decrease on blood pH in the acidotic group compared to either ADX or Sham-operated groups.

- ADX + acidosis + B: Animals were adrenalectomized 48 h prior to stress treatment. This group was subjected to an acid overload (10 ml of 200 mM HCl) and an intramuscular injection of 68 μg B/100 g of body weight, 2 h before the removal of the kidneys. This treatment brings corticosterone levels to maximal circulating values [Damasco et al., 1979, 1990; Igarreta et al., 1997; Igarreta et al., 1998].
- ADX + cannula: Animals were adrenalectomized 48 h prior to stress experiments. To this group the acid overload was simulated (placement of only the rubber catheter) 2 h before removal of the kidneys.
- Intact: Undisturbed animals (neither operated nor stressed).

Corticosterone administration. ADX rats were injected intramuscularly, with vehicle, as control group (ADX + vehicle) or with, 1 μg B/100 g body weight (ADX + 1 μg), 4 μg B/100 g body weight (ADX + 4 μg), 68 μg B/100 g body weight (ADX + 68 μg), or 308 μg B/100 g body weight (ADX + 308 μg), 2 h before kidney removal and microsomal preparation. The hormone was dissolved in a mixture of absolute ethanol:propyleneglycol:0.9% NaCl (0.1:0.2:3.7), immediately before injection.

Plasma Levels of Corticosterone

Plasma corticosterone levels were determined by radioimmunoassay according to the method of Gomez-Sanchez et al. [1975].

Preparation of Renal Microsomes

Kidneys were perfused with 0.9% NaCl, excised, decapsulated, and sliced. Slices were resuspended in 0.1 M sodium phosphate buffer containing 1.5 mM $MgCl_2$, pH = 7.4 (MG buffer) (25 ml MG buffer/gram of tissue) and homogenized with a Potter Teflon homogenizer. Homogenates were centrifuged at 10,000*g* for 30 min. The supernatant was, then, centrifuged at 105,000*g* for 60 min. The microsomal fraction, thus obtained, was resuspended in MG buffer and total protein was determined by the method of Bradford [1976].

Kinetic Studies

Kinetics parameters were determined by measuring the conversion rate of [$^3H_{1,2,6,7}$]corticosterone into [$^3H_{1,2,6,7}$]11-dehydrocorticosterone. Microsomal suspensions (250 μg protein/ml) were incubated in 250 μl of MG buffer containing 3 nM, 7 nM of [$^3H_{1,2,6,7}$]corti-

costerone or 14 nM of [$^3H_{1,2,6,7}$]corticosterone plus 0–220 nM of radioinert corticosterone and 400 μM NAD^+, for 10 min at 37°C. The reaction was stopped by the addition of ethyl acetate and an excess of unlabelled steroids. Steroids were analyzed by thin layer chromatography as previously described [Igarreta et al., 1999].

Single-Point HSD2 Activity Studies

Single-point HSD2 activity was measured in kidney microsomes according to Leckie et al. [1995], with modifications. Briefly, 250 μg protein/ml, were incubated in 250 μl of MG buffer containing 14 nM of [$^3H_{1,2,6,7}$]corticosterone and 400 μM NAD^+ as cofactor. Incubation and steroid analysis were performed as described for the kinetic studies.

Western-Blot

Microsomes corresponding to 70 μg protein were resuspended in 20 μl of sample buffer and loaded on a discontinuous gel system with a 10% polyacrylamide separating gel. After the run, proteins were transferred to a nitrocellulose membrane. The efficiency of protein transfer was evaluated with 0.2% Ponceau Red S. Then, the membrane was blocked with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBST) plus 5% non-fat milk, for 1 h at room temperature. The blot was, then, incubated overnight with a sheep anti-rat HSD2 polyclonal antibody diluted 1:8,000 in PBST + BSA 1% [Gomez-Sanchez et al., 2001], followed by a rabbit antisheep IgG peroxidase-labeled antibody diluted 1:4,000 in PBST + BSA 1%, for 1 h at room temperature. Finally, the membrane was developed with chemiluminescence reagent. The autoradiograph was scanned and the band intensities were quantified using the MD Image Quant Software (version 3.3).

Statistical Analysis

All values are presented as mean ± standard error (SE). Differences between multiple groups were evaluated by analysis of variance (ANOVA) followed by the Dunnett's test or by the Tukey–Kramer's test, as indicated. Statistical significance was set at $P < 0.05$.

The n value for HSD2 activity studies corresponds to three independent experiments while the n value for HSD2 abundance studies corresponds to two independent experiments. The groups in each independent experiment included five animals, with the exception of the acidotic groups (ADX + acidosis and ADX + acidosis + B), which included eight rats each.

For the determination of corticosterone plasma levels, the n value corresponds to the number of animals used in each group.

RESULTS

Stress Studies

Kinetic studies. Figure 1 shows the results of kinetic studies performed for the different experimental groups.

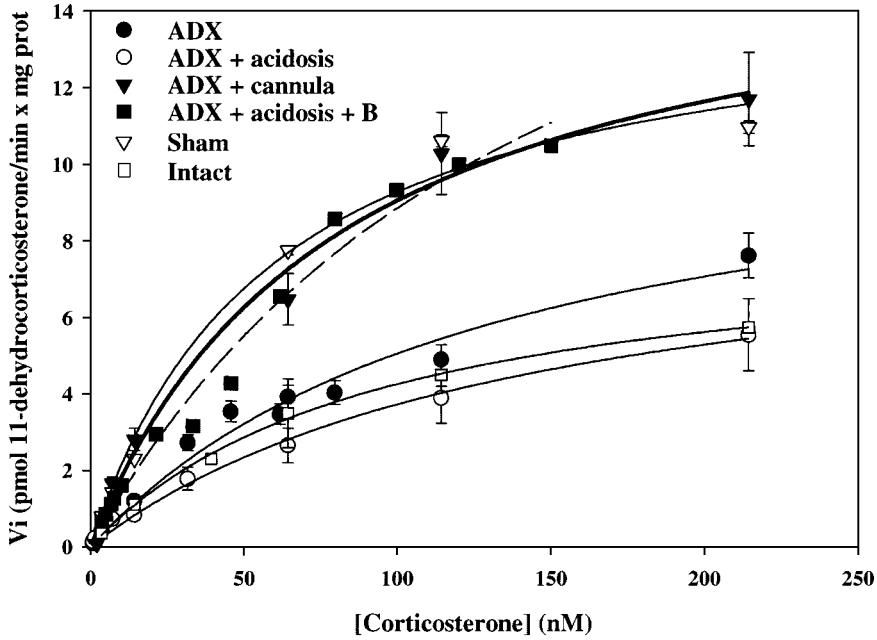
No differences in HSD2 activity were found between ADX and Intact rats. However, the Sham-operated animals, showed a significant increase in Vmax. These results indicate a possible involvement of an adrenal factor on the regulation of enzyme activity.

Both, Sham-operated animals and ADX animals with an oropharyngeal cannula (ADX + cannula), almost tripled Vmax for HSD2. This last group repeated the results already observed in Intact animals subjected to the same treatment [Igarreta et al., 1999], where a twofold increase in Vmax was observed. These results could suggest that the stimulation of enzyme activity in this group is independent of an intact adrenal gland or, that adrenal factors are not the only ones regulating the enzymatic response to this kind of stress.

In the case of ADX animals subjected to metabolic acidosis (ADX + acidosis), this group did not show any variation in enzyme activity, compared to ADX and Intact animals. However, when along with the HCl overload, a physiological dose of corticosterone was administered to the ADX animals (ADX + acidosis + B); there was a recovery of enzyme activity, suggesting the need of physiological levels of circulating glucocorticoids for a response to metabolic acidosis. This assumption could be supported by observations in Intact acidotic animals, where a correspondence between the increase in Vmax and circulating glucocorticoids was observed [Igarreta et al., 1999 and data not shown].

No significant differences were observed in K_M in any of the experimental groups. All the observed K_M were in the nanomolar range, in accordance to previously reported values [Igarreta et al., 1999; Agarwal, 2000; Odermatt et al., 2001; Seckl and Walker, 2001].

Plasma corticosterone levels. As expected after 48 h post-adrenalectomy, ADX



Treatment	Vmax (pmol 11dehydrocorticosterone/mg prot x min)	K_M (nM)
ADX	5.2 ± 0.4	74 ± 9
Intact	5.8 ± 1.2	64 ± 9
Sham	13.7 ± 0.2***+++	61 ± 2
ADX + cannula	15.4 ± 1.6***+++	73 ± 9
ADX + acidosis	5.8 ± 1.0	61 ± 7
ADX + acidosis + B	14.0 ± 0.2***+++	85 ± 5

Fig. 1. Kinetic analysis of HSD2 from animals subjected to stress. Kinetic studies were performed on renal microsomal proteins, obtained from Intact, Sham operated, ADX animals or ADX animals under different acute stress situations. Two hundred and fifty micrograms of protein/ml were incubated in 250 µl of phosphate buffer containing 14 nM [$^3H_{1,2,6,7}$]corticosterone plus 0–220 nM of radioinert corticosterone and 400 µM NAD^+, during 10 min at 37°C. After incubation, steroids were separated by TLC and radioactivity was measured by liquid scintillation. Results are expressed as mean ± SE of three independent experiments performed in triplicate. ***$P < 0.001$ versus ADX, $^{+++}P < 0.001$ versus Intact, Tukey–Kramer's test. ADX, adrenalectomized; B, corticosterone.

animals showed very low levels of circulating corticosterone (Table I).

In Sham-operated animals, the increase in enzyme activity was accompanied by a significant increase in circulating corticosterone levels, compared to both, the ADX and Intact groups. This 10-fold increase was similar to those found in Intact stressed animals (data not shown).

The ADX acidotic group, injected with corticosterone, showed physiological levels of circulating corticosterone (Table I) [Akana and Dallman, 1997; Igarreta et al., 1997, 1998; Audigé et al., 2002].

HSD2 protein expression. In correlation with the results shown in kinetic studies, there were no differences in HSD2 protein expression between ADX and Intact rats, while the Sham-operated group showed a significant increase in HSD2 abundance (Fig. 2).

The adrenalectomized animals subjected to a simulated acid overload (ADX + cannula) showed a significant increase in enzyme abundance, which correlated with stimulation of enzyme activity.

Contrarily, ADX animals with metabolic acidosis showed an increase in protein abun-

TABLE I. Plasma Corticosterone Levels

Treatment	Corticosterone (ng/ml of plasma)
Intact	$36 \pm 3^{++}$
ADX basal	$5 \pm 1^{**}$
Sham	$310 \pm 2^{**,++}$
ADX + cannula	$9 \pm 2^{**}$
ADX + acidosis	$5 \pm 1^{**}$
ADX + acidosis + B	$79 \pm 8^{**,++}$

Prior to kidney removal, blood was drawn from the aorta of Sham-operated, Intact, ADX animals, or ADX animals under different acute stress situations. Corticosterone levels were determined by specific radioimmunoassay. Values are expressed as mean ± SE (n = 5).
$^{**}P < 0.01$ versus Intact.
$^{++}P < 0.01$ versus ADX, Dunnett's test.

dance, with no correlation to kinetic studies, whereas those also injected with corticosterone (ADX + acidosis + B) showed no modifications in protein expression, as compared to Intact or ADX animals. Considering both the activity and protein expression results for these last two groups, it could be hypothesized that, in a situation of metabolic acidosis without circulating glucocorticoids, most of the enzyme is in a latent inactive form, with an unsuccessful attempt to compensate this situation by increasing protein abundance. On the other hand, the injection of a physiological dose of corticosterone, rising the circulating levels to those found during the circadian trough [Audigé et al., 2002], improves enzyme activity by increasing the active form, without changes in its protein expression.

Studies on Adrenalectomized Animals Injected With Corticosterone

Single-point HSD2 activity and plasma corticosterone levels. Only the dose of 68 μg of B/100 g body weight, which brought the circulating levels to those maintaining the normal physiological functions of glucocorticoids (Table II) [thymus weight, fat depot,

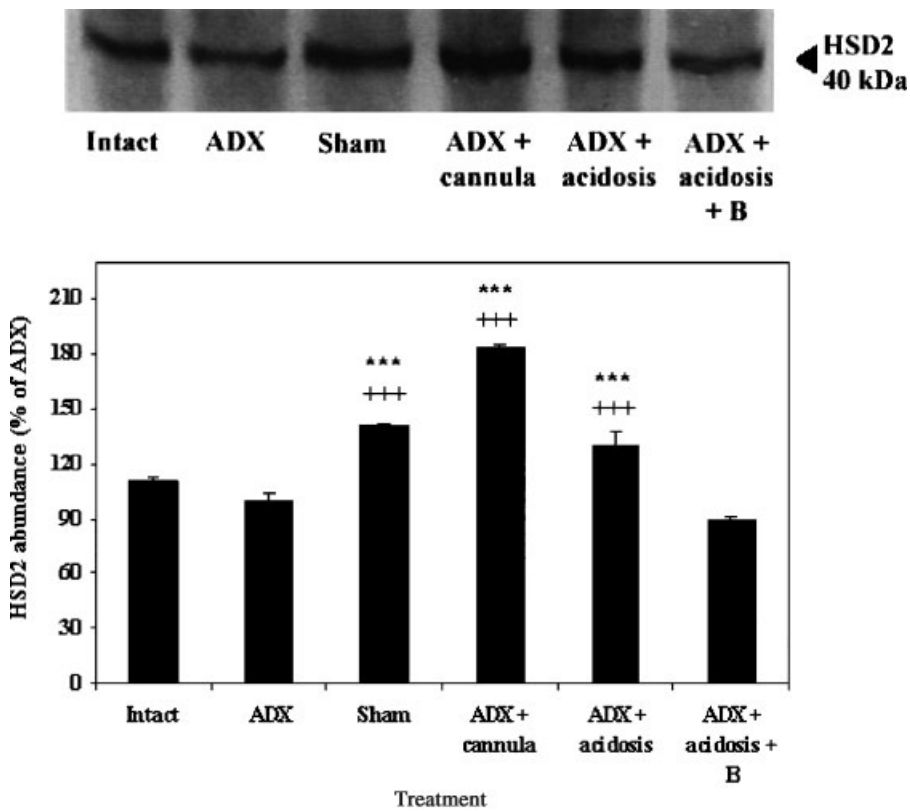


Fig. 2. HSD2 expression from animals subjected to stress. Western blot was performed on proteins from renal microsomes, obtained from Intact, Sham operated, ADX animals, or ADX animals under different acute stress situations. Results are expressed as mean ± SE of two independent experiments performed in quadruplicate. $^{***}P < 0.001$ versus ADX, $^{+++}P < 0.001$ versus Intact, Tukey–Kramer's test.

TABLE II. Plasma Corticosterone Levels

Treatment	Corticosterone (ng/ml of plasma)
ADX + vehicle	2 ± 1
ADX + 1 μg	9 ± 1
ADX + 4 μg	19 ± 1
ADX + 68 μg	74 ± 8**
ADX + 308 μg	174 ± 10**

Prior to kidney removal, blood was drawn from the aorta of ADX animals injected with vehicle or increasing doses of corticosterone. Corticosterone levels were determined with a specific radioimmunoassay. Results are expressed as mean ± SE (n = 5). **$P < 0.01$ versus ADX + vehicle, Dunnett's test.

insulin concentration, blood pressure, etc., Akana and Dallman, 1997], showed a significant increase in HSD2 activity (Fig. 3).

The dose of 308 μg of B/100 g of body weight, which brings the levels of circulating glucocorticoids to those observed during the circadian peak (Table II) [Dallman et al., 1987; Audigé et al., 2002], did not produce any variation in enzyme activity, when compared to control values (ADX + vehicle).

These results could reflect both permissive (observed at low concentration, 68 μg B) and suppressive (observed at high concentration, 308 μg B) actions of glucocorticoids [Munck and Náray-Fejes-Tóth, 1992, 1994; Sapolsky et al., 2000]. Permissive effects, generally stimulatory and mediated by MR, would activate renal HSD2 in order to prevent mineralocorticoid action of corticosterone. Suppressive effects, mainly mediated by glucocorticoid receptors (GR), by inhibiting renal HSD2, would allow the additional glucocorticoid actions, observed during the circadian peak.

Expression of HSD2. Considering the doses of 1 μg, 4 μg, and 68 μg of B/100 g body weight, the protein expression of HSD2 showed a dose-dependent tendency to increase, being this increment only significant at the higher dose (Fig. 4). These results are in accordance with enzyme activity observed for each dose, where activation of HSD2 was only significant for the ADX + 68 μg group.

The dose of ADX + 308 μg B did not produce any variation on HSD2 expression, compared to

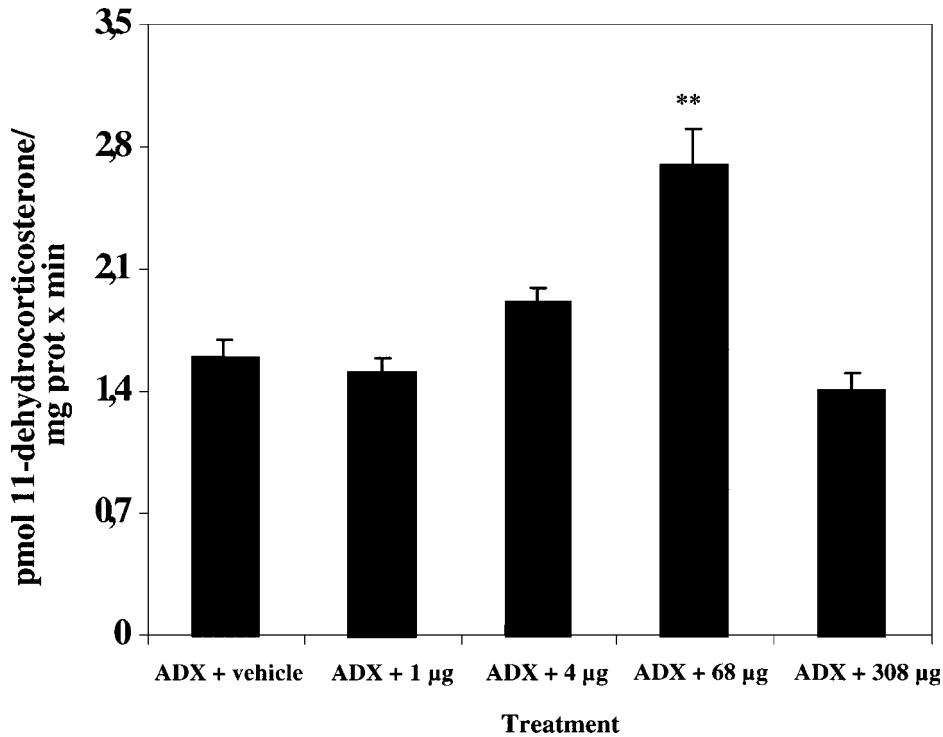


Fig. 3. HSD2 activity in ADX animals injected with increasing doses of corticosterone. Renal microsomes obtained from ADX animals injected with vehicle or increasing doses of corticosterone were incubated in 250 μl of phosphate buffer containing 14 nM [$^3H_{1,2,6,7}$]corticosterone and 400 μM NAD^+, during 10 min at 37°C. After incubation, steroids were separated by TLC and radioactivity was measured by liquid scintillation. Results are expressed as mean ± SE of three independent experiments performed in quintuplicate. **$P < 0.01$ versus ADX + vehicle, Dunnett's test.

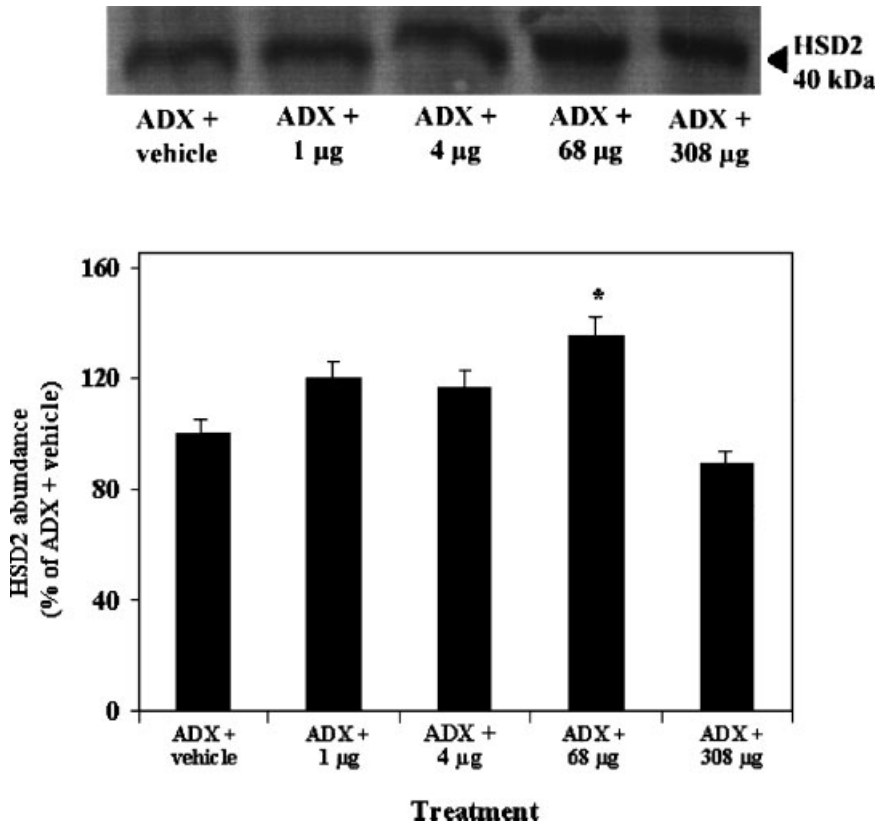


Fig. 4. HSD2 expression in ADX animals injected with increasing doses of corticosterone. Western blot was performed on proteins from renal microsomes, obtained from ADX animals injected with vehicle or increasing doses of corticosterone. Results are expressed as mean ± SE of two independent experiments performed in quadruplicate. $^*P < 0.05$ versus ADX + vehicle, Dunnett's test.

the ADX + vehicle group, accordingly to what happened with the enzyme activity.

DISCUSSION

HSD2 activity and abundance are regulated by corticosteroid hormones, but also by other factors such as epidermal growth factor and arginine vasopressin [Alfaidy et al., 1997; Darnel et al., 1999; Brooks et al., 2003]. Considering that stress stimulates the HPA axis and leads to an increase in glucocorticoid synthesis and secretion [Sapolsky et al., 2000; Pacak and Palkovits, 2001], we decided to study the effect of stress and corticosterone on the regulation of renal HSD2.

Previous results from our laboratory [Igarreta et al., 1999] showed an increase in Vmax, in Intact animals under different situations of acute stress (oropharyngeal cannulation and metabolic acidosis generated with an overload of HCl which, in turn, produced an increase in aldosterone secretion). In the present study, we evaluated the role of the adrenal gland and one of its products, corticosterone, on such stimulation using adrenalectomized animals.

Sham-operated animals showed an increase in Vmax, HSD2 protein expression and circulating corticosterone, results similar to those observed for the Intact stressed animals [Igarreta et al., 1999]. The effects observed on the Sham-operated group, would be dependent on adrenal factors involved in a response to stress. This is supported by the observation on ADX animals, which also underwent surgery, and the Intact ones, with no increase in enzyme activity. Although we cannot rule out some other stress-related factor (i.e., mineralocorticoids, since it has been shown that aldosterone may stimulate HSD2 activity in ADX animals and potentiate arginine vasopressin effects [Alfaidy et al., 1995; Hawk et al., 1996; Farman and Bocchi, 2000]), in the regulation of enzyme activity in

Sham-operated animals, the results presented herein as well as those by others [Li et al., 1996; Darnel et al., 1999; Koyama and Krozowski, 2001; Suzuki et al., 2003] seem to indicate the involvement of glucocorticoids in this enzyme regulation.

ADX animals, subjected to stress by introduction of an oropharyngeal cannula (ADX + cannula), showed an increase in Vmax, coincident with an increase in protein expression, suggesting that stimulation of HSD2 under this condition is independent of the presence of an intact adrenal gland, or that adrenal factors are not the only ones regulating the enzymatic response to this kind of stress. This stimulation could be mediated by stress-related extra-adrenal factors, such as norepinephrine, which is present in the kidney [Jacobson and Kokko, 1985; Kurokawa, 1985; Tanioka et al., 2002; Ye et al., 2002], thus affecting HSD2 activity in a paracrine manner.

In the case of adrenalectomized rats with metabolic acidosis (ADX + acidosis and ADX + acidosis + B), the presence of physiological levels of circulating glucocorticoids seems to be sufficient to produce stimulation of the enzymatic activity. Only in the acidotic ADX animals, receiving corticosterone, we observed a significant increase in HSD2 Vmax.

The lack of stimulation of enzyme activity in the case of the ADX acidotic animals (ADX + acidosis) compared to ADX and Intact ones, regardless of the significant increase in enzyme expression, could be explained if the enzyme is present as an inactive dimer. The lack of adrenals and their factors, prevents the recovery from the metabolic acidosis, leading to a decrease in enzyme activity, probably due to an increase in the inactive dimer, with a corresponding decrease in the active monomer. This increase in protein expression cannot compensate for the absence of stimulation of enzyme activity, probably because HSD2 remains in a latent state with no change in Vmax. Corticosterone reverses this situation (ADX + acidosis + B), increasing Vmax to levels similar to those found in Sham-operated and ADX + cannula, without a concomitant increase in HSD2 protein expression. Glucocorticoids could favor a shift in the dimer-monomer equilibrium, towards the active form with no increase in total protein expression.

In accordance with our results, Li et al. [1996] found a negative correlation between glucocorticoid effect and the activity and expression of HSD2. These authors suggest that the observed stimulation of enzyme activity, although with a concomitant decrease of its mRNA abundance, would be mediated by an activation of a latent form of the renal enzyme by a direct or an indirect glucocorticoid effect.

As a consequence of these results, and in order to evaluate the effects of glucocorticoids under basal conditions (absence of stress), studies on enzyme activity were performed on animals injected with increasing doses of corticosterone. Doses within the range of 1–68 μg B/100 g body weight, showed a tendency to increase both enzyme activity as well as protein expression. Nevertheless, these variations were significant only for the higher dose (ADX + 68 μg), showing a weak up regulation of HSD2 by glucocorticoids. This is the same dose used for recovery of enzyme activity, under metabolic acidosis, but contrarily to the ADX animals injected with corticosterone, there was a correspondence between the increase in activity and protein expression in the ADX + 68 μg group. This differential response between acidotic ADX animals, injected with corticosterone (ADX + acidosis + B) and those non-acidotic ADX animals, injected with the same dose (ADX + 68 μg), could be due to the acidosis itself, to some other factors that were altered under this stress situation, or a combination of both.

The highest dose of corticosterone (ADX + 308 μg), produced values of HSD2 activity and abundance similar to the ADX + vehicle group.

The results obtained with the doses of 1, 4, and 68 μg of B, partially confirmed those recently published by Suzuki et al. [2003]. They observed an increase in HSD2 activity and abundance when cells, derived from bronchial epithelium, were incubated with increasing concentrations of the synthetic glucocorticoid, dexamethasone.

It is known that glucocorticoids exert both permissive and suppressive effects at physiological basal or diurnal peaks, respectively. We determined the circulating levels of corticosterone in each of the experimental groups. A dose of 68 μg B corresponded to basal physiological levels, accounting for permissive actions on renal HSD2. Upon increase in circulating corticosterone (ADX + 308 μg B), suppressive actions become more important, with an inhibition of HSD2. In this situation, the increase in

corticosterone, essential for the physiological functions normally found during the circadian peak, would not be counteracted with stimulation in HSD2 activity, in a self-regulatory action. Lower doses of corticosterone (1 and 4 μg) had no effect.

In conclusion, we have demonstrated that, Sham-operated and adrenalectomized animals under different stress situations, or injected with different doses of corticosterone, presented changes in renal HSD2, leading, in some cases to stimulation of enzyme activity. This stimulation in HSD2 activity suggests the involvement of different factors.

In the case of Sham-operated and ADX + acidosis + B, these factors involved the adrenal glands, as alteration of HSD2 activity was observed only in the presence of the whole gland (Sham-operated versus ADX animals) and after hormone replacement with physiological doses of corticosterone (ADX + acidosis + B versus ADX + vehicle). Glucocorticoid effect seems to be indirect, probably mediated by some other stress-related factor, as shown in the absence of stressor (Intact animals), where no variation was observed in both activity and abundance of HSD2, even in the presence of physiological levels of circulating glucocorticoids.

Animals subjected to a simulated gavage (ADX + cannula) showed stimulation of HSD2 probably mediated, at least in part, by extra-adrenal factors such as catecholamines, which are present in the kidney [Jacobson and Kokko, 1985; Kurokawa, 1985; Tanioka et al., 2002; Ye et al., 2002] and involved in the response to stress [Sapolsky et al., 2000; Pacak and Palkovits, 2001]. These factors could act in a autocrine/paracrine manner regulating renal HSD2 activity.

In the case of ADX animals injected with different doses of corticosterone, although there are several studies on HSD2 regulation by natural and synthetic glucocorticoids [Alfaidy et al., 1995; Diederich et al., 1996; Li et al., 1996; Koyama and Krozowski, 2001; Audigé et al., 2002; Suzuki et al., 2003], these seem to be, in some cases, contradictory, depending on the models utilized. Also, a tissue-specific regulatory pattern cannot be ruled out. In rats, our ex vivo studies revealed a weak up regulation of HSD2 activity and abundance by a "permissive" dose of corticosterone (ADX + 68 μg).

Considering that the animals were killed and the kidneys removed 2 h after the stressors were applied or after glucocorticoid injection and, taking into account that a glucocorticoid-responsive element has not yet been described in the HSD2 promoter region [Nawrocki et al., 2002], we could suggest, an indirect and non-genomic role for glucocorticoids on HSD2, probably through post-transcriptional changes.

Knowing that stress is multifactorial, and so are the responses in the organism, leading to stimulation or inhibition of certain pathways, and considering that the pathways involved are dependent on the kind of stress [Akana and Dallman, 1997; Sapolsky et al., 2000; Pacak and Palkovits, 2001], it is not unlikely to find a differential regulation of HSD2, depending on the stress applied.

It could be concluded that, under a determined stressful situation or during glucocorticoid-circadian rhythm, the organism modulates renal HSD2 through different mechanisms involving adrenal and extra-adrenal factors and ultimately, this modulation could regulate both paracrine and autocrine glucocorticoid and mineralocorticoid activities.

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