Sexual dimorphism of ornithine decarboxylase in the mouse adrenal: influence of polyamine deprivation on catecholamine and corticoid levels

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Bastida CM, Cremades A, Castells MT, López-Contreras AJ, López-García C, Sánchez-Mas J, Peñafiel R. Sexual dimorphism of ornithine decarboxylase in the mouse adrenal: influence of polyamine deprivation on catecholamine and corticoid levels. Am J Physiol Endocrinol Metab 292: E1010–E1017, 2007. First published December 5, 2006; doi:10.1152/ajpendo.00316.2006.—Adrenal sexual dimorphism is thought to be important in explaining sex-related differences regarding prevalent diseases and the responses to stress and drugs. We report here that in CD1 mice there is marked sexual dimorphism affecting not only gland size and corticoid hormone secretion but also adrenal ornithine decarboxylase (ODC), polyamine, and catecholamine levels in which testosterone appears to be a major determinant. Our results show that adrenal weight, ODC activity, and corticosterone and aldosterone secretion were higher in female than in male mice and that orchidectomy brought these male parameters closer to the values found in females. mRNA levels of steroidogenic proteins SF-1, Dax-1, steroid 21-hydroxylase, and aldosterone synthase appeared to be slightly higher in female than in male adrenals. Immunocytochemical analysis of adrenal ODC revealed that immunoreactivity was higher in females than in males and was located mainly in the cortical cells, and especially in zona glomerulosa, whereas no sex differences in ODC mRNA levels were observed. These results suggest that sex-associated differences in the expression of ODC in the mouse adrenal gland appear to be related mainly to posttranscriptional mechanisms. Combination treatment of mice with α-difluoromethylornithine (a suicide inhibitor of ODC) and a polyamine-deficient diet produced a marked decrease in adrenal polyamine and catecholamine levels and a significant reduction in plasma corticosterone and aldosterone concentrations that were not associated with a decrease in the mRNA levels of steroidogenic proteins. All of these data suggest a relevant role for testosterone, ODC, and polyamines in the mouse adrenal function.

mice; α-difluoromethylornithine; corticosterone; aldosterone; steroidogenesis

THE ADRENAL GLAND plays an important role in stress response, with the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenomedulatory system as the principal components (41). Considerable evidence has accumulated indicating that gonadal factors act as regulators of the hypothalamic-pituitary-adrenal axis and adrenal function. In the rat there is a marked sexual dimorphism in adrenal weight, plasma ACTH, and corticosterone concentrations, with females having higher values than males (1, 25, 27, 30, 45). This adrenal sexual dimorphism is thought to be an important factor in explaining sex differences in relation to prevalent diseases associated with hypothalamic-pituitary-adrenal axis reactivity, such as cardiovascular disease, inflammation, diabetes, and hypertension (19, 55). On the other hand, it has been suggested that a transient increase in adrenal decarboxylase (ODC) activity and tissue polyamines, termed the polyamine stress response, may be considered as an integral component of the cellular stress program (18), and, in consequence, sex-dependent differences in the ODC/polyamine system in the adrenals could be, in part, responsible for the sex dependence of the stress response.

Polyamines are small aliphatic cations that are essential for the growth, differentiation, and function of normal cells (22, 36, 47, 55, 58). The cellular polyamine content is precisely regulated by multiple pathways that include transport mechanisms and biosynthesis from amino acid precursors. ODC, a key rate-limiting enzyme in the polyamine biosynthetic pathway converting L-ornithine into putrescine, is finely tuned by many hormones and factors, acting at transcriptional, translational, and posttranslational levels (13, 26, 38, 49). Although it has been known for many years that pituitary and gonadal hormones induce ODC in target tissues (2, 24, 59), the physiological function of ODC induction in response to these factors still remains largely unknown. In this respect, we (7, 8) recently found that the induction of ovarian ODC mediated by luteinizing hormone is necessary for folliculogenesis and luteinization. These studies suggested that ODC induction may be relevant for the regulation of the steroidogenic activity in the mouse ovary. On the other hand, although it is known that in the rat adrenal ODC is affected by ACTH and different stressful conditions (2, 3, 29, 31, 42, 50), the significance of ODC induction in adrenal physiology and the influence of gonadal factors in the regulation of adrenal polyamine metabolism are poorly understood. In mice, the existence of a marked extragenital sex-dependent dimorphism, affecting mainly the kidney, liver, and skeletal muscle, is widely recognized (5). Despite having reported sex differences in the mouse adrenal weight (14, 52), little is known about the contribution of this sexual dimorphism in the physiology of the mouse adrenal and its influence on polyamine metabolism of this gland.

In the present study we have analyzed 1) the influence of sex on ODC and polyamine levels in the mouse adrenal and the possible differences in adrenal corticoid and catecholamine levels between male and female CD1 mice and 2) the effect of polyamine deprivation induced by feeding mice a polyamine-deficient diet and α-difluoromethylornithine (DFMO; a specific inhibitor of ODC) treatment on the secretory capacity of...
this gland. Our results indicate the existence of marked sexual dimorphism in the mouse adrenal and that polyamine deprivation reduces corticoid secretion.

MATERIALS AND METHODS

Animals and treatments. Adult male and female Swiss CD1 mice were used in these experiments. Animals were fed with standard chow (UAR A03; Panlab, Barcelona, Spain) and water ad libitum. Animals were maintained at 22°C ambient temperature and 50% relative humidity under a controlled 12:12-h light-dark cycle (lights on at 0800). Blood samples were collected under light ether anesthesia by cardiac puncture at 0900–1000 (1 puncture/animal). Plasma was obtained by centrifugation at 4°C and was kept frozen at −70°C until analysis. Animals were killed by cervical dislocation, and the adrenals were quickly removed, weighed, and processed. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the University of Murcia that are in compliance with national (RD 1201/2005) and international laws and policies (European Economic Community Council Directive 86/609 and National Research Council Guide for the Care and Use of Laboratory Animals (34a)).

To study the influence of chronic exposure to testosterone on adrenal ODC activity, adult mice were injected with 0.01 ml/g body wt of testosterone propionate dissolved in olive oil (20 mg/kg sc, every other day) and killed after 2 wk of treatment. The influence of ACTH on adrenal ODC induction was studied by intraperitoneal injection of 0.01 ml/g body wt of ACTH (2 mg/kg, dissolved in 0.9% NaCl) to adult mice between 0900 and 1000 and having the animals killed 5 h after injection. Testosterone propionate was supplied by Sigma-Aldrich (St. Louis, MO). hACTH 1–24 was obtained from Calbiochem (La Jolla, CA). Three-month-old mice were gonadectomized under ether anesthesia and were used 15 days after surgery. Hypophysectomized (HX) mice were obtained from Charles River (Portage, MI) and received 5% glucose in the drinking water.

To assess the influence of polyamine deprivation on adrenal parameters, the ODC inhibitor DFMO, obtained from Ilex Products (San Antonio, TX), was given to 15 male and 15 female adult mice as a 2% solution in the drinking water (pH adjusted to 7.0), associated with feeding with a polyamine-deficient chow for 20 days. The polyamine deficient diet (ICN Biomedicals, Aurora, OH) contained 46.4% sucrose, 23.1% corn starch, 10% corn oil, 0.5% American Institute of Nutrition (AIN) 76 vitamin mix, 5% AIN 76 mineral mix, 0.2% choline bitartrate, and 15.0% amino acid mixture. The analysis of the pellet showed that putrescine, spermidine, and spermine were absent from the diet.

Enzymatic measurements. For enzyme determination, pools of two to three adrenals were homogenized with the aid of a Polytron homogenizer in a buffer containing 25 mM Tris (pH 7.2), 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, and 0.25 M sucrose, using a ratio of 1:40 (wt/vol). The homogenate was centrifuged at 20,000 g for 20 min, and ODC activity was determined in the supernatant. ODC activity was determined basically as described elsewhere (43) by measuring 14CO2 release from L-[1-14C]ornithine. The incubation mixture contained 20 mM Tris, pH 7.2, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, 2 mM diithreitol, 0.4 mM L-[1-14C]ornithine (specific activity 2.6 Ci/mmol; New England Nuclear, Boston, MA) in a total volume of 62.5 μl. The samples were incubated at 37°C for 30 min, and the reaction was stopped by adding 0.5 ml of 2 M citric acid. Activity was expressed as nanomoles of CO2 produced per hour and per gram of wet weight tissue.

Polyamine analysis. Several pools of 3–4 adrenal glands were homogenized in 0.4 M perchloric acid (1:20 wt/vol), and, after centrifugation at 10,000 g for 5 min, the polyamines from the supernatant were dansylated according to standard method (46). Dansylated polyamines were separated by HPLC using a Lichrosorb 10-RP-18 column (4.6 × 250 mm) and acetonitrile/water mixtures (running from 70:30 to 96:4 ratios during 25 min of analysis) as mobile phase. 1,6-Hexanediolamide was used as internal standard. Detection of the derivatives was achieved using a fluorescence detector, with a 340-nm excitation filter and a 435-nm emission filter. Polyamine content was expressed as nanomoles per gram wet weight of tissue.

Catecholamine analysis. Several pools of 3–4 adrenal glands were homogenized with a Polytron homogenizer in 0.1 M perchloric acid containing 2 mM EDTA (1:20 wt/vol), and, after centrifugation at 10,000 g for 5 min, the supernatant was filtered through a 0.22-μm filter (Millex-GV; Millipore). Catecholamine analysis was performed by means of a 5-μm C18 reverse-phase column (Waters), using a mobile phase consisting of 95:5 (vol/vol) mixture of water and methanol with 50 mM sodium acetate, 20 mM citric acid, 3.75 mM 1-octyl sodium sulphonate, 1 mM di-n-butylamine, and 0.135 mM EDTA, pH adjusted to 4.3. Electrochemical detection was accomplished with a glassy carbon electrode set at a potential of +0.65 V vs. the silver-silver chloride reference electrode. 3,4-Dihydroxybenzylamine was used as internal standard. Catecholamine content was expressed as nanomoles per gram wet weight of tissue.

RT-PCR. RNA was extracted from several pools of 3–4 adrenals with the Gen Elute total RNA miniprep kit (Sigma-Aldrich) and purified, following the manufacturer’s specifications. Total RNA was reverse transcribed using oligo(dT)_18 as primer and Moloney murine leukemia virus reverse transcriptase (Ambion, Austin, TX). Products were amplified by means of Taq polymerase (Sigma Chemical, St. Louis, MO), using specific primer pairs within the linear range for each gene product. Twenty-five cycles (denaturation for 1 min at 95°C, annealing for 2 min at 55°C, and extension for 2 min at 72°C, followed by a final 10-min extension at 72°C) were performed using adrenal cDNA as template. Amplified products were resolved by electrophoresis in 2% agarose gel containing 40 mM Tris-acetate and 1 mM EDTA (pH 8.0) in a horizontal slab gel apparatus using 1X Tris-acetate plus 2 mM EDTA buffer. The gel was stained with ethidium bromide (0.2 μg/ml for 15 min), photographed by UV transillumination using a Gel Doc system camera (Bio-Rad), and the bands quantified using the Multi-Analyt software and expressed as percentage of the β-actin band. The size of the specific bands matched with the predicted length of the amplicons. Primers used (from Sigma-Genosys, Suffolk, UK) for the different mouse genes analyzed are given in Table 1.

Northern blot. Total RNA from several pools of three to four adrenals from different mice was isolated with guanidinium thiocyanate, denatured with glyoxal and DMSO, electrophoresed on 1.5% agarose gels in 10 mM sodium phosphate buffer, pH 7.0, and transferred to Hybond nylon membrane. Prehybridization and hybridization were performed as described (44). The random priming 32P-labeled probe used was 550-bp ODC fragment (primers GGATT-TGACTGTTGACAACG and GAGTCTGATGGGAAGTAC) and 282-bp GAPDH fragment (primers CGTCTCTCACCACCATGGA and CCGCATCAGCGCACGTTT). Signal intensities were quantified by phosphorimaging and normalized to the GAPDH signal.

Hormone analysis. Costicosterone and aldosterone were determined in plasma of control and treated mice by means of commercial kits (Corticosterone Radioimmunoassay; ICN Biomedicals, Costa Mesa, CA, and Aldosterone Radioimmunoassay, Diagnostic Systems Laboratories, Webster, TX), following the manufacturer instructions. Histology and cytochemistry. Adrenals from male and female mice were removed and fixed in 10% formalin in PBS (0.01 M PBS, pH 7.4) for 10 h. After being washed in PBS, samples were processed routinely, embedded in paraffin, and cut in 5-μm sections. Sections were stained with haematoxycin-eosine and immunocytochemistry.

For immunocytochemistry, a two-step method was applied. Tissue sections from paraffin blocks were deparaffinized in xylene and hydrated in a graded ethanol series. Endogenous peroxidase activity was destroyed by treatment with 3% hydrogen peroxide in PBS for 30 min. Sections were washed in three 5-min changes of PBS and then
incubated with normal goat serum (1:30) for 1 h and then with rabbit polyclonal antibody to ODC (1:100; Euro-Diagnostica, Malmö, Sweden) overnight at 4°C. After being washed in PBS, they were incubated with peroxidase conjugated goat anti-rabbit immunoglobulin (1:100; Chemicon International) for 1 h and developed with 0.05% 3,3-diaminobenzidine tetrahydrochloride and 0.015% hydrogen peroxide. After immunostaining, sections were counterstained with haematoxylin. In the control, anti-ODC was substituted by PBS.

Statistics. Results are given as means ± SD. The significance of the differences observed was assessed by ANOVA, followed by the post hoc Newman-Keuls multiple range test, or by the nonparametric Mann-Whitney test. \( P < 0.05 \) was considered statistically significant.

RESULTS

Figure 1 shows the levels of ODC activity found in the adrenal glands at specific times of the day in both male and female mice. There was a highly significant circadian variation in the enzyme activity that increased from 1030 to 1830, especially in males, although it was always about 56% increase in adrenal ODC activity was observed, whereas in the castrated females the enzyme activity remained unchanged (Fig. 3). Testosterone treatment induced a decrease in adrenal ODC activity that was more important in the females than in males, where the activity fell to 29% of control values (Fig. 3). The adrenal weight was also affected by testosterone since gonadectomy markedly increased the weight of the adrenal in castrated males (−2-fold), whereas testosterone propionate produced a significant regression in the adrenal weight, especially in the female mice (−45% of control; Table 2). Figure 4 shows that, after 5 h of ACTH treatment, adrenal ODC activity increased about threefold above basal levels in both male and female mice, whereas hypophysectomy produced a clear diminution in adrenal ODC activity, reaching values of −20–30% of control animals. ACTH treatment of the HX mice induced a marked increase in enzyme activity in both sexes that was prevented by prior treatment with testosterone. Treatment of intact mice with olive oil used as vehicle did not affect adrenal ODC activity (results not shown).

The analysis of ODC mRNA from the adrenal glands of male and female mice by means of Northern blot and RT-PCR revealed that there were no significant differences between male and female mice by means of Northern blot and RT-PCR revealed that there were no significant differences between the two sexes. The results are shown in Table 2.

Table 1. Primers used in RT-PCR experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Amplicon, bp</th>
<th>Primer Set (5′→3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>NM_007393</td>
<td>535</td>
<td>TGCTGCTGGACCTGCTG</td>
</tr>
<tr>
<td>ODC</td>
<td>D00658</td>
<td>409</td>
<td>CTTGAAGGAGATGCAAGT</td>
</tr>
<tr>
<td>AZ1</td>
<td>M538267</td>
<td>496</td>
<td>AGCTGTTACAGAAACCTG</td>
</tr>
<tr>
<td>AZ2</td>
<td>NM_019779</td>
<td>684</td>
<td>TGATCCCTCTACTAGATC</td>
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<tr>
<td>AZ3</td>
<td>AF511594</td>
<td>290</td>
<td>ACTACATGTACTACCTG</td>
</tr>
<tr>
<td>AZI</td>
<td>NM_018745</td>
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<td>CTCAACTGGAAGCAACCTG</td>
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<tr>
<td>PMF1</td>
<td>BCO49223</td>
<td>381</td>
<td>CCATCCGAGGTGAGAAA</td>
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<tr>
<td>StAR</td>
<td>L36062</td>
<td>654</td>
<td>CTGAAAGGACGAACCTG</td>
</tr>
<tr>
<td>CYP11A</td>
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</tr>
<tr>
<td>CYP11B2</td>
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<td>920</td>
<td>ACTCCGGACCTTATGGG</td>
</tr>
<tr>
<td>CYP21</td>
<td>NM_009995</td>
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</tr>
<tr>
<td>3β-HSD1</td>
<td>NM_008293</td>
<td>689</td>
<td>TGTGCTCTACACCTG</td>
</tr>
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<td>SF-1</td>
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<td>Dax-1</td>
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</tr>
<tr>
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<td>699</td>
<td>ACTCGGAGCTTATGG</td>
</tr>
<tr>
<td>AQ27</td>
<td>AB109631</td>
<td>269</td>
<td>TGGAACAGTGGAGACCTC</td>
</tr>
<tr>
<td>AQ27</td>
<td>AB109631</td>
<td>349</td>
<td>ACTGAAAGGAGGAAGCTC</td>
</tr>
</tbody>
</table>

Table 2. Influence of sex hormones on adrenal weight

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>Castrated</th>
<th>+TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.89±0.78 (28)</td>
<td>3.98±0.77*(10)</td>
<td>1.99±0.47 (5)</td>
</tr>
<tr>
<td>Female</td>
<td>5.31±0.96*(18)</td>
<td>5.81±0.82 (6)</td>
<td>2.08±0.57†(13)</td>
</tr>
</tbody>
</table>

Results are means ± SD. Animals were gonadectomized under ether anesthesia and killed 15 days later. Testosterone propionate (TP) was given every other day (20 mg/kg), and animals were killed 15 days after treatment. All animals were killed between 0900 and 1000. The weight is expressed as milligrams per gland. The number of animals per group is given in parentheses. Statistical significance (ANOVA): *P < 0.001 vs. control male; †P < 0.001 vs. control female. No significant differences in the adrenal weight between left and right adrenals were observed.
sexes in the levels of ODC mRNA in this tissue (Fig. 5). To know whether the sex-dependent ODC activity in the adrenals of mice could be related to differences in the expression of several ODC-related genes, mRNA levels of ODC antizymes (AZ1, AZ2, and AZ3), antizyme protein inhibitor (AZI), and polyamine-modulated factor (PMF) were determined by semi-quantitative RT-PCR. Figure 6 shows no significant differences in the levels of the mRNAs assayed between the adrenals of male and female mice. The immunocytochemical analysis of ODC protein in the adrenals, by means of techniques using antibodies directed against mouse ODC, showed a higher level of reactivity in the adrenals of female mice, particularly in the cortical zone (Fig. 2, c and d). A more detailed analysis indicated that ODC immunoreactivity was more intense in zona glomerulosa (G) than in zona fasciculata (F) or zona reticularis (R). g: negative control of the technique; section incubated only with secondary antibody (no reaction). Bars: 1 mm (a–d), 80 μm (e), 20 μm (f and g). All animals were killed between 0900 and 1000.

Fig. 3. Influence of sex steroids on adrenal ODC activity. Mice were gonadectomized under ether anesthesia and killed 15 days after surgery. Testosterone propionate (TP; 20 mg/kg sc) was given every other day, and mice were killed 15 days after the first injection. All animals were killed between 0900 and 1000. Results are means ± SD of several pools of 2–3 adrenals (4–6 animals/group). Statistical significance (ANOVA): $^aP < 0.01$ vs. control male; $^bP < 0.001$ vs. control male; $^cP < 0.001$ vs. control female; $^dP < 0.001$ vs. intact female + TP.

Fig. 4. Effect of ACTH and hypophysectomy on adrenal ODC activity. Intact and hypophysectomized (HX) animals were treated with ACTH (2 mg/kg ip) and killed 5 h after the injection. In 1 group, HX mice were treated with TP (20 mg/kg sc, 15 days) before ACTH administration. Intact control and treated animals were killed between 1400 and 1500. Results are means ± SD of several pools of 2–3 adrenals (4–6 animals/group). Statistical significance (ANOVA): $^aP < 0.001$ vs. their respective sex control group; $^bP < 0.001$ vs. their respective sex HX group; $^cP < 0.001$ vs. their respective sex HX + ACTH group.
Sex-dependent dimorphism was also evident in the plasma values of corticosterone and aldosterone, which were two- to threefold higher in females than in males (Table 3). Castration of male mice markedly increased plasma aldosterone and corticosterone levels ≥346 ± 71 and 406 ± 76 ng/ml, respectively, reaching values similar to those found in female mice. The analysis by semiquantitative RT-PCR of mRNAs of different genes related to the steroidogenic process expressed in the adrenals of male and female mice is given in Fig. 7. Although mRNA levels of steroidogenic acute regulatory protein, cytochrome P450 cholesterol side chain cleavage, and 3ß-hydroxysteroid dehydrogenase appeared to be slightly higher in males, the differences were not statistically significant. However, mRNA levels of genes coding for cytochrome P450 21-hydroxylase (CYP21) and several transcription factors related to the adrenal function, such as steroidogenic factor-1, transcription factor Dax-1, and androgen receptor (AR), were significantly lower in the adrenal of males. Almost nondetectable levels of expression of AQ27, the receptor of the peptide pyroglutamylated arginine-phenylalanine-amide peptide (QRFP) that has been related with aldosterone synthesis in the rat (17), were observed in the mouse adrenals, even using two different pair of primers (Table 1) and different PCR conditions, in contrast to the marked level of expression measured in rat adrenals (data not shown).

To study the possible influence of ODC and polyamines on adrenal function, DFMO, an irreversible and specific inhibitor of ODC (33), was given to the animals in combination with feeding of a polyamine deficient diet, treatment that has been reported to cause a marked decrease in both polyamine levels and growth rate of different experimental tumors (48). The weights of the adrenals were not significantly affected by the treatment (2.36 ± 0.43 mg in the males and 5.85 ± 1.14 mg in the females). DFMO treatment did not affect body weight in the females but slightly decreased body weight in the males (<5%). However, as shown in Table 4, there was a marked decrease in the concentration of putrescine in both sexes (reaching values ~30% of control levels) and a moderate diminution in spermidine (to values ~51–62% of control levels). The concentrations of the catecholamines norepineph-

### Table 3. Effect of polyamine deprivation on plasma corticosterone and aldosterone levels in male and female mice

<table>
<thead>
<tr>
<th>Corticosterone, ng/ml</th>
<th>Aldosterone, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>Treated</strong></td>
</tr>
<tr>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>148±43 (12)</td>
<td>152±31 (12)</td>
</tr>
<tr>
<td>69±27† (15)</td>
<td>84±30† (15)</td>
</tr>
<tr>
<td>414±97±(12)</td>
<td>342±39*(12)</td>
</tr>
<tr>
<td>132±39† (15)</td>
<td>264±75‡ (15)</td>
</tr>
</tbody>
</table>

Results are means ± SD. Adult animals were treated with 2% α-difluoromethylornithine (DFMO) given in the drinking water and fed a polyamine deficient diet for 20 days. All animals were killed between 0900 and 1000. The number of animals per group is given in parentheses. Statistical significance (ANOVA): *P < 0.001 vs. respective control mice; †P < 0.001 vs. control male; ‡P < 0.01 vs. respective control mice.
Table 4. Influence of polyamine deprivation on the content of polyamines and catecholamines in the adrenal gland of male and female mice

<table>
<thead>
<tr>
<th></th>
<th>Polyamines</th>
<th>Catecholamines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PUT</td>
<td>SPD</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>81±26</td>
<td>653±116</td>
</tr>
<tr>
<td>Females</td>
<td>127±33a</td>
<td>615±158</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>20±6b</td>
<td>337±101b</td>
</tr>
<tr>
<td>Females</td>
<td>45±29c</td>
<td>383±68c</td>
</tr>
</tbody>
</table>

Results are means ± SD of several pools of 3–4 adrenals from 6–7 animals/group and in nmol/g. PUT, putrescine; SPD, spermidine; SPN, spermine; NE, norepinephrine; E, epinephrine; D, dopamine. Adult animals were treated with 2% DFMO given in the drinking water and fed a polyamine deficient diet for 20 days. All animals were killed between 0900 and 1000. Statistical significance (ANOVA): aP < 0.05 vs. control males; bP < 0.01 vs. control males; cP < 0.001 vs. control females; dP < 0.01 vs. control females; eP < 0.001 vs. control females.

In mice, the existence of extragenital sexual dimorphism was reported in the brain, kidney, and skeletal muscle (5) and more recently in the adrenal size (14, 52). In addition, our results reveal a marked sexual dimorphism in the adrenal glands of CD1 mice, affecting not only the weight and size of the gland but also the secretion of corticosterone and aldosterone, steroid hormones that are regulated mainly by ACTH secretion and by the renin-angiotensin system and plasma potassium concentration, respectively (16, 34, 51). This sex-dependent dimorphism in the murine adrenals, with corticosterone and aldosterone values higher in females than in males, is in agreement with what found in rats and humans (1, 23, 25, 27, 36, 45, 56). This finding could be related to the higher mRNA levels of CYP21 (21-hydroxylase) and CYP11B2 (aldosterone synthase) found in the female adrenal, since these two enzymes regulate the last steroidogenic steps in the mouse adrenal gland (12).

Our results also show, for the first time, that ODC activity in the adrenals of female mice was considerably higher than that found in males, with testosterone being a major regulator. However, whereas in other rodent tissues such as mouse kidney (15, 28) or rat prostate (39) the enzyme was stimulated by androgens, in the mouse adrenal ODC activity was downregulated by testosterone. The molecular mechanisms, by which testosterone influences ODC expression, have not been characterized in detail. Although in mouse kidney (15, 28), rat (39), and human prostatic cells (4, 10) the transcription of ODC gene is stimulated by testosterone, probably by the binding of AR to an androgen-responsive element-like sequence located in the ODC promoter (15), our present results indicate that the action of testosterone on adrenal ODC activity appears to be exerted at the translational level, since no differences in adrenal ODC mRNA levels between sexes were observed, whereas the amount of ODC protein responsive to ODC antibodies was higher in the adrenal cortex of female mice. The almost identical level of expression of mRNAs of several ODC-related proteins such as AZ1, AZ2, AZI, and PMF found in the adrenal gland also support the contention that sex differences in ODC expression in the adrenal of mice are related mainly to the translational control. In this regard, translational and posttranslational mechanisms controlling ODC expression mediated by different factors, including androgens, have been reported in different cells and tissues (9, 49). Since ARs are expressed in the murine adrenal, the opposite responses of ODC to testosterone observed in kidney and adrenal of mice suggest that different tissue-specific mediators might be implicated in androgen action on ODC in these tissues. Since it is known that ACTH is a major regulator of adrenal ODC, it is possible that the effect of testosterone on adrenal ODC activity could be indirect as a consequence of the inhibitory action of androgens on hypothalamic corticotropin-releasing hormone release (11, 32) that may decrease ACTH secretion and its action on adrenal ODC induction. However, the inhibitory action elicited by testosterone on the induction of adrenal ODC by ACTH in HX mice supports the contention of a direct effect of testosterone on the mouse adrenal.

Regarding the physiological relevance of the sex-related differences in adrenal ODC activity reported here, the parallelism between adrenal ODC activity and gland size observed in CD1 mice would be in accordance with the fact that ODC is directly implicated in cell growth (37, 53). However, the apparent lack of effect of the inhibition of adrenal ODC activity on adrenal size would not support this possibility. This situation is similar to that found in the mouse kidney, where the renal hypertrophy elicited by androgens observed in males was
not affected by DFMO treatment despite the dramatic fall in renal ODC activity (54). Despite the fact that the decrease in polyamine content produced by DFMO treatment in the adrenal gland did not affect the gland size, our results clearly show that the DFMO treatment is associated with a marked decrease in catecholamine content and corticosteroid secretion in the gland adrenal, which suggests that the levels of ODC activity appear to be relevant for adrenal function. Moreover, the parallelism between adrenal ODC activity and corticoid secretion between males and females and the effect of DFMO on adrenal polyamine content and plasma corticoid levels support the contention that ODC activity, through its influence on polyamine levels, may be an important factor in steroid secretion.

On the other hand, although the experiments with DFMO support a certain role of ODC in the secretory functions of the medulla and adrenal cortex, the preferential localization and distribution of ODC in the cortex suggests a more complex role of ODC in the cortical region. The fact that the treatment with DFMO affected corticosterone more than aldosterone values, despite the fact that a higher ODC immunoreactivity was found in the capsule and zona glomerulosa, suggests that in these regions ODC may be implicated in cellular processes other than aldosterone synthesis and secretion. In this regard, although it is generally accepted that the primary function of the zona glomerulosa is the secretion of aldosterone, immunocytochemical studies have revealed that comparatively few glomerulosa cells contain aldosterone synthase (6, 57). Moreover, in recent years new information (57) has accumulated supporting other functions not directly related with steroid biosynthesis for cells of the zona glomerulosa. According to these claims, glomerulosa would be the major site of adrenal cell proliferation, although it has also been suggested that stem cells from the adrenal capsula may differentiate into adrenal cortex cells (21, 40). Since ODC and polyamine levels have been related with cell differentiation (20, 35), one may speculate that the reduction of the relatively higher values of ODC activity present in the capsula and zona glomerulosa of murine female adrenals produced by DFMO would decrease the rate of formation of cells in zona fasciculata and zona reticularis and, hence, could diminish corticosterone biosynthesis and secretion. The absence of expression of the AQ27 receptor in the mouse adrenal appears to exclude the possibility found in rats that aldosterone secretion may be regulated by the release of the hypothalamic peptide QRFP (17). The small difference found between sexes in the levels of mRNA of adrenal steroidogenic proteins and the lack of effect of DFMO treatment in these values suggest that the influence of the system ODC/polyamines on the steroidogenic proteins appears to be exerted at translational or posttranslational levels.

In conclusion, our findings demonstrate that in CD1 mice there is marked sexual dimorphism in the adrenal gland affecting not only polyamine metabolism but other parameters, such as gland size and secretory pattern. Furthermore, polyamine deprivation provokes a decrease in adrenal catecholamine levels and a reduction in the secretion of corticosterone, the major glucocorticoid in mice, and of the mineral corticoid aldosterone, suggesting a relevant role of the ODC/polyamine system in adrenal function. This sexual dimorphism may contribute to explain the differences observed between males and females with regard to different prevalent diseases and the response to stress.

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