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Androgen and estrogen stimulation of ornithine decarboxylase activity in mouse kidney

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Abstract

In the present work, the activity of mouse renal ornithine decarboxilase (ODC) from CBA female mice was used as a biological marker to detect (anti)androgenic activity of different groups of endocrine disruptors and steroids. Daily injections of testosterone or dihydrotestosterone (DHT) into 60 day old female mice for 4 days increased renal ODC activity in a dose-dependent manner that reached up to 100-fold (testosterone) or 250-fold (DHT) above the baseline when the highest dose, 200 μg/mouse, was used. Administration of flutamide concurrently with testosterone (75 μg/mouse) caused a potent decrease of ODC induction in a dose-dependent manner, suppressing the enzyme activity at the doses of 0.1 and 0.5 mg/mouse by about 88 and 95%, respectively. In contrast, estradiol at the doses of 0.5 and 1 mg/mouse induced a significant stimulation of renal ODC activity in a dose-dependent manner when it was given alone or in combination with testosterone. Using a sensitive increase in ODC activity in response to androgens as an end point, we did not detect an antiandrogenic effect of several antiandrogens, such as cyproterone acetate, spironolactone, *p,p′*DDE and vinclozolin. Also, none of these antiandrogens were able to change the basal level of renal ODC activity, with the exception of cyproterone acetate that at a dose of 0.1 mg/mouse stimulated ODC activity. The data obtained suggest that mouse renal ODC from CBA females is not strictly androgen-specific and cannot be used for estimation of antiandrogenic effects of compounds having an affinity to different types of receptors.

Keywords: Cyproterone acetate; Dihydrotestosterone; Estradiol; Mouse renal ornithine decarboxylase activity; *p,p′*-DDE; spironolactone; Testosterone; Vinclozolin

1. Introduction

It has been suggested that certain industrial pollutants, pesticides and fungicides in the environment, can impair male fertility and/or sexual development [1,2], and the main attention has been focused on environmental estrogens [3,4]. However, most of the suspected negative effects on male reproduction reported to be caused by environmental pollutants could be explained by a lack of androgenic effects on the male organism. Although estrogens at very high doses can have some antiandrogenic effects, it seems that more efforts should be made to search for possible environmental antiandrogens or androgens.

Thus, it is important to develop bioassays that are appropriate for detection of potential endocrine disruptors for (anti)androgenic activity. Mouse renal ornithine decarboxylase (ODC) is under testosterone control, and potent stimulation of the enzyme by androgens is well known [5]. Thus, this enzyme seems to be appropriate for the estimation of the (anti)androgenic effects of unknown compounds.

In this work, we have evaluated (anti)androgenic effects of different groups of endocrine disruptors and steroids in the kidney of female mice treated with exogenous testosterone, measuring renal ODC activity as an end point for detection of (anti)androgenic activity.

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2. Materials and methods

2.1. Reagents

[1-^{14}C]-L-Ornithine hydrochloride (1.92 GBq/mmol) was purchased from the Radiochemical Centre, Amersham, UK. Testosterone anhydrous, 17β-estradiol, flutamide, spironolactone, cyproterone acetate and L-ornithine were obtained from Sigma (St. Louis, MO). *p,p′*-DDE and vinclozolin were bought from Riedel-de Häen (Seelze, Germany).

2.2. Treatment of animals

Mature female mice of the CBA strain were obtained from the B&K Laboratories, Sollentuna, Sweden. They were fed a standard pellet diet and water ad libitum. The antiandrogens and steroids were dissolved in 10% (v/v) ethanol/sesame oil and were administered (alone or in combination with testosterone) for 4 days with daily subcutaneous injections. Control animals received sesame oil only.

2.3. Determination of ornithine decarboxylase activity

Twenty-four hours after the last injection of testosterone, the mice were killed, and the kidneys were rapidly removed and dissected free of fat and connective tissue. For enzyme assay, kidneys were homogenized in seven volumes (w/v) of ice-cold 0.1 M Tris–HCl buffer (pH 7.4 at 20°C), 2.5 mM dithiothreitol and 0.1 mM EDTA. The extracts were centrifuged at 15 000 *g* for 20 min at 4°C, and the supernatant was used to determine ornithine decarboxylase (ODC) activity. The extracts were incubated with 5 mM pyridoxal-5-phosphate, 25 mM L-ornithine and 0.05 μCi [1-^{14}C]-L-ornithine in a total volume of 1 ml. The tubes were capped with rubber stoppers equipped with polypropylene center wells (Kontes Glass Co., Vineland, NJ). After incubation for 45 min, the enzyme reaction was stopped by the injection of 0.5 ml of 2 M trichloroacetic acid through the stopper. The expelled $^{14}CO_2$ was trapped into 100 μl of hydroxide of Hyamine 10-x in the center well. Incubation was continued for another 45 min to allow the absorption of the released $^{14}CO_2$ by the Hyamine 10-x. The center wells were then transferred to counting vials with 3 ml of Emulsifier-safe scintillation fluid (Packard BioScience Co., Netherlands) and counted in a Beckman (LS 5000CE) scintillation counter. The enzyme activity was expressed as nmoles of released CO_2/mg protein/h.

Protein measurements were performed using the method of Bradford [6] with bovine serum albumin as the standard. Means in different experimental groups were compared using Student's *t*-test (*t*-independent). Significance in all cases was assumed with $P < 0.05$.

3. Results

Fig. 1 shows the effect of testosterone and dihydrotestosterone (DHT) on mouse renal ODC activity. Daily injections of any of these androgens for 4 days increased renal ODC activity in a dose-dependent manner. Treatment with testosterone at the highest dose, 200 μg/mouse, resulted in a 100-fold increase in renal ODC activity, while DHT at a dose of 100 and 200 μg/mouse caused a 250-fold increase in ODC activity (this steroid was about six times more potent than testosterone). The dose of testosterone, 75 μg/mouse, was chosen for all further experiments.

Fig. 2 represents the effect of a well-known non-steroidal antiandrogen flutamide [7] on renal ODC activity induced by testosterone in the mouse kidney. Flutamide has been shown to reduce ODC induction dramatically in a dose-dependent manner, acting as a very potent antiandrogen. Administration of flutamide concurrently with testosterone at the doses of 0.1 and 0.5 mg/mouse inhibited renal ODC activity by about 88 and 95%, respectively, while the highest dose, 1 mg/mouse, completely blocked renal ODC induction.

The action of 17β-estradiol on renal ODC activity in mice was also studied (Fig. 3), since antiandrogenic effects of estrogens at high doses are well known [8,9]. In view of previous observations that, in rats, the kidney requires a higher dose of estradiol than other organs to achieve a maximal increase in ODC activity [10], the doses of estradiol used were saturating. The doses were chosen to maximize the possible effect of estradiol on mouse renal ODC activity. 17β-Estradiol was found to induce stimulation of renal ODC activity in a dose-dependent manner when it was given alone or

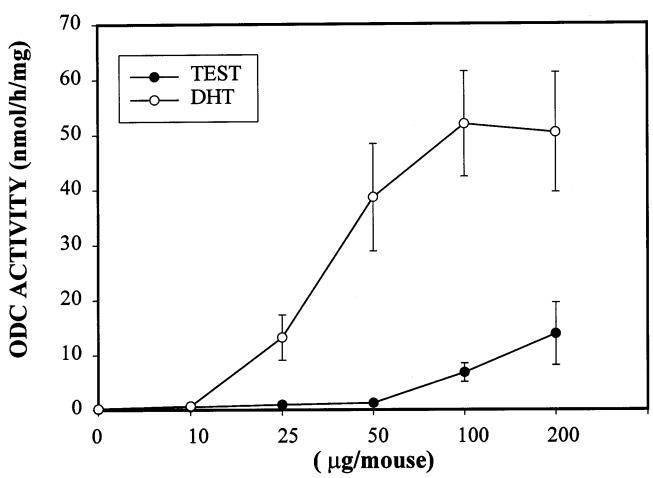

Fig. 1. Androgen-stimulated dose–response curve of mouse renal ODC activity. CBA females were given four daily s.c. injections of the androgens at the doses shown. The animals were sacrificed 24 h after the last treatment. The kidney was removed for the determination of ODC activity, as described in Section 2. Values are the mean ± S.E.M from four mice.

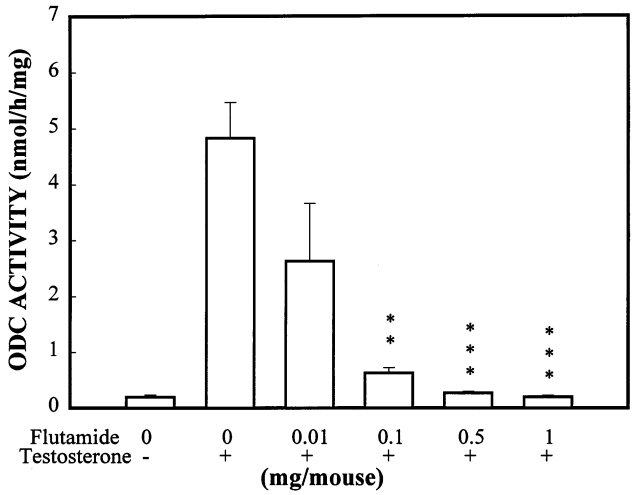

Fig. 2. Effect of flutamide on renal ODC activity induced by testosterone treatment of CBA mice. CBA females were given four daily s.c. injections of 75 μg of testosterone plus flutamide at the doses shown. The animals were sacrificed 24 h after the last treatment. The kidney was removed for the determination of ODC activity, as described in Section 2. Each bar shows the mean ± S.E.M for five or six animals. $^{**}P < 0.01$; $^{***}P < 0.001$ versus control.

in combination with testosterone. A synergistic effect of estrogen and androgen on renal ODC activity was established. The data support the fact that the mouse kidney contains estrogen receptors [11] and indicate that these can be involved in the regulation of ODC activity.

In the next experiments, different groups of antiandrogens were tested for their capacity to inhibit renal ODC activity induced by androgen. The results showed

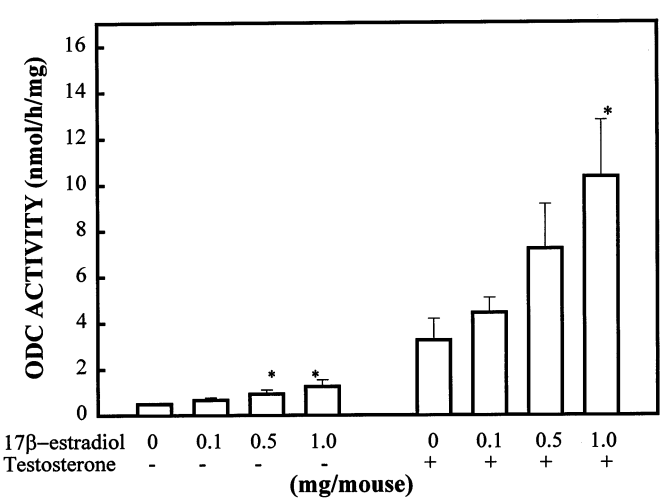

Fig. 3. Effect of 17β-estradiol on basal and testosterone-stimulated renal ODC activity in the mouse kidney. CBA females were given four daily s.c. injections of 17β-estradiol alone or plus 75 μg of testosterone at the doses shown. The animals were sacrificed 24 h after the last treatment. The kidney was removed for the determination of ODC activity, as described in Section 2. Each bar shows the mean ± S.E.M for five or six animals. $^{*}P < 0.05$ versus control.

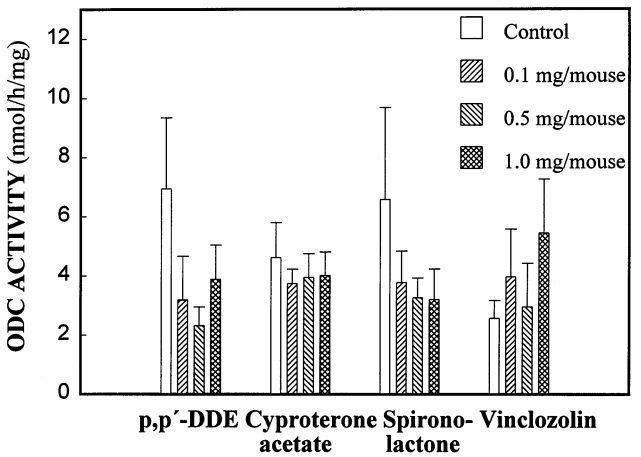

Fig. 4. Effect of different antiandrogens on testosterone stimulated renal ODC activity in mouse kidney. CBA females were given four daily s.c. injections of 75 μg testosterone plus the different antiandrogens at the doses shown. The animals were sacrificed 24 h after the last treatment. The kidney was removed for the determination of ODC activity, as described in Section 2. Each bar shows the mean ± S.E.M for five or six animals.

that none of the antiandrogens used produced a statistically significant change in renal ODC activity, even after 4 days of treatment (Fig. 4). Here, only *p,p′*DDE and spironolactone had a tendency to inhibit renal ODC activity stimulated by testosterone, but the effects were not significant.

In order to elucidate possible effects of the above antiandrogens on the basal level of renal ODC activity, each of the antiandrogens alone was given at the doses of 0.1 and 1 mg/mouse. Fig. 5 shows that none of the antiandrogens were able to change the basal level of

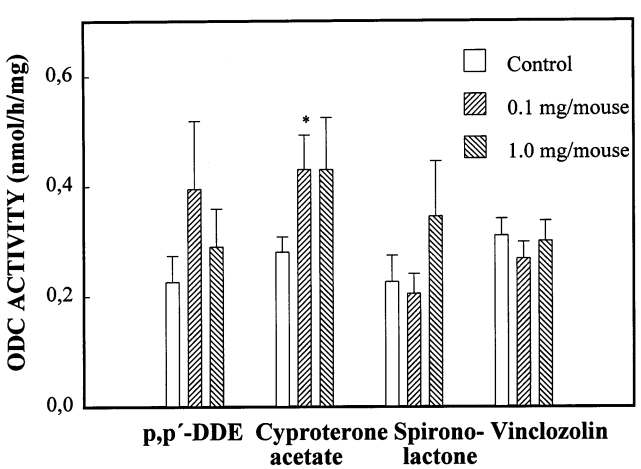

Fig. 5. Effect of different antiandrogens on the basal level of renal ODC activity in mouse kidney. CBA females were given four daily sc injections of the different antiandrogens at the doses shown. The animals were sacrificed 24 h after the last treatment. The kidney was removed for the determination of ODC activity, as described in Section 2. Each bar shows the mean ± S.E.M for five or six animals. $^{*}P < 0.05$ versus control.

renal ODC activity, with the exception of cyproterone acetate. At a dose of 0.1 mg/mouse, this antiandrogen produced significant stimulation of the basal renal ODC activity.

4. Discussion

The present study aimed at developing a sensitive in-vivo bioassay for the detection of (anti)androgenic activity of compounds using mouse renal ODC response to androgen as a biological marker. Mouse renal ODC has been shown to be extremely responsive to exogenous androgen [12,13], induction of the enzyme in this tissue has been considered to be specific for androgenic steroids [14], and functional androgen receptors are needed to stimulate the enzyme [14,15]. All these data suggested that mouse renal ODC activity could be an appropriate end point for the estimation of the (anti)androgenic properties of compounds. Using this approach, antiandrogenic effects of RU 486 [16] and RU 56187 [17] have been established. Our data have confirmed the earlier reports [13,14] that androgens can stimulate a large increase in mouse renal ODC activity in a dose-dependent manner. Androgen-induced renal ODC stimulation has been abolished in a dose-dependent manner, when the pure non-steroidal antiandrogen flutamide has been used in combination with testosterone. Inhibition of the androgen-induced increase in the mouse renal ODC activity by flutamide has been shown to occur via decreased accumulation of ODC-mRNA [18].

In contrast to flutamide suppression of testosterone-induced ODC activity in the mouse kidney, 17β-estradiol alone or in combination with testosterone produced a significant stimulation of the enzyme activity in a dose-dependent manner. This indicates that in this in-vivo setting, mouse renal ODC activation was not strictly specific for androgen, as was reported previously [13,17]. The potentiating effect of 17β-estradiol on the testosterone-stimulated mouse renal ODC activity is in contrast to the proposed antiandrogenic effect of estrogens [3]. Although mouse kidney contains estrogen receptors [11], mouse renal ODC activity did not change after treatment with estrogen in acute experiments [13], in contrast to a clear stimulation by estradiol in rat kidneys [10,19]. The results of the present investigation differ from those of Pajunen et al. [14], who found that estrogen had a slight antiandrogenic action when it was given concurrently with androgen. One possible explanation for these discrepancies is that another kind of androgen (5α-dihydrotestosterone) and different doses of androgen and estrogen were used in these experiments. In view of the fact that genetic factors (the strain of mice used) also influence ODC regulation in the mouse kidney [20], it cannot be excluded that the enzyme from CBA females is under control of both androgen and estrogen receptors, underlining the problems involved in extrapolating conclusions from one species to another. In retrospect, knowing the stimulatory effects of estradiol on ODC activity, it cannot be excluded that some of the testosterone effects might be due to aromatization of testosterone to estrogens. However, the observed strong effect of DHT, that cannot be aromatized, speaks strongly against such a hypothesis. In addition, 17β-estradiol could have a stimulatory effect on ODC activity, altering the metabolism of testosterone by stimulating the conversion of testosterone to dihydrotestosterone. This possibility should be examined in further experiments.

The present data have shown that except for flutamide, none of the antiandrogens used produced a marked change in androgen-stimulated mouse renal ODC activity, even after long-term treatment. With the exception of cyproterone acetate (which had a slight but significant androgenic, rather than antiandrogenic, effect), the known antiandrogens did not influence the basal level of the enzyme activity. In acute experiments, cyproterone acetate has been found not to stimulate mouse renal ODC activity but rather to abolish the acute effects of testosterone on the enzyme activity [13]. One of the possible reasons for the discrepancies between the studies might be the fact that in our experiments, treatment with cyproterone acetate was for a longer period of time. Furthermore, genetic factors might influence the ODC response. A dose-dependent androgenic activity of cyproterone acetate has been demonstrated when mouse renal β-glucuronidase was used as a marker for androgenicity [21].

Another important finding in our investigation is the lack of antiandrogenic action of spironolactone, vinclozolin and *p,p′*-DDE, when mouse renal ODC was used as a marker for androgenicity. The data indicate that these antiandrogens cannot reach a complete inhibition of the stimulating effect of androgen on renal ODC achieved with the pure antiandrogen flutamide, suggesting that they possess mixed agonistic–antagonistic activity. Spironolactone has been shown to be a partial androgen agonist–antagonist [22], possessing a significant level of androgenic activity, when androgen-sensitive cells were used as an end point for androgenicity. The fact that such well-known antiandrogens as *p,p′*-DDE and vinclozolin [2] did not significantly affect the androgen-stimulated mouse renal ODC activity suggests that the mode of antiandrogenic action of these endocrine disruptors may vary in different tissues. Furthermore, it should be taken into account that vinclozolin itself is a poor inhibitor, and its effects are mediated via the formation of active metabolites [23]. Thus, the poor inhibition found with vinclozolin may be due to incomplete convertion to the metabolically

active forms having antiandrogenic effects in CBA mouse kidney.

Thus, the present data demonstrate that in CBA female mice, renal ODC response is not strictly androgen-specific and cannot be used for estimation of overall antiandrogenic effects. The only compound (among those tested) that has a clear, potent antiandrogenic effect is the pure non-steroidal antiandrogen flutamide. Furthermore, the present results underline the problems inherent to extrapolation of the effects of suspected endocrine disruptors from one tissue to another, and from acute to chronic exposure.

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