Effect of cyproterone acetate on alpha1-adrenoceptor subtypes in rat vas deferens

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Abstract

Gonadal hormones regulate the expression of α₁-adrenoceptor subtypes in several tissues. The present study was carried out to determine whether or not cyproterone acetate, an anti-androgenic agent, regulates the α₁-adrenoceptor subtypes that mediate contractions of the rat vas deferens in response to noradrenaline. The actions of subtype selective α₁-antagonists were investigated in vas deferens from control and cyproterone acetate-treated rats (10 mg/day, sc, for 7 days). Prazosin (pA₂ = 9.5), phentolamine (pA₂ = 8.3) and yohimbine (pA₂ = 6.7) presented competitive antagonism consistent with activation of α₁-adrenoceptors in vas deferens from both control and treated rats. The pA₂ values estimated for WB 4101 (≈ 9.5), benoxathian (≈ 9.7), 5-methylurapidil (≈ 8.5), indoramin (≈ 8.7) and BMY 7378 (≈ 6.8) indicate that α₁A-adrenoceptors are involved in the contractions of the vas deferens from control and cyproterone acetate-treated rats. Treatment of the vas deferens from control rats with the α₁B/α₁D-adrenoceptor alkylating agent chloroethylclonidine had no effect on noradrenaline contractions, supporting the involvement of the α₁A-subtype. However, this agent partially inhibited the contractions of vas deferens from cyproterone acetate-treated rats, suggesting involvement of multiple receptor subtypes. To further investigate this, the actions of WB 4101 and chloroethylclonidine were reevaluated in the vas deferens from rats treated with cyproterone acetate for 14 days. In these organs WB 4101 presented complex antagonism characterized by a Schild plot with a slope different from unity (0.65 ± 0.05). After treatment with chloroethylclonidine, the complex antagonism presented by WB 4101 was converted into classical competitive antagonism, consistent with participation of α₁A-adrenoceptors as well as α₁B-adrenoceptors. These results suggest that cyproterone acetate induces plasticity in the α₁-adrenoceptor subtypes involved in the contractions of the vas deferens.

Introduction

Three different α₁-adrenoceptor subtypes, α₁A-, α₁B- and α₁D-adrenoceptors, have been cloned (1). Several drugs interact selectively with one or more of these subtypes in functional and/or radioligand binding studies (2). Additional α₁-adrenoceptor heterogeneity has been suggested by functional studies in which prazosin showed low potency in inhibiting

Key words
• Alpha1-adrenoceptors
• Vas deferens
• Cyproterone acetate
contractions of certain vascular tissues in response to adrenergic agonists (2). However, prazosin also shows low potency for inhibition of [3H]-inositol phosphate formation in cell lines expressing each of the human α1A-adrenoceptor splice variants, suggesting that these additional subtypes may represent low affinity state(s) of the α1A-adrenoceptors and not a different protein encoded by a different gene (3,4).

Although some information is available concerning the mechanisms that regulate acute expression of α1-adrenoceptors such as desensitization and internalization (5), much less is known about the factors that regulate the expression of α1-adrenoceptor subtypes for longer periods of time. However, some studies have shown that gonadal hormones differentially regulate the expression of α1-adrenoceptor subtypes in several tissues (6-12). For instance, previous data from our laboratory have shown that castration changes the α1-adrenoceptor subtypes involved in the contractions of the rat vas deferens in response to noradrenaline (8) and that testosterone replacement treatment of castrated rats prevents this plasticity (12).

The present study was carried out to determine whether or not cyproterone acetate, an anti-androgenic drug, induces such plasticity in the α1-adrenoceptor subtypes involved in the contractions of the rat vas deferens in response to noradrenaline. To this end, we determined the actions of α1-selective antagonists against the contractions induced by noradrenaline in vas deferens from cyproterone acetate-treated and control untreated rats.

Material and Methods

Cyproterone acetate treatment and vas deferens isolation

Male Wistar rats weighing 280-360 g (16-20 weeks old) were treated with 10 mg/day cyproterone acetate (sc) for 7 or 14 days and were killed at the end of the treatment. The vasa deferentia from cyproterone acetate-treated and age-matched control rats (untreated) were excised, separated from surrounding tissue, weighed, and immediately mounted for contractility studies. To check the anti-androgenic action of the treatment, the wet weights of the ventral prostate and seminal vesicle were also determined. Previous experiments using larger doses of cyproterone acetate (up to 35 mg/day for 7 days) did not induce additional significant wet weight reductions, suggesting that 10 mg/day was the maximal effective dose (measurements on 5 rats, P > 0.05). The experimental procedures were approved by the Ethics Committee for Animal Research from UNESP, Botucatu, SP, Brazil.

Functional studies

For the study of contractility the vasa deferentia were mounted under 9.80 mN of tension in 10 ml organ baths containing a nutrient solution of the following composition: 138 mM NaCl, 5.7 mM KCl, 1.8 mM CaCl2, 0.36 mM NaH2PO4, 15 mM NaHCO3, and 5.5 mM dextrose, prepared in glass-distilled, deionized water and maintained at 30ºC, pH 7.4 (8). Vasa deferentia from control or cyproterone acetate-treated rats were equilibrated for 30 min before the beginning of the experiments. After this period, two or three cumulative concentration-response curves for noradrenaline were obtained, and then 6 µM cocaine, 10 µM corticosterone and 0.1 µM propranolol were added to the organ bath in order to block neuronal and extraneuronal uptake and β-adrenoceptor, respectively. The interval between concentration-response curves was 45 min. Competitive antagonists were added to the bath 45 min before and during the contractile responses to noradrenaline. After incubation with 100 µM chloroethylclonidine for 45 min the preparation was washed repeatedly (at least ten times) for 30 min before the
concentration-response curve to noradrenaline.

**Calculation of pA₂ values**

The pA₂ values for the competitive antagonists were calculated by Schild regression analysis (13). The ratios between the half-maximal concentrations of noradrenaline (concentration-ratios, r) were calculated only when the maximal amplitude of the concentration-response curve in the presence of the competitive antagonists was similar to that obtained in its absence. Data were plotted as log antagonist concentrations (M) vs log (r - 1). For calculation purposes the slope parameter was constrained to 1.0 when not statistically different from unity.

**Drugs**

Drugs were obtained from the following sources: cyproterone acetate (Androcur®; Berlimed, São Paulo, SP, Brazil, or Galena, Campinas, SP, Brazil); cocaine (Cocainum Hydrochloricum puriss.; C.H. Boehringer, Ingelheim, Germany). Corticosterone and noradrenaline [(±)-arterenol HCl] were from Sigma (St. Louis, MO, USA). Benoxathian HCl, chloroethylclonidine 2 HCl, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4,5] decane-7, 9-dione dihydrochloride), 5-methylurapidil HCl, prazosin HCl, phentolamine HCl, (±)-propranolol HCl, WB 4101 (2-(2,6-dimethoxyphenoxymethyl) aminomethyl-1, 4-benzodioxane hydrochloride), and yohimbine HCl were from Research Biochemicals Inc. (RBI/Sigma), Natick, MA, USA. Indoramin hydrochloride was a gift from Wyeth-Fontoura (São Paulo, SP, Brazil). Drugs were dissolved in distilled water or 1 mM dimethylsulfoxide, kept frozen and discarded after 20 days. Noradrenaline was dissolved in 10 mM HCl each day shortly before the experiments. Cyproterone acetate was solubilized in soybean oil.

**Statistical analysis**

All data are reported as means ± SEM for N experiments. Differences between mean values were tested for statistical significance (P < 0.05) using paired or unpaired Student t-tests or analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple comparisons.

**Results**

**Effects of treatment with cyproterone acetate for 7 days on the wet weight of the vas deferens, ventral prostate and seminal vesicle**

Since no significant effect of cyproterone acetate on total rat weight was observed, a direct comparison of the wet weight of the vas deferens, ventral prostate and seminal vesicle was used to determine the efficacy of the anti-androgenic treatment. The treatment induced significant reductions of the wet weights of the vas deferens, ventral prostate and seminal vesicle (Table 1). The wet weights of the same organs from surgically castrated rats (orchiectomized 7 days before) are shown in Table 1 for comparison. Surgical castration induced more marked reductions of the weights of the organs (P < 0.05).

**Effects of noradrenaline on vas deferens from control rats and from rats treated with cyproterone acetate for 7 days**

Nearly rhythmic spontaneous contractions of low magnitude were observed in a few vasa deferentia from rats treated with cyproterone acetate for 7 days, while none of the organs from control rats presented spontaneous contractions.

Noradrenaline induced concentration-dependent contractions of the vas deferens from control rats and from rats treated with cyproterone acetate for 7 days (Figure 1). The maximal contraction induced by nor-
adrenaline in the vas deferens from cyproterone acetate-treated rats (19.7 ± 0.5 mN, N = 24) was significantly lower than in vas deferens from control rats (23.2 ± 0.5 mN, N = 31; P < 0.05). However, the potency of noradrenaline (assessed by the pD2 values) in the vas deferens from control rats (7.0 ± 0.1, N = 31) was not different from that in vas deferens from cyproterone acetate-treated rats (7.0 ± 0.1, N = 24).

Effects of selective α-adrenoceptor antagonists on noradrenaline contractions

The concentration-response curves for noradrenaline applied to the vas deferens from control rats and from rats treated with cyproterone acetate for 7 days were competitively antagonized by prazosin (α1-selective), phentolamine (non-α1/α2-selective) and yohimbine (α2-selective) as characterized by the slopes in the Schild plots not different from unity and by the lack of effect of these antagonists on the maximal responses to noradrenaline (Figure 2 and Table 2). The rank order of potency found for these antagonists (prazosin > phentolamine > yohimbine) indicates that the contractions induced by noradrenaline in vas deferens from control and cyproterone acetate-treated rats are mediated by α1-adrenoceptors.

Effects of subtype-selective ααααα-adrenoceptor antagonists on noradrenaline contractions

In vas deferens from control rats and from rats treated with cyproterone acetate for 7 days, the antagonists WB 4101 and benoxathian (α1A/α1D-selective), indoramin and 5-methylurapidil (α1A-selective), and BMY 7378 (α1D-selective) inhibited noradrenaline contractions showing competitive antagonisms (Figure 3 and Table 2). The slopes of the Schild plots for these antagonists did not differ from unity.

Effect of chloroethylclonidine on noradrenaline contractions

The incubation of vas deferens from control rats with the α1B/α1D-adrenoceptor alkylating agent chloroethylclonidine (100 µM, 45 min) resulted in no significant change in the concentration-response curves for noradrenaline (Figure 4A, Table 3). However, this same treatment resulted in a 3-fold rightward shift in the concentration-response curve for noradrenaline in vas deferens from cyproterone acetate-treated rats associated with an ≈30% reduction of the maximal response (Figure 4B, Table 3).

Effects of longer cyproterone acetate treatments on tissue wet weight and on the antagonism of noradrenaline by WB 4101 and chloroethylclonidine

The fact that chloroethylclonidine was effective on the vas deferens from rats treated with cyproterone acetate for 7 days sug-
Figure 2. Concentration-response curves for noradrenaline in the absence and presence of increasing concentrations of prazosin (A and B), phentolamine (C and D) and yohimbine (E and F) in vas deferens from control (A, C and E) and cyproterone acetate-treated rats (B, D and F). The Schild plots obtained for prazosin, phentolamine and yohimbine are presented in G. Each symbol indicates the mean and the vertical line, when larger than the symbol, the SEM of 4-12 determinations for 4 to 6 rats in each group. When appropriate, the rats received cyproterone acetate (10 mg/day, sc) for 7 days.
Table 2. Effect of cyproterone acetate treatment on the pA2 and slope values of α-adrenoceptor antagonists against noradrenaline-induced contractions of vas deferens.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Control</th>
<th>Cyproterone acetate</th>
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<tbody>
<tr>
<td></td>
<td>pA2</td>
<td>slope</td>
</tr>
<tr>
<td>Prazosin</td>
<td>9.51 ± 0.19</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>8.25 ± 0.10</td>
<td>1.14 ± 0.06</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>6.67 ± 0.24</td>
<td>0.99 ± 0.07</td>
</tr>
<tr>
<td>WB 4101</td>
<td>9.61 ± 0.03</td>
<td>1.01 ± 0.03</td>
</tr>
<tr>
<td>Benoxathian</td>
<td>9.83 ± 0.04</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>Indoramin</td>
<td>8.79 ± 0.07</td>
<td>0.92 ± 0.06</td>
</tr>
<tr>
<td>5-Methylurapidil</td>
<td>8.58 ± 0.06</td>
<td>0.89 ± 0.06</td>
</tr>
<tr>
<td>BMY 7378</td>
<td>6.89 ± 0.05</td>
<td>1.11 ± 0.07</td>
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Rats received cyproterone acetate (10 mg/day, sc) for 7 days. Data are reported as means ± SEM of 4 to 12 determinations on tissue from 4 to 6 rats in each group.

Discussed that the anti-androgenic treatment was not of long enough duration to allow detection of alterations in the actions of α1-adrenoceptor selective antagonists such as WB 4101. Therefore, the actions of WB 4101 were determined against the contractions induced by noradrenaline in vas deferens from rats treated with cyproterone acetate for 14 days. Treatment of the rats with cyproterone acetate for 14 days induced more marked reductions in the wet weights of the vas deferens (47 ± 1.0 mg, N = 5; P < 0.05), ventral prostate (98 ± 3.0 mg, N = 5; P < 0.05) and seminal vesicle (115 ± 4.0 mg, N = 5; P < 0.05) than the treatment for 7 days (Table 1). Noradrenaline induced concentration-dependent contractions in vas deferens from rats treated with cyproterone acetate for 14 days with the same potency (pD2 = 7.2 ± 0.2, N = 5) as observed in vas deferens from control rats or from rats treated with cyproterone acetate for 7 days. However, the maximal contraction induced by noradrenaline in vas deferens from rats treated with cyproterone acetate for 14 days (17.7 ± 0.5 mN, N = 5) was smaller than that induced in vas deferens from control rats but not different from that induced in vasa from rats treated for 7 days. The antagonist WB 4101 also presented competitive antagonism of the contractions induced by noradrenaline in vas deferens from rats treated with cyproterone acetate for 14 days, as indicated by the slope of the Schild plot not different from the theoretical unity (pA2 = 9.31 ± 0.10, slope = 0.88 ± 0.05; see the solid line in Figure 5B). However, a closer inspection of this antagonism shows that the slope of the line in the Schild plot did not differ from the theoretical unity only if a relatively wide range of antagonist concentrations (1 to 100 nM) was considered. Alternatively, if only the first four concentrations of WB 4101 were used to construct the Schild plot a slope of 0.65 ± 0.05 was obtained, which was smaller than 1.0 (P > 0.05; see the broken line in Figure 5C).

In order to determine whether this complex antagonism presented by WB 4101 and the effectiveness of chloroethylclonidine share common mechanisms, the actions of WB 4101 were reevaluated against the contractions in response to noradrenaline that were resistant to this alkylating agent (Figure 5A). The antagonism presented by WB 4101 after chloroethylclonidine treatment is consistent with classic competitive antagonism regardless of the concentrations of antagonist used to construct the Schild plot (pA2 = 9.54 ± 0.04; slope = 1.08 ± 0.03, N = 5; Figure 5). This suggests that the complex antagonism presented by WB 4101 is related to a component of the response to noradrenaline which is sensitive to chloroethylclonidine.

Discussion

We investigated the effects of treatment with the anti-androgen cyproterone acetate on α1-adrenoceptor subtypes involved in the contractions of the rat vas deferens in response to noradrenaline using subtype-selective competitive antagonists. Treatment of the rats with cyproterone acetate reduced the wet weights of the vas deferens, ventral prostate and seminal vesicle as expected according to the anti-androgenic action of this drug. However, surgical castration induced
Figure 3. Concentration-response curves for noradrenaline in the absence and presence of increasing concentrations of WB 4101 (A and B), benoxathian (D and E), indoramin (G and H), 5-methylurapidil (J and K), and BMY 7378 (M and N), in vas deferens from control (A, D, G, J and M) and cyproterone acetate-treated rats (B, E, H, K and N). The Schild plots obtained for WB 4101, benoxathian, indoramin, BMY 7378, and 5-methylurapidil are presented in C, F, I, L, and O, respectively. Each symbol indicates the mean and the vertical line, when larger than the symbol, the SEM of 4-12 determinations for 4 to 6 rats in each group. The rats received cyproterone acetate (10 mg/day, sc) for 7 days.
more pronounced reductions in the wet weights of these organs, showing that treatment with cyproterone acetate had only a partial anti-androgenic action in comparison to bilateral orchietomy.

Cyproterone acetate treatment reduced the maximal contraction induced by noradrenaline in the vas deferens without changing the potency of this agonist, as indicated by the pD2 values. Interestingly, cyproterone acetate treatment resulted in atrophy of the vas deferens as characterized by the loss of approximately 20% of the wet weight of the organ. Therefore, it is tempting to associate

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**Table 3. Effects of chloroethylclonidine on pD2 and maximal contractions in response to noradrenaline acting on vas deferens from control and cyproterone acetate-treated rats.**

<table>
<thead>
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<th></th>
<th>Control</th>
<th>Cyproterone acetate</th>
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<tbody>
<tr>
<td></td>
<td>pD2</td>
<td>Emax (%)</td>
</tr>
<tr>
<td>Before treatment</td>
<td>7.1 ± 0.1</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>After treatment</td>
<td>7.0 ± 0.1</td>
<td>95 ± 9</td>
</tr>
</tbody>
</table>

The tissue was treated with 100 µM chloroethylclonidine for 45 min and extensively washed (at least ten times) before the measurement. Data are reported as means ± SEM of 5 to 8 determinations on tissue from 5 to 8 rats in each group. The rats were treated with cyproterone acetate (10 mg/day, sc) for 7 days. Emax is reported as percent of maximal response before chloroethylclonidine treatment.

<sup>a</sup>P < 0.05 compared to the respective value before treatment (Student t-test).
the reduction in the maximal contraction in response to noradrenaline with atrophy of the organ. However, other factors may be involved. For example, it is known that treatment of the rats with cyproterone acetate induces a drastic down-regulation of L-type voltage-dependent calcium channels in the vas deferens (14), as does bilateral orchiectomy (15). This may contribute to the reduced responsiveness of the vas deferens to noradrenaline since the contractions induced by this agonist are dependent on calcium influx through L-type voltage-dependent calcium channels (16,17).

The actions of a series of subtype-selective antagonists were determined against the contractions induced by noradrenaline to determine the effects of cyproterone acetate treatment on $\alpha_1$-adrenoceptor subtypes. The results obtained with these reversible competitive antagonists indicate that noradrenaline-induced contractions in vas deferens from both control and cyproterone acetate-treated rats are due to the activation of $\alpha_{1A}$-adrenoceptors as judged by the high $pA_2$ values found for the $\alpha_{1A}$-selective antagonists WB 4101, 5-methylurapidil and indoramin. In addition, the low potency shown by the $\alpha_{1D}$-selective antagonist BMY 7378 against the contractions induced by noradrenaline in both organs further indicates that $\alpha_{1A}$-adrenoceptors are involved in this functional response. The conclusion that $\alpha_{1A}$-adrenoceptors are involved in the contractions of the vas deferens agrees with previous studies from this and other laboratories (8,16-19). Interestingly, the $\alpha_{1A}$-adrenoceptor is not the only $\alpha_1$-subtype expressed in the rat vas deferens since radioligand binding experiments have detected the presence of $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors (20-22) and mRNA species for $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-subtypes have been detected in this organ (23-25, and Pupo AS and Avellar MCW, unpublished observations). The roles of the $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor subtypes in the rat vas deferens remain to be established.

The lack of effect of chloroethylclonidine on vas deferens from control rats also supports the involvement of $\alpha_{1A}$-adrenoceptors in these contractions. However, chloroethylclonidine inhibited, at least in part, the contractions induced by noradrenaline in vas deferens from cyproterone acetate-treated rats. Since chloroethylclonidine alkylates $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptors, this result suggests that multiple subtypes may participate in the contractions of the vas deferens from cyproterone acetate-treated rats. This led us to test the effects of longer treatments with cyproterone acetate. WB 4101 showed complex antagonism against the contractions of the vas deferens from rats treated with cyproterone acetate for 14 days in response to noradrenaline. The complex antagonism of WB 4101 was observed with the effects of the concentrations of 1 to 30 nM which resulted in a slope parameter in the Schild plot much lower than theoretical unity, also indicating that mixed receptor populations may be involved in the contractions induced by noradrenaline. Accordingly, after treatment with chloroethylclonidine of the vas deferens from rats treated with cyproterone acetate for 14 days, the complex antagonism presented by WB 4101 was converted into classical competitive antagonism resulting in a high $pA_2$ consistent with the interaction of this antagonist with $\alpha_{1A}$-adrenoceptors.

Previous studies have shown that the expression of $\alpha_1$-adrenoceptor subtypes is differentially regulated by gonadal hormones. We have observed that bilateral orchiectomy induces a plasticity similar to that described in the present study (8) and that testosterone replacement treatment of castrated rats prevents its appearance (12). Recently, Homma et al. (9) observed a specific down-regulation of the mRNA for $\alpha_{1A}$-adrenoceptors associated with a reduced potency of phentolamine in the rat prostate after androgen deprivation by surgical castration. Sexual maturation, and supposedly the accompanying increase in plasma testosterone levels,
reduces the mRNA for $\alpha_{1A}$-adrenoceptors in the caput epididymis and increases the mRNA for $\alpha_{1D}$-adrenoceptors in the cauda epididymis of the rat (10). Estradiol, on the other hand, has been shown to increase selectively the expression of $\alpha_{1B}$-adrenoceptor binding sites and signaling in the hypothalamus and preoptic area of the female rat (6,7,11).

The results presented here indicate that the contractions of the vas deferens from control rats in response to noradrenaline are mediated by $\alpha_{1A}$-adrenoceptors. However, in addition to $\alpha_{1A}$-adrenoceptors, the $\alpha_{1B}$-subtype also participates in the contractions of the vas deferens from cyproterone acetate-treated rats, indicating that this anti-androgen induces some plasticity in the functional $\alpha_{1}$-adrenoceptor subtypes in the rat vas deferens.

Acknowledgments

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