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Effects of castration and of testosterone replacement on α_1-adrenoceptor subtypes in the rat vas deferens

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Abstract

The contractions of the rat vas deferens in response to noradrenaline are mediated through α_{1A}-adrenoceptors. We observed participation of α_{1B}-adrenoceptors in these contractions after castration. We now investigated the time course of this plasticity and the effects of testosterone by determining the actions of competitive antagonists on noradrenaline-induced contractions after 7, 14, 21 and 30 days of castration. BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) antagonised noradrenaline-induced contractions in control and castrated rats with low pA_2 values ($\cong$6.8). In control vas deferens, WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) had a slope in the Schild plot no different from 1.0, while slopes lower than 1.0 ($\approx$ 0.6) were observed for vas deferens from castrated rats. Chloroethylclonidine was ineffective in the control vas while it inhibited noradrenaline-induced contractions in vasa from castrated rats and converted the complex antagonism by WB 4101 into simple competitive antagonism. Treatment of castrated rats with testosterone prevented the effects of castration. The results suggest that α_{1B}-adrenoceptors are detectable in vas deferens from at least the 7th through the 30th day after castration and that testosterone prevents this plasticity.

Keywords: α_1-Adrenoceptor; Vas deferens; (Rat); Castration; Testosterone

1. Introduction

Three different α_1-adrenoceptor subtypes have been cloned and named α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (for review, see Zhong and Minneman, 1999). The existence of three different α_1-adrenoceptors has also been evidenced pharmacologically since several drugs have been shown to interact selectively with one or more of these subtypes in functional and or radioligand binding studies (for review, see Docherty, 1998). Additional α_1-adrenoceptor heterogeneity is suggested by functional studies in which prazosin shows low potency to inhibit contractions of certain vascular tissues in response to adrenoceptor agonists. However, prazosin also shows low potency for inhibition of [^{3}H]inositol phosphates formation in cell lines expressing human α_{1A}-adrenoceptors, suggesting that these additional subtypes may represent low affinity state(s) of the α_{1A}-adrenoceptors and not a separate protein encoded by a different gene (Ford et al., 1997; Daniels et al., 1999).

The α_1-adrenoceptor subtype involved in the contractions of the rat vas deferens in response to adrenoceptor agonists has been identified as α_{1A}-adrenoceptors. This is based on the high potencies shown by selective antagonists such as prazosin, WB 4101 and 5-methylurapidil, the low potency of the α_{1D}-adrenoceptor-selective antagonist, BMY 7378, and on the absence of effect of the α_{1B}/α_{1D}-adrenoceptor alkylating agent, chloroethylclonidine (Aboud et al., 1993; Burt et al., 1995; Pupo, 1998; Pupo et al., 1999). However, we found a significant participation of α_{1B}-adrenoceptors in the contractions in response to noradrenaline after 30-day castration. This was indicated by the effectiveness of chloroethylclonidine to inhibit these contractions, by the complex antagonism shown by WB 4101, and by the fact that after treatment of the vas deferens from castrated rats with chloroethylclonidine the complex antagonism by WB 4101 was converted into classical competitive antagonism (Pupo, 1998). Therefore, the α_1-adrenoceptors involved in the contractions of the rat vas deferens are subject to some degree of plasticity.

In the present study, we further investigated the plasticity induced by castration in the α_1-adrenoceptor subtypes in the rat vas deferens by determining the time course of the effects of castration and the effects of testosterone replace-

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ment in castrated rats. To this end, the actions of selective competitive antagonists were investigated in vas deferens from rats castrated for 7, 14, 21 and 30 days, and in 7 days castrated rats treated with testosterone.

2. Methods

2.1. Castration, testosterone replacement and vas deferens isolation

Male Wistar rats weighing between 280 and 360 g (16–20 weeks old) were maintained under ether anaesthesia and a 2-cm suprapubic incision was done. The testes were localised, major vessels were ligated to prevent bleeding, and bilateral orchidectomy was done. The incision was sutured and the animals were killed by ether inhalation 7, 14, 21 or 30 days after castration. Some castrated rats received testosterone propionate (1 mg/day, s.c.) for 7 days starting on the day of the surgery and were killed at the end of the treatment. The vasa deferentia were excised, cleaned from surrounding tissues, weighed, and immediately set up for contractile studies. The experimental procedures were approved by the *Ethics Committee for the Use of Experimental Animals* from UNESP-Botucatu.

2.2. Functional studies

For contractile studies, the vasa deferentia were set up under 9.80-mN tension in 10-ml organ baths containing a nutrient solution of the following composition (mM): NaCl 138; KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15, dextrose 5.5, prepared in glass-distilled, deionized water and maintained at 30 °C, pH 7.4. The bath solution was set at 30 °C because, at this temperature, both frequency and amplitude of the spontaneous contractions of vas deferens from castrated rats were lower than those at 37 °C (Pupo, 1998). Vas deferens from control or castrated rats was equilibrated for 30 min before the start of the experiments. After this period, two or three cumulative concentration–response curves for noradrenaline were obtained, and then cocaine (6 μM), corticosterone (10 μM) and propranolol (0.1 μM) were added to the incubate in order to block neuronal and extraneuronal uptake and β-adrenoceptors, respectively. The interval between each concentration–response curve was 45 min. Competitive antagonists were incubated 45 min before and during the contractile responses to noradrenaline. Chloroethylclonidine (100 μM) was incubated for 45 min and at the end of this period, the preparation was washed repeatedly (at least 10 times) for 30 min before the concentration–response curve to noradrenaline.

Part of the results obtained with vas deferens from rats castrated for 30 days have been published (Pupo, 1998) and are included to facilitate comparisons.

2.3. Data analysis

The pA_2 values for competitive antagonists were calculated by Schild regression analysis (Arunlakshana and Schild, 1959). 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 compet-

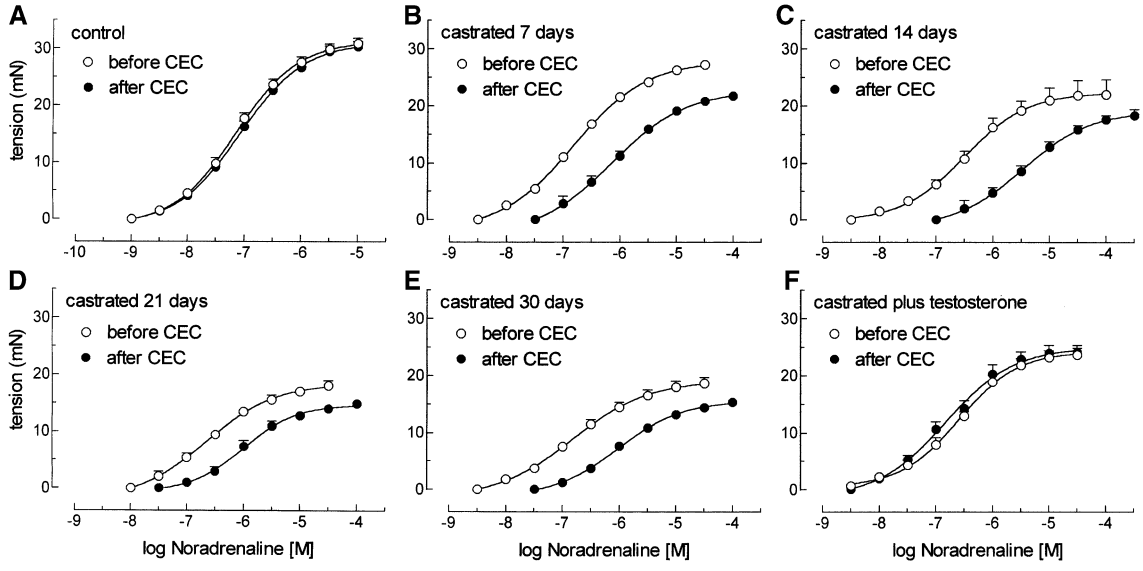

Fig. 1. Concentration–response curves for noradrenaline before (○) and after (●) chloroethylclonidine (CEC 100 μM, 45 min) in vas deferens from control rats (A) and from rats castrated for 7 (B), 14 (C), 21 (D) and 30 days (E), and from castrated rats treated with testosterone propionate (1 mg/day) (F). Each symbol represents the mean and the vertical line, when greater than the symbol, the S.E.M. of five to eight experiments. Data for rats castrated for 30 days were from Pupo (1998).

Table 1
pD_2 values and maximal effects (E_{max}, in mN)[a] for noradrenaline in vas deferens from control and castrated rats before and after chloroethylclonidine treatment (CEC, 100 μM, 45 min)

	pD_2			E_{max} (mN)		
	Before CEC	After CEC	Ratio[b]	Before CEC	After CEC	Inhibition (%)[c]
Control	7.15 ± 0.09	7.08 ± 0.08	–	30.80 ± 1.67	30.25 ± 1.37	–
Castrated 7 days	6.80 ± 0.06[d]	6.14 ± 0.07[e]	4.6	26.93 ± 0.70[d]	21.69 ± 0.60[e]	19
Castrated 14 days	6.50 ± 0.06[d]	5.47 ± 0.05[e]	10.7	21.94 ± 2.70[d]	18.26 ± 1.18	–
Castrated 21 days	6.61 ± 0.05[d]	6.06 ± 0.08[e]	3.6	18.02 ± 0.97[d]	14.83 ± 0.42[e]	18
Castrated 30 days	6.83 ± 0.06[d]	6.03 ± 0.05[e]	6.3	18.61 ± 1.12[d]	15.30 ± 0.37[e]	18
Castrated + testosterone	6.60 ± 0.08[d]	6.83 ± 0.07	–	23.66 ± 1.14[d]	24.02 ± 1.34	–

[a] Each value represents the mean and the S.E.M. of five to eight experiments.
[b] Antilog (pD_2 before − pD_2 after CEC).
[c] Percent inhibition of the E_{max} induced by CEC treatment.
[d] Different from the respective value found in vas deferens from control rats ($P<0.05$).
[e] Different from the respective value found before CEC treatment ($P<0.05$).

itive 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 statistically no different from unity.

All values are shown as means ± standard error of mean (S.E.M.) of n experiments. Differences between mean values were tested for statistical significance ($P<0.05$) using Student's paired or unpaired t-tests or analysis of variance (ANOVA) followed by Newmann–Keuls test for multiple comparisons.

2.4. Drugs

Drugs were obtained from the following sources: cocaine (Cocainum Hydrochloricum puriss., C.H. Boehringer, Germany); corticosterone, noradrenaline [(±)-arterenol HCl]; testosterone propionate from Sigma, USA; BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]-decane-7,9-dione dihydrochloride), chloroethylclonidine 2 HCl, prazosin HCl, (±)-propranolol HCl; WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), yohimbine HCl from Research Biochemicals, Natick, MA, USA Drugs were dissolved in distilled water or dimethylsulfoxide (1 mM), kept frozen and discarded after 20 days. Noradrenaline solutions were dissolved in 0.01 N HCl each day shortly before the experiments. Testosterone propionate was dissolved in corn oil.

3. Results

3.1. Vasa deferentia wet weights and noradrenaline-induced contractions

There were significant and time-dependent reductions in the wet weights of vasa deferentia after castration ($P<0.05$). These wet weights decreased from 76.8 ± 2.3 mg in control vasa ($n=50$) to 59.7 ± 1.5 mg after 7 days ($n=38$); 44.6 ± 2.4 mg after 14 days ($n=44$), 37.6 ± 1.2 mg after 21 days ($n=46$) and 31.6 ± 1.1 mg after 30 days ($n=50$). The wet weight of the vas deferens from castrated rats treated with testosterone propionate was similar to that of the vas from control rats (82.1 ± 4.5 mg; $n=24$).

Noradrenaline induced concentration-dependent contractions of vas deferens from control rats, from rats castrated for 7, 14, 21 and 30 days and from castrated rats treated with testosterone propionate (Fig. 1). The potency of (pD_2), and maximal contraction (E_{max}) induced by noradrenaline were reduced after 7, 14, 21 and 30 days of castration (Fig. 1; Table 1). Noradrenaline also had a reduced potency and induced a smaller maximal contraction in the vas deferens of

Table 2
pA_2 and slope values[a] for competitive antagonists against noradrenaline contractions in vas deferens from control and castrated rats

	Prazosin		Phentolamine		Yohimbine		BMY 7378	
	pA_2	slope	pA_2	slope	pA_2	slope	pA_2	slope
Control	9.59 ± 0.07	1.03 ± 0.05	8.39 ± 0.04	1.08 ± 0.07	7.16 ± 0.09	0.99 ± 0.06	6.72 ± 0.04	0.99 ± 0.04
Castrated 7 days	9.65 ± 0.07	0.91 ± 0.03	8.36 ± 0.12	1.09 ± 0.11	6.91 ± 0.10	0.89 ± 0.08	6.60 ± 0.10	0.88 ± 0.10
Castrated 14 days	9.43 ± 0.06	1.07 ± 0.04	8.52 ± 0.06	0.97 ± 0.04	7.12 ± 0.10	0.90 ± 0.04	6.55 ± 0.07	0.97 ± 0.06
Castrated 21 days	9.55 ± 0.09	1.08 ± 0.05	8.43 ± 0.04	1.00 ± 0.04	6.99 ± 0.09	1.11 ± 0.06	6.84 ± 0.05	1.02 ± 0.06
Castrated 30 days	9.48 ± 0.07	0.99 ± 0.04	8.48 ± 0.12	0.98 ± 0.04	6.96 ± 0.08	0.92 ± 0.07	6.59 ± 0.05	0.93 ± 0.05
Castrated + testosterone	9.69 ± 0.06	0.91 ± 0.04	8.45 ± 0.07	1.02 ± 0.06	7.19 ± 0.12	1.05 ± 0.10	6.53 ± 0.08	0.99 ± 0.07

[a] Each value represents the mean and the S.E.M. of five to eight experiments.

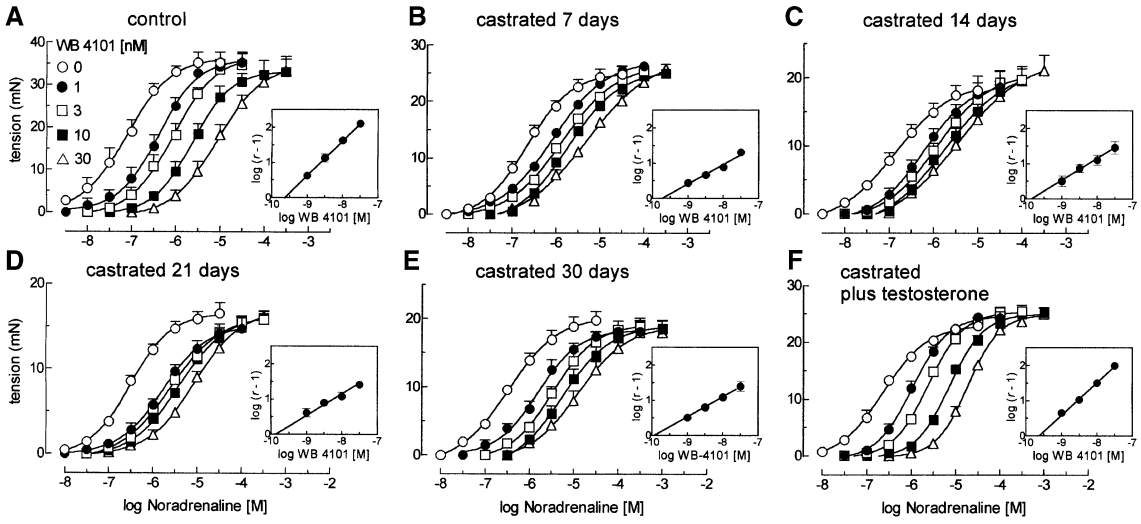

Fig. 2. Concentration–response curves for noradrenaline in vas deferens from control rats (A) and from rats castrated for 7 (B), 14 (C), 21 (D) and 30 days (E), and from castrated rats treated with testosterone propionate (1 mg/day) (F) in the absence (○) and presence of WB 4101 1.0 (●), 3.0 (□), 10 (■) and 30 nM (△). The insets show the respective Schild plots for WB 4101. Each symbol represents the mean and the vertical bar, when greater than the symbol, the S.E.M. of five to eight experiments. Data for rats castrated for 30 days were from Pupo (1998).

castrated rats receiving testosterone propionate (Fig. 1; Table 1).

3.2. Effects of chloroethylclonidine on noradrenaline contractions

The α_{1B}/α_{1D}-adrenoceptor alkylating agent, chloroethylclonidine (100 μM, 45 min), was ineffective against the contractions induced by noradrenaline in vas deferens from control rats (Fig. 1A; Table 1). On the other hand, chloroethylclonidine (100 μM, 45 min) caused either significant reductions of the maximal contractions induced by noradrenaline or rightward shifts in the concentration–response curves in vas deferens from rats castrated for 7, 14, 21 and 30 days (Fig. 1B–E; Table 1). Chloroethylclonidine was also ineffective against the contractions induced by noradrenaline in the vas deferens of castrated rats treated with testosterone propionate (Fig. 1F; Table 1).

3.3. Effects of competitive antagonists on noradrenaline contractions

The α-adrenoceptor competitive antagonists, prazosin, phentolamine and yohimbine, inhibited the contractions induced by noradrenaline in vas deferens from control rats, from rats castrated for 7, 14, 21 and 30 days and from castrated rats treated with testosterone propionate, showing classical competitive antagonism; i.e., they induced parallel leftward shifts without affecting the maximal contractions (data not shown). The rank order of potency for both control and castrated vasa was prazosin>phentolamine>yohimbine (Table 2).

The selective α_{1D}-adrenoceptor antagonist, BMY 7378, antagonised competitively the contractions induced by noradrenaline in vas deferens from control, castrated rats and castrated rats treated with testosterone propionate (Table 2).

The selective α_1-adrenoceptor antagonist, WB 4101, showed classical competitive antagonism against noradrenaline contractions in vas deferens from control rats (Fig. 2A; Table 3). However, in vasa deferentia from rats castrated for 7, 14, 21 and 30 days, WB 4101 showed complex antagonism characterised by slopes in the Schild plots lower than the theoretical unity (Fig. 2B–E; Table 3). WB 4101 antagonised competitively the contractions induced by noradrenaline in the vas deferens from castrated rats treated with testosterone propionate (Fig. 2F; Table 3).

Table 3
pA_2 and slope values[a] for WB 4101 against noradrenaline-induced contractions in vas deferens from control and castrated rats before and after chloroethylclonidine treatment (CEC, 100 μM, 45 min)

	WB 4101 antagonism			
	Before CEC (see Fig. 2)		After CEC (see Fig. 3)	
	pA_2	slope	pA_2	slope
Control	9.60 ± 0.15	0.98 ± 0.06	n.d.	n.d.
Castrated 7 days	n.c.	0.57 ± 0.06[b]	9.38 ± 0.06	0.98 ± 0.03
Castrated 14 days	n.c.	0.60 ± 0.08[b]	9.26 ± 0.13	0.89 ± 0.06
Castrated 21 days	n.c.	0.52 ± 0.03[b]	9.48 ± 0.13	0.94 ± 0.05
Castrated 30 days	n.c.	0.59 ± 0.08[b]	9.41 ± 0.06	0.98 ± 0.03
Castrated + testosterone	9.65 ± 0.10	0.92 ± 0.04	n.d.	n.d.

The pA_2 values were not calculated (n.c.) for these organs because the slopes in the Schild plots were lower than unity ($P<0.05$). The antagonist action of WB 4101 was not determined (n.d.) because CEC was ineffective in these organs.

[a] Each value represents the mean and the S.E.M. of five to eight experiments.

[b] Significantly less than 1.0 ($P<0.05$).

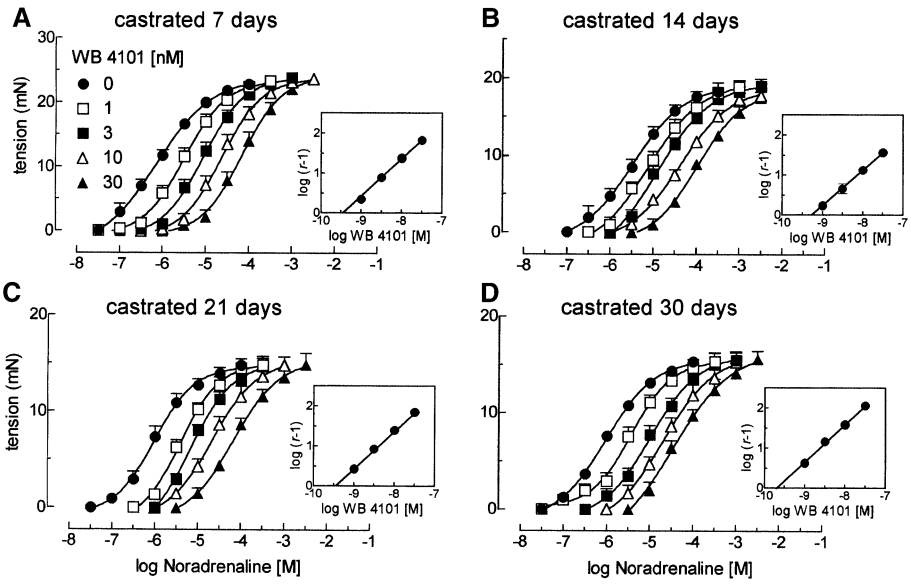

Fig. 3. Concentration–response curves for noradrenaline (after treatment with chloroethylclonidine 100 μM, 45 min) for vas deferens from rats castrated for 7 (A), 14 (B), 21 (C) and 30 days (D) in the absence (●) and presence of WB 4101 1.0 (□), 3.0 (■), 10 (△) and 30 nM (▲). The insets show the respective Schild plots for WB 4101. Each symbol represents the mean and the vertical bar, when greater than the symbol, the S.E.M. of five to eight experiments. Data for rats castrated for 30 days were from Pupo (1998).

3.4. Effect of WB 4101 after chloroethylclonidine treatment

The effects of WB 4101 were reevaluated against the contractions in response to noradrenaline that were resistant to the treatment with chloroethylclonidine. Pretreatment of vas deferens from rats castrated for 7, 14, 21 and 30 days with chloroethylclonidine converted the complex antagonism showed by WB 4101 into a classical competitive antagonism (Fig. 3A–D; Table 3). The Schild plots had slopes no different from unity, resulting in high pA_2 values.

4. Discussion

The present study investigated the time course of the effects of castration on α_1-adrenoceptors subtypes involved in the contractions of the rat vas deferens in response to noradrenaline. The effects of testosterone replacement in castrated rats were also studied.

Castration affected differently the potency and maximal contractions of noradrenaline in the vas deferens. The potency of noradrenaline decreased progressively after 7 and 14 days of castration and tended to revert to control values after 21 and 30 days. There were progressive decreases in the maximal contractions of the vas deferens in response to noradrenaline after 7, 14 and 21 days of castration although no further reduction was observed after 30 days. Interestingly, castration induced progressive atrophy of the vas deferens, resulting in loss of almost 60% of the wet weight after 30 days of castration. Therefore, it is tempting to associate the reductions in the maximal contractions induced by noradrenaline with the progressive organ atrophy. However, other factors may be involved. For example, it is known that castration induces a drastic down-regulation of L-type voltage-dependent Ca^{2+} channels in the rat vas deferens (Castillo et al., 1992). This could contribute to the reduced responsiveness of the vas deferens to noradrenaline, since the contractions induced by this agonist are dependent on Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (Aboud et al., 1993; Pupo et al., 1997). It is also known that the reduced responsiveness of the vas deferens from castrated rats to noradrenaline can be restored to levels comparable to those of organs from control rats if the concentration of Ca^{2+} in the nutrient solution is increased above normal values (Jurkiewicz et al., 1977). Additionally, the fact that testosterone replacement in castrated rats prevented the vas deferens atrophy but not the reduced responsiveness to noradrenaline also suggests that other factors may be involved.

The results obtained in the present study with the reversible competitive antagonists, prazosin, phentolamine and yohimbine, indicate that the contractions induced by noradrenaline in vas deferens from control and castrated rats are due to the activation of α_1-adrenoceptors and that there is no involvement of α_2-adrenoceptors as judged by the rank order of potency found for these antagonists (prazosin> phentolamine>yohimbine). Additionally, the low pA_2 values found for the α_{1D}-adrenoceptor-selective antagonist, BMY 7378, in vas deferens from control and castrated rats suggest that the α_{1D}-subtype is not involved in the contractions induced by noradrenaline.

The contractions of the vas deferens from control rats in response to noradrenaline were resistant to the α_{1B}/α_{1D}-adrenoceptor alkylating agent, chloroethylclonidine. The

lack of effect of chloroethylclonidine allied to the high potency exhibited by WB 4101 and the low potency exhibited by BMY 7378 suggest that the contractions induced by noradrenaline in the vas deferens from control rats are mediated by α_{1A}-adrenoceptors. This is in agreement with results of previous studies where a more extensive list of selective drugs was investigated (Aboud et al., 1993; Burt et al., 1995; Pupo, 1998; Pupo et al., 1999). Interestingly, the α_{1A}-adrenoceptor is not the unique α_1-subtype expressed in the rat vas deferens, since radioligand binding experiments have detected the presence of α_{1A}- and α_{1B}-adrenoceptors (Hanft and Gross, 1989; Salles and Badia, 1991; Vivas et al., 1997), and mRNA species for α_{1A}-, α_{1B}- and α_{1D}-subtypes have been detected in this organ (Faure et al., 1994; Laz et al., 1994; Scofield et al., 1995; Pupo and Avellar, unpublished observations). The roles of α_{1B}- and α_{1D}-adrenoceptors in the rat vas deferens remain to be established.

In contrast to the lack of effect of chloroethylclonidine on vas deferens from control rats, this selective α_{1B}/α_{1D}-adrenoceptor alkylating agent inhibited the noradrenaline-induced contractions of the vas deferens from rats castrated for 7, 14, 21 and 30 days. However, chloroethylclonidine affected differently the potency of and maximal response to noradrenaline depending on the castration period investigated. For example, chloroethylclonidine treatment reduced more effectively the potency in vas deferens from rats castrated for 14 days ($\approx$ 10-fold reduction) although the maximal response was not affected in this organ. The maximal contractions induced by noradrenaline were similarly inhibited by $\approx$ 18% after treatment of the vas deferens from rats castrated from 7, 21 and 30 days with chloroethylclonidine while the potency in these organs was reduced 3.6- to 6.6-fold. Therefore, there was no progressive effect of the time of castration on the effectiveness of chloroethylclonidine.

As mentioned before, WB 4101 antagonised competitively and with high potency the contractions of the vas deferens from control rats in response to noradrenaline. In contrast, WB 4101 showed complex antagonism against the noradrenaline-induced contractions of the vas deferens from rats castrated for 7, 14, 21 and 30 days. The complex antagonisms were characterised by slopes in the Schild plots much lower than the theoretical unity. These results, in conjunction with the low potency of BMY 7378 and the effectiveness of the α_{1B}/α_{1D}-adrenoceptor alkylating agent, chloroethylclonidine, suggest that a heterogeneous population of α_1-adrenoceptors comprised of α_{1A}- and α_{1B}-subtypes participates in the noradrenaline-induced contractions of the vas deferens from rats castrated for 7, 14, 21 and 30 days. After the treatment of vas deferens from rats castrated for 7, 14, 21 and 30 days with chloroethylclonidine, the complex antagonism by WB 4101 was converted into classical competitive antagonism resulting in a high pA_2 consistent with the interaction of this antagonist with α_{1A}-adrenoceptors. Again, no clear progressive effect of the time of castration was observed, since WB 4101 exhibited similar complex antagonism against noradrenaline in the vas deferens from rats castrated for 7, 14, 21 and 30 days. Therefore, these results suggest that, from at least the 7th through the 30th day after castration, α_{1A}- and α_{1B}-adrenoceptors participate in the contractile responses of the rat vas deferens to noradrenaline.

Interestingly, chloroethylclonidine was ineffective against the contractions induced by noradrenaline in the vas deferens from castrated rats treated with testosterone propionate. Additionally, WB 4101 behaved as a classic competitive antagonist against noradrenaline in this organ. These results raise the possibility that the major factor responsible for the chloroethylclonidine-sensitive, WB 4101-low affinity, component in the contractile responses during castration is the absence of testosterone. Previous studies have shown that gonadal hormones control the expression of α_1-adrenoceptor subtypes. Recently, Homma et al. (2000) observed a specific down-regulation of the mRNA for α_{1A}-adrenoceptors associated with a reduced potency of phenylephrine in the rat prostate after androgen deprivation. Sexual maturation, and supposedly the accompanying increase in testosterone plasma levels, reduces the mRNA for α_{1A}-adrenoceptors in the caput epididymis and increases the mRNA for α_{1D}-adrenoceptors in the cauda epididymis of the rat (Queiroz et al., 2002). Estradiol, on the other hand, has been shown to increase selectively the expression of α_{1B}-adrenoceptor binding sites and signalling in the hypothalamus and preoptic area of the female rat (Petitti et al., 1992; Karkanias et al., 1996; Quesada and Etgen, 2002).

In conclusion, we suggest that α_{1B}-adrenoceptors are functionally detected in vas deferens from at least the 7th through the 30th day after castration and that the absence of testosterone plays a role in the surge of this α_{1B}-adrenoceptor-mediated component in the contractions of the vas deferens in response to noradrenaline.

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