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Functional effects of castration on α_1-adrenoceptors in rat vas deferens

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Abstract

The effects of castration on α_1-adrenoceptors in rat vas deferens were investigated by determining the actions of selective antagonists against the contractions induced by noradrenaline. The results obtained in vas deferens from control rats suggest participation of α_{1A}-adrenoceptors as judged by the pA_2 values for prazosin (9.6), benoxathian (9.5), 2(2,6-dimethoxyphenoxyethyl) amino-methyl-1,4-benzodioxone hydrochloride) (WB 4101) (9.6), phentolamine (8.4), 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5] decane-7,9-dionedihydrochloride (BMY 7378) (6.7) and by the insensitivity to chloroethylclonidine (100 μM, 45 min). In vas deferens from castrated rats, WB 4101 and spiperone showed slopes lower than 1.0 in the Schild plots, suggesting participation of multiple receptors. In these organs, noradrenaline contractions were partially inhibited by chloroethylclonidine (100 μM, 45 min), indicating participation of α_{1B}-adrenoceptors. After chloroethylclonidine treatment, WB 4101 showed a slope not different from 1.0 in the Schild plot, resulting in a pA_2 of 9.4, which indicates an interaction with α_{1A}-adrenoceptors. It is suggested that castration modifies the functional α_1-adrenoceptors subtypes in rat vas deferens. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Vas deferens; α_1-Adrenoceptor; Castration

1. Introduction

Studies based on functional, radioligand binding and molecular biological techniques showed that α_1-adrenoceptors are a heterogeneous class of receptors. Current classification recognizes three functionally distinct subtypes showing high affinity for prazosin and which have been designated α_{1A}, α_{1B}, (Morrow and Creese, 1986; Han et al., 1987) and α_{1D}-adrenoceptors (Piascik et al., 1995). The corresponding cloned gene products are identified with lower case letters as α_{1a} (previously named α_{1c}, Schwinn et al., 1990; Laz et al., 1994), α_{1b} (Cottecchia et al., 1988) and α_{1d} (previously named $\alpha_{1a/d}$, Lomasney et al., 1991; Perez et al., 1991; see for review Hieble et al., 1995). These subtypes can be pharmacologically distinguished by selective antagonists. The drugs, 2(2,6-dimethoxyphenoxyethyl)amino-methyl-1,4-benzodioxone hydrochloride (WB 4101), phentolamine, benoxathian and 5-methylurapidil, are competitive antagonists that show selectivity for α_{1A}-adrenoceptors (Morrow and Creese, 1986; Gross et al., 1988; Hanft and Gross, 1989). Functional and binding studies showed that chloroethylclonidine is a selective alkylating agent of α_{1B}-adrenoceptors (Han et al., 1987; Minneman, 1988; Aboud et al., 1993; Eltze, 1994; Burt et al., 1995) and that spiperone has some selectivity for this subtype (Michel et al., 1989; Taddei et al., 1993; Eltze, 1994). Recent data indicate that 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4.5] decane-7, 9-dione dihydrochloride (BMY 7378) is a selective α_{1D}-adrenoceptor competitive antagonist (Goetz et al., 1995; Piascik et al., 1995).

The α_1-adrenoceptors involved in the contractions of the rat vas deferens in response to noradrenaline have been identified as α_{1A} (Aboud et al., 1993; Kenny et al., 1994; Burt et al., 1995). However, it was shown recently that the α_1-adrenoceptor population in rat vas deferens has some degree of plasticity as indicated by the participation of α_{1B}-adrenoceptors in the contractions in response to noradrenaline after transplantation of the organ to the intestinal wall for 7 days (Pupo et al., 1997).

Castration is able to modify the expression of several neurotransmitter-related receptors. For example, in rat prostate after castration, muscarinic cholinergic receptors

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are reduced (Shapiro et al., 1985) while benzodiazepine receptors (Batra and Alenfall, 1992) and imidazoline I_2 receptors (Regunathan et al., 1996) are up-regulated. In castrated spontaneously hypertensive rats, the number of α_{2B}-adrenoceptors in renal tissue is reduced (Gong et al., 1994). In rat vas deferens, castration induces down-regulation of L-type voltage-dependent calcium channels (Castillo et al., 1992). Besides the reduction of the number of calcium channels, castration also induces severe alterations in the contractile responses of the rat vas deferens to adrenergic agonists (MacDonald and McGrath, 1980). However, as there is no information about the effects of castration on α_1-adrenoceptors subtypes in the rat vas deferens, the present study was designed to investigate these effects. To this end, the determination of pA_2 values for selective antagonists and of the effects of the alkylating agent chloroehtylclonidine was carried out.

2. Materials and methods

2.1. Animals and castration

Male Wistar rats from our own colony, weighing between 280–360 g (16 to 20 weeks old), were anesthetized by ether inhalation and a 2-cm suprapubic incision was done. Major vessels were ligated to prevent bleeding and both testes were removed. After this procedure, the incision was sutured and the animals were killed 29 to 31 days after surgery. The experimental procedures were approved by the Ethics Committee for the Use of Experimental Animals from UNESP, campus of Botucatu.

2.2. Isolated vas deferens

Control and castrated rats were killed by ether inhalation. The vasa were removed and the lumen was washed with the help of a syringe and a needle. The associated blood vessels and mesentery were dissected away. For the recording of isometric contractions, the whole vasa deferentia from control or castrated rats were mounted under 1 g of 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, because at this temperature both the frequency and amplitude of the spontaneous contractions of vas deferens from castrated rats were lower than those observed at 37°C.

2.3. Functional experiments

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 incubated in order to block neuronal and extraneuronal uptake and β-adrenoceptors, respectively. The interval between each concentration–response curve was 45 min. Preliminary experiments demonstrated no significant difference in sensitivity to noradrenaline between at least five concentration-effect curves. α-Adrenoceptor 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.

2.4. Calculation of pA_2 values

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 antagonist was similar to that obtained in its absence. Data were plotted as log antagonist concentrations (M) vs. log ($r-1$). It is assumed that when the slope value of the regression line in

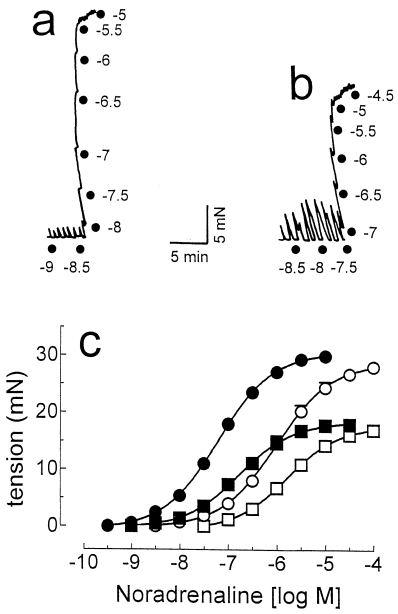

Fig. 1. Representative tracing showing the isometric contractions in response to noradrenaline (log [M]) in vas deferens from control (a) and castrated rats (b) in the presence of cocaine (6 μM), corticosterone (10 μM) and propranolol (0.1 μM). In (c) are shown the mean concentration–response curves obtained in vasa from control (○,●, $n=38$) and castrated rats (□, ■, $n=41$) in the absence (open symbols) and presence (filled symbols) of the above mentioned concentrations of cocaine, corticosterone and propranolol. Vertical bars, when larger than the symbols, represent the S.E.M.

Table 1
pD_2 and maximal effect (E_{max})[a] of noradrenaline in vas deferens from control and castrated rats in the absence and presence of the cocktail containing cocaine (6 μM), corticosterone (10 μM) and propranolol (0.1 μM)

	Absence of cocktail		Presence of cocktail	
	pD_2	E_{max} (mN)	pD_2	E_{max} (mN)
Control	6.01 ± 0.07	28.81 ± 1.43	7.21 ± 0.09	30.02 ± 1.35
Castrated	5.70 ± 0.09[b]	17.75 ± 1.18[b]	6.77 ± 0.08[b]	18.08 ± 1.12[b]

[a] Data are means ± S.E.M. of 38–41 experiments.
[b] Different from the respective value found in control vas ($P < 0.05$).

the Schild plot is not statistically different from 1.0, the pA_2 value represents the dissociation constant of the antagonist (pK_B). For calculation purposes, the slopes were constrained to 1.0 when statistically not different from unity.

2.5. Statistical analysis

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-test.

2.6. Drugs

Drugs were obtained from the following sources: cocaine (Cocainum Hydrochloricum puriss., C.H. Boehringer, Germany; corticosterone, noradrenaline [(±)-arterenol HCl]; from Sigma, USA; idazoxan HCl, chloroethylclonidine 2 HCl, WB 4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride), benoxathian HCl, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), prazosin HCl, phentolamine HCl, (±)-propranolol HCl and yohimbine HCl from Research Biochem-

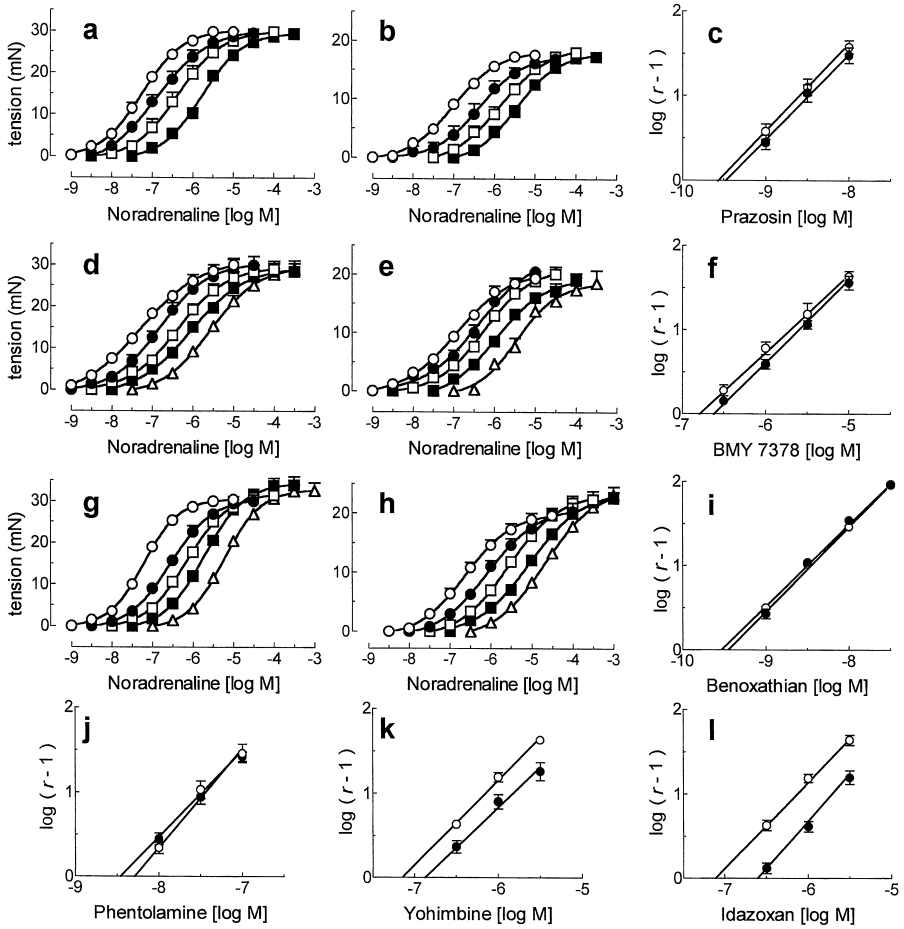

Fig. 2. Concentration–response curves obtained in vas deferens from control (a, d, g) and castrated rats (b, e, h) with noradrenaline in the absence (○) and presence of (a,b) 1 (●), 3 (□) and 10 nM (■) prazosin; (d, e) 0.3 (●), 1 (□), 3 (■) and 10 μM (△) BMY 7378; (g, h) 1 (●), 3 (□), 10 (■) and 30 nM (△) benoxathian. (c, f, i, j, k, l) Schild plots for the interaction between noradrenaline and prazosin (c), BMY 7378 (f), benoxathian (i), phentolamine (j), yohimbine (k) and idazoxan (l) in vas deferens from control (○) and castrated rats (●). Each point represents the mean, and the vertical bars, when larger than the symbols, the S.E.M. of 5–8 experiments.

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

	Control		Castrated	
	pA_2	slope	pA_2	slope
Prazosin	9.59±0.07	1.03±0.05	9.48±0.07	0.99±0.04
BMY 7378	6.72±0.04	0.91±0.05	6.59±0.05	0.93±0.05
Benoxathian	9.51±0.05	0.97±0.04	9.47±0.05	1.02±0.04
Phentolamine	8.39±0.04	1.08±0.07	8.48±0.12	0.98±0.04
Yohimbine	7.16±0.09	0.99±0.06	6.83±0.08[b]	0.89±0.12
Idazoxan	7.21±0.08	0.98±0.06	6.60±0.10[b]	0.96±0.12
Spiperone	7.66±0.05	1.09±0.06	n.d.	0.63±0.08[c]
WB 4101	9.60±0.11	0.94±0.04	n.d.	0.59±0.08[c]
WB 4101 after CEC[d]	n.d.	n.d.	9.41±0.06	0.98±0.03

[a] Data are means±S.E.M. of 5–8 experiments.
[b] Different from the respective value found in Control ($P < 0.05$).
[c] Significantly less than 1.0 ($P < 0.05$).
[d] CEC, Chloroethylclonidine (100 μM, 45 min).
n.d. = not determined.

icals International (Natick, MA, USA). Drugs were dissolved in distilled water or dimethyl sulfoxide (DMSO, 1 mM), kept frozen and discarded after 20 days. Noradrenaline solutions were dissolved in 0.01 M HCl each day shortly before the experiments.

3. Results

3.1. Effect of castration on noradrenaline-induced contractions

Noradrenaline produced sustained and concentration-dependent contractions of vas deferens from control and castrated rats (Fig. 1). Intense spontaneous contractions with phasic characteristics were observed in the vas deferens from castrated rats (Fig. 1b). The addition of a cocktail containing cocaine (6 μM), corticosterone (10 μM) and propranolol (0.1 μM) shifted to the left the concentration–response curves for noradrenaline in tissues taken from both control and castrated rats (Fig. 1c and Table 1). In the absence or presence of the above cocktail, both pD_2 values and the maximal contractions of vas deferens from castrated rats were lower than those in control vas (Table 1).

3.2. Effect of α-adrenoceptor antagonists on noradrenaline-induced contractions

In vasa deferentia from control and castrated rats, the antagonists, prazosin, benoxathian, BMY 7378, phento-

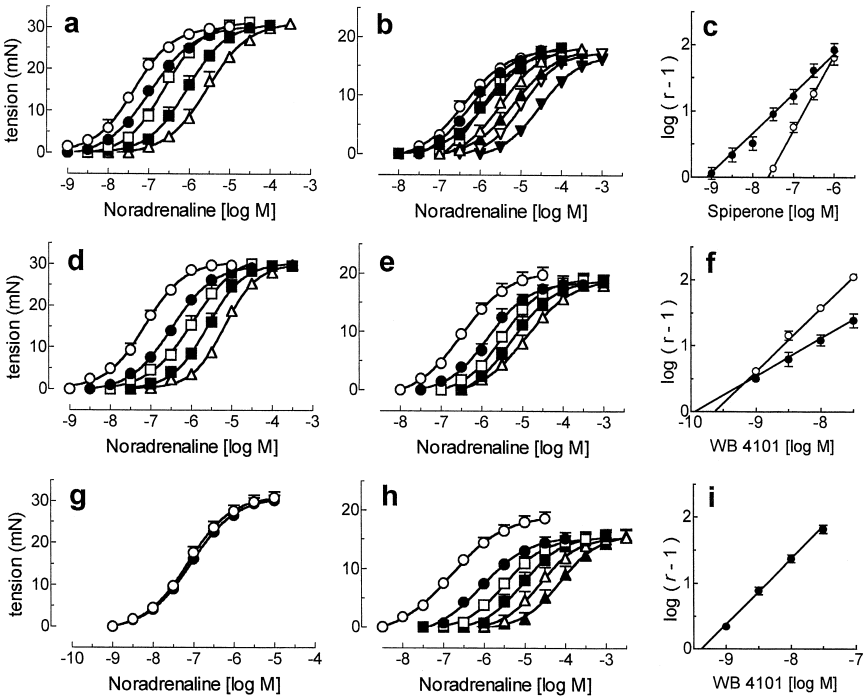

Fig. 3. Concentration–response curves obtained in vas deferens from control (a, d) and castrated rats (b, e) with noradrenaline in the absence (○) and presence of (a) 0.03 (●), 0.1 (□) 0.3 (■) and 1 μM (△) spiperone; (b) 1 (●), 3 (□), 10 (■), 30 (△), 100 (▲), 300 (▽) and 1000 nM (▼) spiperone;(d,e) 1 (●), 3 (□), 10 (■) and 30 nM (▲) WB 4101. (g, h) Concentration–response curves for noradrenaline before (○) and after (●) the treatment of vas deferens from control (g) and castrated rats (h) with chloroethylclonidine (100 μM, 45 min). Also in h are shown the effects of 1 (□), 3 (■), 10 (△) and 30 nM (▲) WB 4101 on chloroethylclonidine-resistant contractions. (c, f, i) Schild plots for spiperone (c) and WB 4101 (f) against noradrenaline in vas deferens from control (○) and castrated rats (●) and (i), against chloroethylclonidine-resistant noradrenaline contractions in vas deferens from castrated rats (●). Each point represents the mean, and the vertical bars, when larger than the symbols, the S.E.M. of 5–8 experiments.

lamine, yohimbine, and idazoxan, blocked noradrenaline contractions showing competitive antagonism (Fig. 2). The Schild plots had slopes statistically not different from 1.0 (Table 2). There was a reduction in the pA_2 values of the α_2-adrenoceptor antagonists, idazoxan and yohimbine, following castration (Fig. 2k,l and Table 2).

Spiperone and WB 4101 also behaved as competitive antagonists in control vas deferens (Fig. 3a,d). However, in vas deferens from castrated rats, WB 4101 and spiperone acted in a manner inconsistent with competitive antagonism (Fig. 3b,e) since the slopes in the Schild plots were lower than 1.0 (Fig. 3c,f and Table 2).

3.3. Effect of chloroethylclonidine on noradrenaline-induced contractions and on WB 4101 antagonism

Treatment of the vas deferens from control rats with chloroethylclonidine (100 μM, 45 min) did not affect the contractions induced by noradrenaline (Fig. 3g). The pD_2 value for noradrenaline before chloroethylclonidine treatment (7.14 ± 0.09; $n = 5$) was similar to that found after the treatment (7.07 ± 0.08; $n = 5$). The maximal contraction determined before (30.79 ± 1.45 mN; $n = 5$) and that after the treatment (30.20 ± 1.22 mN; $n = 5$) were similar. However, treatment of the vas from castrated rats with chloroethylclonidine (100 μM, 45 min) caused a 5-fold rightward shift in the concentration–response curve to noradrenaline (pD_2 before $= 6.82 \pm 0.04$ and pD_2 after $= 6.07 \pm 0.07$; $n = 8$, $P < 0.05$) and a 20% reduction of the maximal contraction (maximal effect before $= 18.61 \pm 1.12$ mN, $n = 8$, and maximal effect after $= 15.17 \pm 1.09$ mN; $n = 8$; $P < 0.05$) (Fig. 3h). After the treatment with chloroethylclonidine, the complex antagonism previously observed with WB 4101 in vas deferens from castrated rats was converted to a competitive antagonism with a slope in the Schild plot not significantly different from 1.0 (Fig. 3h,i and Table 2).

4. Discussion

The present study investigated the effects of α-adrenoceptor competitive antagonists in an attempt to identify the α_1-adrenoceptors subtypes involved in the contractile responses of vas deferens from control and castrated rats to noradrenaline.

The vas deferens from castrated rats yielded intense spontaneous contractile activity. MacDonald and McGrath (1980) also reported the presence of spontaneous activity in vas deferens from castrated rats. These authors found that noradrenaline no longer produced 'tonic' contractions, but increased the 'phasic' component of the spontaneous activity. However, 'tonic' contractions in response to noradrenaline in vas deferens from castrated rats were detected in the present work. It should be noted that MacDonald and McGrath (1980) used rats castrated 84 days (12 weeks) previously while in the present work, the rats were used 29–31 days after castration. It is known that, after castration, the number of L-type voltage-dependent calcium channels in rat vas deferens is reduced by about 85% (Castillo et al., 1992). This reduction could be related to the lower maximal contraction found for noradrenaline in the vas deferens from castrated rats, since these contractions are extremely sensitive to dihydropyridine calcium channels antagonists (Aboud et al., 1993; Pupo et al., 1997). At this moment, there is no clear explanation for the lower pD_2 value for noradrenaline found after castration, but it could be related to a reduction in receptor density, reserve, coupling efficiency or even to a reduction in the affinity of the receptor for the agonist.

The results obtained in vas deferens from control rats indicate that noradrenaline-induced contractions are due to an interaction with α_{1A}-adrenoceptors, as judged by the high pA_2 values found for WB 4101, phentolamine, benoxathian and by the absence of an effect of chloroethylclonidine. The conclusion that contractions to noradrenaline in control vas deferens are mediated by α_{1A}-adrenoceptors is in agreement with the work of other groups (Aboud et al., 1993; Kenny et al., 1994; Burt et al., 1995) since the affinities (pA_2 values) for several antagonists now found both compare well with the values obtained by these authors and confirm the ineffectiveness of chloroethylclonidine to inhibit these contractions. It was not possible to detect participation of α_{1D}-adrenoceptors in these contractions, since the selective α_{1D}-adrenoceptor antagonist, BMY 7378, showed low pA_2 values, not corresponding to an interaction with this subtype.

The identification of the α_1-adrenoceptor subtype involved in the contractions of the vas deferens from castrated rats is a complex task. This complexity is caused by the antagonism showed by WB 4101 and spiperone, which resulted in slopes in the Schild plots much lower than 1.0. There are at least three clear explanations for a slope lower than unity: (1) presence of an uptake mechanism of the agonist from the medium (Furchgott, 1972), (2) multiple drug properties or chemical interference (Kenakin, 1997) and (3) presence of a heterogeneous receptor population (Kenakin, 1985; Milnor, 1986). The first and the second explanation can be discarded because the experiments were done in the presence of cocaine and corticosterone and in vas deferens from control rats slopes lower than 1.0 were not observed with these blockers. The third hypothesis, the presence of a heterogeneous receptor population, was more profoundly investigated by using the alkylating agent, chloroethylclonidine.

Contrary to the observations made with control vas deferens, chloroethylclonidine was able to inhibit, at least partially, the noradrenaline-induced contractions in vas deferens from castrated rats. Since chloroethylclonidine is an irreversible competitive antagonist, selective for α_{1B}-

adrenoceptors (Han et al., 1987; Minneman, 1988; Aboud et al., 1993; Eltze, 1994), this result suggests participation of α_{1B}-adrenoceptors in the contractile responses of the vas deferens from castrated rats to noradrenaline. This suggestion is supported by the fact that, after chloroethylclonidine treatment, the slope in the Schild plot for WB 4101 was not significantly different from 1.0 and resulted in a high pA_2 value, consistent with an interaction with α_{1A}-adrenoceptors. Therefore, it is suggested that, in contrast to what was observed in vas deferens from control rats, α_{1A}- and α_{1B}-adrenoceptors play a role in the contractions of the vas deferens from castrated rats.

Molecular biological studies have identified mRNA species for α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors in the rat vas deferens (Faure et al., 1994; Scofield et al., 1995) and radioligand binding experiments showed α_{1A}- and α_{1B}-adrenoceptor binding sites in this organ (Hanft and Gross, 1989; Sallés and Badia, 1991; Vivas et al., 1997). Also, it is known that castration can modulate the expression of several neurotransmitter-related receptors (Shapiro et al., 1985; Batra and Alenfall, 1992; Regunathan et al., 1996) and that α_{1B}-adrenoceptors can couple to different signal transduction systems (Perez et al., 1993; Minneman and Esbenshade, 1994; Esbenshade and Minneman, 1995). It could, therefore, be speculated that castration induces a change in the expression of receptor subtypes or, alternatively, a change in the signal transduction system to which the α_{1B}-adrenoceptors are coupled, enabling its participation in the contractile responses to noradrenaline. More experiments are needed to test these suggestions.

The pA_2 values for yohimbine and idazoxan in vas deferens from castrated rats were lower than the values found for control rats. Although statistically significant, this reduction has uncertain pharmacological relevance because there is no clear indication in the literature that idazoxan shows selectivity for one of the α_1-adrenoceptors subtypes, and the small difference observed for yohimbine does not allow an assumption to be made.

In conclusion, it is proposed that castration is able to change the subtypes of functional α_1-adrenoceptors involved in the contractile responses of the rat vas deferens to noradrenaline, as indicated by the participation of α_{1B}-adrenoceptors in the contractions of the vas deferens from castrated rats.

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