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Effects of clonidine in the isolated rat testicular capsule

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ABSTRACT

The testicular capsule contracts in response to noradrenaline and adrenaline, but the effects of adrenoceptor agonists, as for instance clonidine, had not yet been thoroughly evaluated. The testicular capsule from adult male Wistar rats was isolated and mounted in organ bath and cumulative concentration curves were performed for clonidine and other adrenergic agonists in the absence or presence of α-adrenoceptors antagonists. The order of potency for agonists (pD_2) was clonidine = adrenaline > UK 14,304 > noradrenaline > phenylephrine > methoxamine. The consecutive curves for clonidine showed desensitization with 3-fold rightward shift and E_{max} reduction of 40%. The noradrenaline curves were 4.5, 19 and 190-fold less potent after clonidine pretreatment at 10^{-5}, 10^{-4} or 10^{-3} M for 10 min, respectively, added to E_{max} decrease by about 20%. Clonidine (10^{-5} M for 10 min) was unable to alter the noradrenaline curves if the treatment was made in the presence of idazoxan (α_2-adrenoceptor antagonist) whereas prazosin (α_1-adrenoceptor antagonist) was ineffective. The effect of idazoxan 3×10^{-7} M on noradrenaline curves was decreased by 50% after clonidine pretreatment, as reflected by the concentration ratio of 5.2 ± 1.2 (treated tissue) and 10.1 ± 1.0 (untreated tissue). However, the concentration ratio for prazosin 3×10^{-8} M was unchanged. After phenoxybenzamine (irreversible antagonist of α_1-adrenoceptor) pretreatment, the residual noradrenaline contraction was antagonized by idazoxan or prazosin with pK_B values of 7.8 and 5.1, respectively. The results indicate the presence of α_2-adrenoceptors in testicular capsule. Furthermore, these receptors may be desensitized by clonidine, causing a decreased potency of noradrenaline.

1. Introduction

The testicular capsule from humans or rodents is a thin tissue layer surrounding the testis with contractile properties which are mainly regulated by catecholamines released from sympathetic nerve endings (Bell and McLean, 1973; Campos et al., 1990; Jurkiewicz et al., 2006). The contractile activity of testicular capsule promotes the correct transit of the sperm out from the seminiferous tubules to epididymis and its dysfunction can promote a decrease of male fertility (Banks et al., 2006; Qin and Lung, 2000, 2001).

The α-adrenoceptors are differentiated into α_1- and α_2-adrenoceptors and both mediate most excitatory functions in response to the endogenous catecholamines released from sympathetic nerve endings in several tissues, such as arteries, veins, spleen, vas deferens and fundus of stomach (Goldberg and Robertson, 1984; Civantos Calzada, Aleixandre de, 2001; Hermann et al., 2005; Jurkiewicz and Jurkiewicz, 1991; Kenakin and Novak, 1988; MacLennan et al., 1997; Molin and Bendhack, 2004).

Previous studies conducted by our laboratory showed that neuronal and exogenous noradrenaline-evoked contraction in the rat testicular capsule was mainly mediated by α_1-adrenoceptors (Jurkiewicz et al., 2006). However, the participation of postsynaptic α_2-adrenoceptors was not fully investigated in the exogenous agonist-induced contraction in rat testicular capsule.

Apart from the endogenous ligands adrenaline and noradrenaline, several synthetic drugs are available and used to discriminate the populations of α_1- and α_2-adrenoceptors. In this context, clonidine was here chosen because it is a selective agonist of α_2-adrenoceptors (Timmermans and van Zwieten, 1981) and widely used as antihypertensive, sedative and analgesic agent (Arimitsu et al., 1998; Gilsbach and Hein, 2012). Clonidine is able to induce inhibitory effects on sympathetic neurotransmission, involving an interaction with pre-synaptic α_2-adrenoceptors, or smooth muscle contractions related to postsynaptic α_1- or α_2-adrenoceptors (Caricati-Neto et al., 1995; Clark et al., 1985; Drew, 1977; Jurkiewicz and Jurkiewicz, 1991; Weiss, 1991).

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Furthermore, the long-term exposure to clonidine has been reported to cause tolerance by desensitization of α_2-adrenoceptors (Shibata et al., 2000) and promote fertility impairment (Clark et al., 1985; Weiss, 1991). Thus, clonidine might induce the rat testicular capsule contraction and/or affect the exogenous catecholamine responses in this tissue, altering the sperm transport to epididymis and, consequently, the animal fertility.

In the present study, the pharmacological profile of clonidine (selective agonist of α_2-adrenoceptors) was compared with non-selective agonists of α-adrenoceptors (adrenaline and noradrenaline), selective agonists of α_1-adrenoceptors (phenylephrine and methoxamine) and selective agonist of α_2-adrenoceptors (UK 14,304). Moreover, we showed that the repeated exposures to clonidine promote a decrease in its responses as well as diminish the potency of the noradrenaline-induced contractions in rat testicular capsule probably due to a desensitization of α_2-adrenoceptors. These findings afford new information about the effects of clonidine on smooth muscles, particularly in testicular capsule which has been poorly studied and plays a significant role in the male fertility.

2. Material and methods

2.1. Animals and isolation of rat testicular capsule

Male (90–120 days old/ 300–400 g) Wistar rats from our own colony (INFAR) were obtained from the Animal Facility (CEDEME) of National Institute of Pharmacology and Molecular Biology – UNIFESP, and maintained under controlled conditions (25 °C, 12/12 h light/dark cycle). After euthanasia by decapitation, the rat testis were exposed and the whole testicular capsule was carefully isolated from the tissues attachments and used for functional experiments, as previously described (Jurkiewicz et al., 2006).

All experimental procedures were approved by the local Ethics Committee for de Use of Experimental Animals of Federal University of São Paulo (Protocol number 0016/2013) and are in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

2.2. Testicular capsule preparation

The isolated testicular capsule was mounted under 1.0 g tension in a 10 ml standard organ bath containing Tyrode solution with the following composition (mM): 137 NaCl; 5.4 KCl; 1.8 $CaCl_2$; 1 $MgCl_2$; 12 $NaHCO_3$; 0.36 KH_2PO_4; 11 glucose, prepared in glass distilled deionized water, bubbled with air, and maintained at 36–37 °C, pH 7.4 (Jurkiewicz et al., 2006). One end of the testicular capsule was attached to the organ chamber, and the other end attached by means of a silk surgical suture to a force–displacement transducer (CB Science, mod. FT 302, USA) connected through a bridge amplifier to a PowerLab recording system (AD Instruments, Castle Hill, Australia), coupled to a computer and the contractions were recorded and the data stored by means of Chart v 4.2.1 software (AD Instruments, Castle Hill, Australia).

2.3. Functional experiments

2.3.1. Cumulative concentration–response curves for clonidine and other adrenoceptors agonists

The tissues were mounted as described above and after an equilibration period of about 40 min, cumulative concentration–response curves for clonidine (10^{-10} to 10^{-5} M), adrenaline (10^{-10} to 3×10^{-6} M), noradrenaline (10^{-9} to 10^{-4} M), phenylephrine (10^{-8} to 3×10^{-4} M), methoxamine (10^{-6} to 10^{-2} M) or UK 14,304 (10^{-9} to 10^{-5} M) were obtained as described below, and these curves were used for determination of the pharmacological parameters listed below. Then, the preparation was carefully washed out and after 40 min of stabilization a new cumulative concentration–response curve was performed in order to check reproducible responses. Moreover, in some experiments the consecutive curves for noradrenaline were performed in the presence of cocaine 6×10^{-6} M, corticosterone 10^{-5} M and propranolol 10^{-7} M (pre-incubated for 40 min) in order to block neuronal and extraneuronal uptake and β-adrenoceptors, respectively. The curve for each agonist was performed in different tissues of distinct animals.

2.3.2. Effects of the pretreatment with clonidine or other adrenergic agonists in the testicular capsule contractions elicited by noradrenaline

After a period of stabilization (40 min) of the isolation in an organ bath, consecutive cumulative concentration–response curves for noradrenaline (10^{-9} to 10^{-4} M) were obtained with 40 min intervals. In some experiments, the testicular capsules were treated with clonidine (10^{-5} to 10^{-3} M), noradrenaline (10^{-5} M), phenylephrine (10^{-5} M) or UK 14,304 (10^{-5} M) for 10 min, extensively washed out, and 40 min later, a concentration–response curve for noradrenaline (non-selective α-adrenoceptor agonist) were constructed. Additionally, the pretreatment with clonidine (10^{-5} M for 10 min) were performed in the presence of α_1- or α_2-adrenoceptors antagonists (prazosin 3×10^{-8} M or idazoxan 3×10^{-7} M, respectively) incubated 5 min before clonidine. Thereafter, the preparation was carefully washed out, and after 40 min, new concentration–response curves for noradrenaline were made. The effect of α-adrenoceptor antagonists was also evaluated after the pretreatment with clonidine (10^{-5} M for 10 min). Time control tissues (matched controls) were treated in exactly the same manner but not exposed to clonidine, noradrenaline, phenylephrine or UK 14,304. The concentration of antagonists was chosen in accordance of pK_B values reported by literature (idazoxan: pK_B 6.4 to 8.0 for α_2-adrenoceptors; prazosin: pK_B 8.5 to 10 for α_1-adrenoceptors) (Dabire, 1986; Halliday et al., 1991; Oshita et al., 1993; Ramagopal and Leighton, 1989; Ruffolo et al., 1991). In this experiment, we used the highest concentration of prazozin (3×10^{-8} M) or idazoxan (3×10^{-7} M) in order to block the clonidine effects (at 10^{-5} M) without affecting the selectivity of these antagonists for α_1- and α_2-adrenoceptors, respectively.

2.3.3. Differential participation of α-adrenoceptors in the first and second cumulative concentration–response curves for clonidine

Cumulative concentration–response curves for clonidine (10^{-10} to 10^{-5} M) were obtained, after a period of stabilization (40 min), in the presence or absence of α_1- or α_2-adrenoceptors antagonists (prazosin 10^{-8} M or idazoxan10^{-8} M, respectively). In addition, after the first curve for clonidine in the absence of antagonists, the preparation was washed out and 40 min later, new cumulative concentration–response curves for clonidine were constructed in the absence or presence of α_1- or α_2-adrenoceptors antagonists. Time control tissues (matched control) were treated in exactly the same manner but not exposed to any antagonist. The antagonist concentration was chosen in accordance with pK_B values described in the literature (see above, Section 2.2.2) and the less effective concentration of prazosin or idazoxan was used in order to calculate the pK_B values of these antagonists against the clonidine curves.

2.3.4. Effects of phenoxybenzamine pretreatment in the noradrenaline-induced contractions on testicular capsule contraction

After initial cumulative concentration–response curve for noradrenaline, the preparation was washed out and idazoxan 10^{-6} M was added 5 min before the addition of the irreversible

antagonist phenoxybenzamine 3×10^{-6} M and both antagonists were maintained for 30 min (idazoxan was used to prevent an eventual irreversible blockade of α_2-adrenoceptors exerted by phenoxybenzamine). Thereafter, the preparation was washed out at least 10 times and 30 min later cumulative concentration–response curves for noradrenaline were repeated. The effects of α_1- or α_2-adrenoceptors antagonists were also evaluated in the following noradrenaline-induced contraction.

2.4. Pharmacological parameters

The pharmacological parameters E_{max} (maximum contraction induced by an agonist) (Jurkiewicz and Jurkiewicz, 1976) and pD_2, (apparent affinity of the agonists) indicating potency, measured as the negative log of EC_{50} (Jurkiewicz et al., 1977), were determined to allow comparisons between curves of adrenergic agonists. Moreover, the CR ($EC_{50}b/EC_{50}a$; the ratio between EC_{50} of the agonist in the presence of antagonist and EC_{50} of the agonist in the absence of antagonist) and the pK_B (antagonist potency expressed as the negative logarithm of the dissociation constant K_B, which is equal to the molar concentration of the antagonist divided by the concentration-ratio minus one) (Besse and Furchgott, 1976; Neubig et al., 2003) values were also calculated.

2.5. Data and statistical analysis

The data were calculated as a percentage of the maximum response attained in the preparation, by using KCl 80 mM (% KCl contraction), in general added at the end of the experiment. Curve fitting by non-linear regression for the calculation of pD_2, E_{max}, CR and pK_B were performed with Prism v.5 software (GraphPAD Software, San Diego, CA, U.S.A.).

Whenever appropriate, values are presented as means ± S.E.M. Paired *t*-tests were used for the comparisons between two groups. A *P* value of less than 0.05 was considered to be statistically significant. The results were obtained from groups of at least four experiments with different tissues from distinct animals.

2.6. Drugs

The following drugs were used: clonidine, noradrenaline, adrenaline, phenylephrine, methoxamine, UK 14,304, prazosin, idazoxan and phenoxybenzamine from Sigma Chemical Co. (St. Louis, MO, USA). All reagents used for nutrient solutions were from Merck (Brazil).

3. Results

3.1. Cumulative concentration–response curves for clonidine and other adrenergic agonists

Fig. 1 shows the log-concentration–response curves obtained in rat testicular capsule in response to clonidine, noradrenaline, adrenaline, phenylephrine and methoxamine. The order of potency for agonists (pD_2) in testicular capsule contraction was clonidine (6.9 ± 0.12; $N=5$) = adrenaline (6.9 ± 0.10; $N=8$) > UK 14,304 (6.5 ± 0.07; $N=5$) > noradrenaline (5.9 ± 0.07; $N=6$) > phenylephrine (5.0 ± 0.1; $N=7$) > methoxamine (3.5 ± 0.05; $N=4$). However, some differences were observed in the efficacies (E_{max}) or intrinsic activities of clonidine, UK 14,304 and methoxamine, which behaved as partial agonists in relation to noradrenaline.

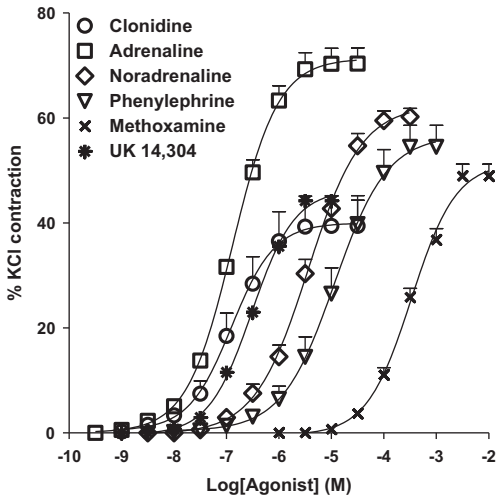

Fig. 1. Non-linear regression of the contractile responses obtained from cumulative concentration–response curves for clonidine (Δ), adrenaline (○), noradrenaline (□), phenylephrine (◊) methoxamine (×) and UK 14,304 (*) expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 4–8 independent experiments performed with tissues from different rats. Values of pD_2 and E_{max} are shown in Table 1.

3.2. Effects of consecutive cumulative concentration–response curves for clonidine

The consecutive curves for clonidine showed desensitization as reflected by about 3-fold rightward shift and a 40% reduction in E_{max} (Fig. 2A). However, the consecutive cumulative concentration–response curves for noradrenaline (Fig. 2B), adrenaline (Fig. 2C), phenylephrine (Fig. 2D), methoxamine (Fig. 2E) and UK 14,304 (Fig. 2F) presented reproducible pD_2 values (Table 1). Thus, the E_{max} values for noradrenaline and adrenaline were slightly increased by about 20% in the second curve (Table 1).

Additionally, consecutive cumulative concentration–response curves for noradrenaline were performed in the presence of cocaine 6×10^{-6} M, corticosterone 10^{-5} M and propranolol 10^{-7} M (pre-incubated for 40 min) in order to block neuronal and extraneuronal uptake and β-adrenoceptor, respectively. We found that consecutive curves for noradrenaline in the presence of these blockers presented reproducible pD_2 (1st curve: 6.5 ± 0.05; 2nd curve: 6.4 ± 0.04; $N=4$) and E_{max} values (1st curve: 115.7 ± 3.39; 2nd curve: 122.4 ± 3.02; $N=4$) (Graph not shown). These results could indicate that the presence of uptake blockers and β-adrenoceptors antagonist did not affect the ability of noradrenaline to induce reproducible responses.

3.3. Effects of the pretreatment with clonidine in the noradrenaline-induced contraction in rat testicular capsule

In order to check whether clonidine induced desensitization in the contractions of rat testicular capsule in response to noradrenaline, the tissues were treated with clonidine at 10^{-5}, 10^{-4} or 10^{-3} M for 10 min, carefully washed out (at least ten times), and after 40 min of interval exposed to noradrenaline. In the tissues pre-exposed to clonidine at 10^{-5}, 10^{-4} or 10^{-3} M (for 10 min), noradrenaline were 4.5, 19 and 190-fold, respectively, less potent than in time controls that had been treated with vehicle (Fig. 3). In addition, the pretreatment with clonidine decreased the E_{max} for noradrenaline by about 20% (in all concentration tested for clonidine) (Fig. 3).

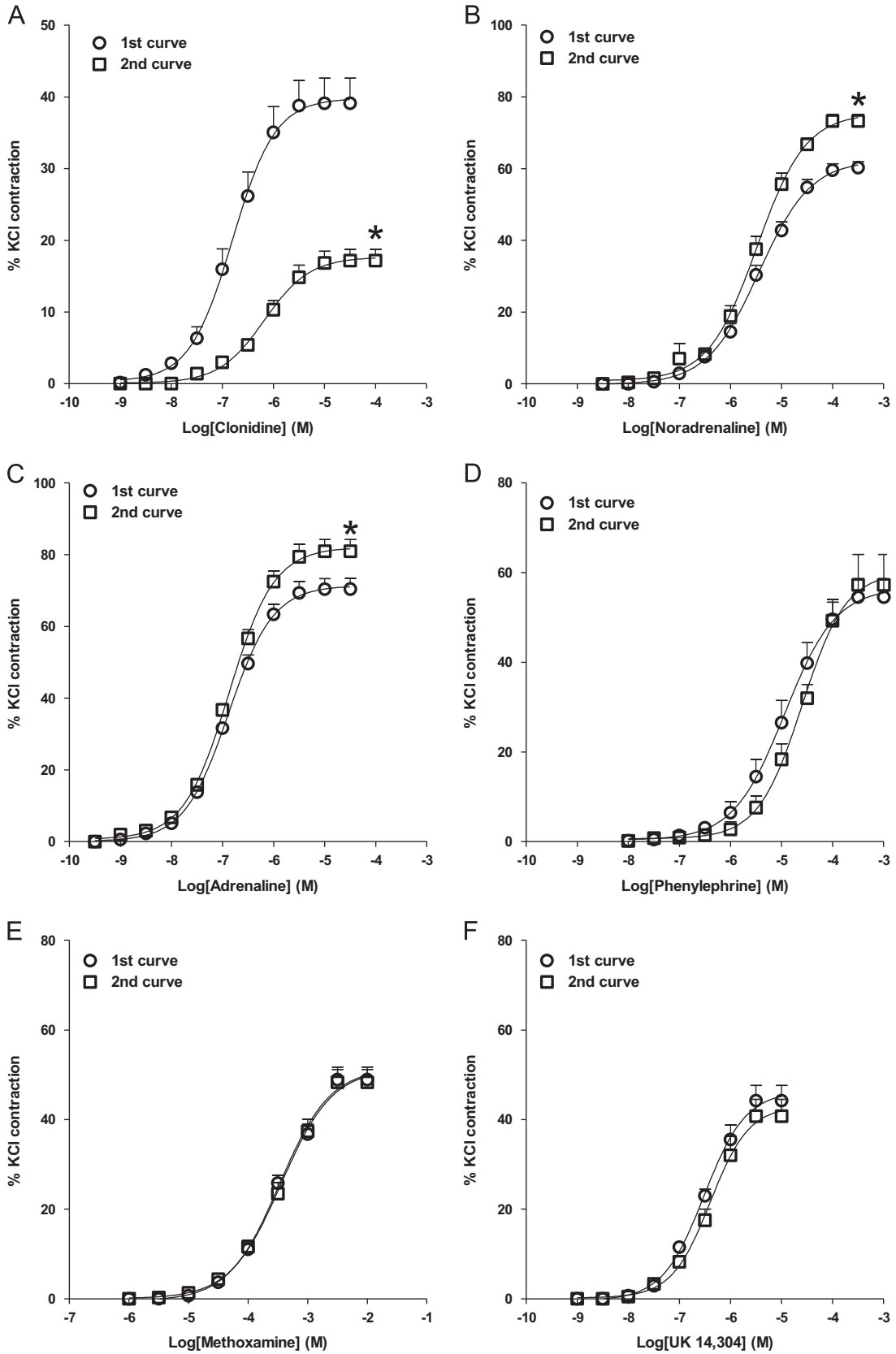

Fig. 2. Non-linear regression of the contractile responses obtained from consecutive cumulative concentration–response curves for clonidine (A), noradrenaline (B), adrenaline (C), phenylephrine (D), methoxamine (E) and UK 14,304 (F) expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 6–10 independent experiments performed with tissues from different rats. *$P < 0.05$ in relation to the E_{max} of the 1st curve. Values of pD_2 and E_{max} are shown in Table 1.

In addition, clonidine (10^{-5} M for 10 min) did not change the pD_2 for phenylephrine (α_1-adrenoceptor agonist), but the E_{max} was depressed by about 50% (data not shown).

3.4. Effects of the pretreatment with noradrenaline, phenylephrine or UK 14,304 in the noradrenaline-induced contraction in rat testicular capsule

The testicular capsule contraction induced by noradrenaline was also evaluated in tissues pre-exposed with noradrenaline, phenylephrine or UK 14,304 for 10 min, extensively washed out, and 40 min later, new curve for noradrenaline was performed. Fig. 4 shows that pretreatment with noradrenaline, phenylephrine or UK 14,304 (all of them at 10^{-5} M for 10 min) did not affect the potency (pD_2) or E_{max} of noradrenaline-induced testicular capsule contraction, compared with time controls which were treated with vehicle (Fig. 4).

Table 1
Maximal contractions (% KCl contraction) and pD_2 values of consecutive curves for clonidine, noradrenaline, adrenaline, phenylephrine and methoxamine in rat testicular capsule.

Agonists	Parameters	Consecutive cumulative-concentration response curve	
		First curve	Second curve
Clonidine ($N=10$)	E_{max}	40.0 ± 2.0	17.6 ± 0.8[a]
	pD_2	6.9 ± 0.1	6.1 ± 0.1[a]
Noradrenaline ($N=5$)	E_{max}	60.6 ± 1.1	74.0 ± 2.1[a]
	pD_2	5.6 ± 0.04	5.5 ± 0.05
Adrenaline ($N=8$)	E_{max}	71.3 ± 1.3	82.0 ± 1.5[a]
	pD_2	6.9 ± 0.05	6.9 ± 0.04
Phenylephrine ($N=5$)	E_{max}	55.2 ± 1.5	60.2 ± 1.5
	pD_2	5.0 ± 0.1	4.6 ± 0.01
Methoxamine ($N=4$)	E_{max}	51.5 ± 1.8	51.1 ± 2.7
	pD_2	3.5 ± 0.05	3.4 ± 0.08
UK 14,304 ($N=5$)	E_{max}	46.3 ± 2.2	43.1 ± 2.7
	pD_2	6.5 ± 0.07	6.4 ± 0.09

E_{max} (maximal contraction) expressed in % of KCl contraction and pD_2 (apparent affinity of the agonists indicating potency, measured as the negative log of EC_{50}) obtained from the non-linear regression curves shown in Fig. 2.

[a] $P<0.05$ in relation to the first curve. N=number of experiments.

3.5. Effects of the pretreatment with clonidine in the presence of α-adrenoceptor antagonists on noradrenaline-induced contraction in rat testicular capsule

The desensitization of noradrenaline curves induced by clonidine (10^{-5} M for 10 min) (shown in Fig. 3) was prevented by the α_2-adrenoceptor antagonist idazoxan 3×10^{-7} M (Fig. 5B) whereas prazosin 3×10^{-8} M (α_1-adrenoceptor antagonist) was ineffective (Fig. 5A), indicating that these effects depend of the α_2-adrenoceptors activation.

3.6. Effects of α-adrenoceptor antagonists after the pretreatment with clonidine in the noradrenaline-induced contraction in rat testicular capsule

The effect of α-adrenoceptor antagonists on noradrenaline curves was also evaluated after clonidine pretreatment (10^{-5} M for 10 min) (Fig. 6). The rightward shift promoted by idazoxan 3×10^{-7} M in the noradrenaline curves was decreased in 50% after clonidine pretreatment compared with untreated tissues, as reflected by the concentration ratio (CR) of 5.2 ± 1.2 (treated tissue) (Fig. 6D) and 10.1 ± 1.0 (untreated tissue) (Fig. 6C). However, the CR values for prazosin 3×10^{-8} M were unchanged, but the E_{max} values for noradrenaline were depressed by about 40% in treated and untreated tissues (Fig. 6A and B). On the other hand, the E_{max} values for noradrenaline were increased in the presence of idazoxan in treated tissues by about 30% (Fig. 6D).

3.7. Differential participation of α-adrenoceptor in the first and second cumulative concentration–response curves for clonidine

The loss of potency in the consecutive curves for clonidine and the effect of the pretreatment of this drug in noradrenaline-induced contraction could indicate a rapid desensitization of α_2-adrenoceptors. Therefore, both α_1- and α_2-adrenoceptors could mediate the first curve and α_1-adrenoceptors could mediate the second. In order to check this possibility, α-adrenoceptor antagonists were incubated before or after the first curve for clonidine. Prazosin 10^{-8} M was able to diminish the E_{max} for the first curve of clonidine by about 50% whereas the pD_2 remained unchanged, indicating a non-competitive antagonism (Fig. 7A). However, idazoxan 10^{-8} M rightward shifted the first curve for clonidine (by about 2.5-fold, $pK_B=8.12\pm0.09$; $N=5$) without altering the E_{max}, indicating a competitive antagonism (Fig. 7B). Surprisingly,

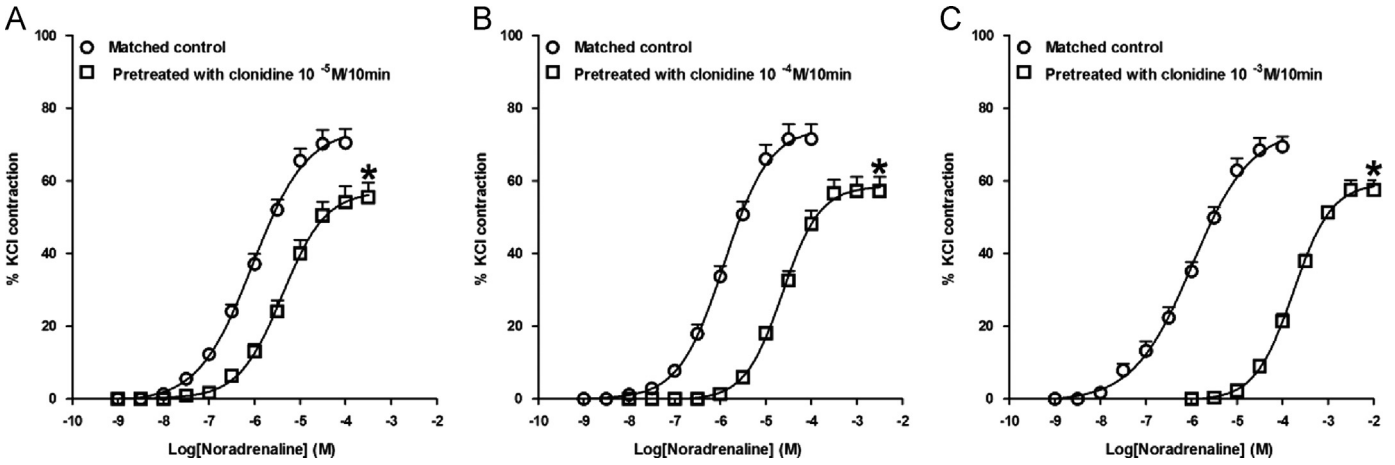

Fig. 3. Non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline after pretreatment with clonidine at 10^{-5} (A), 10^{-4} (B) and 10^{-3} M (C) for 10 min compared with matched control (untreated tissue with clonidine). Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5–7 independent experiments performed with tissues from different rats. *$P<0.05$ in relation to the E_{max} of the matched control.

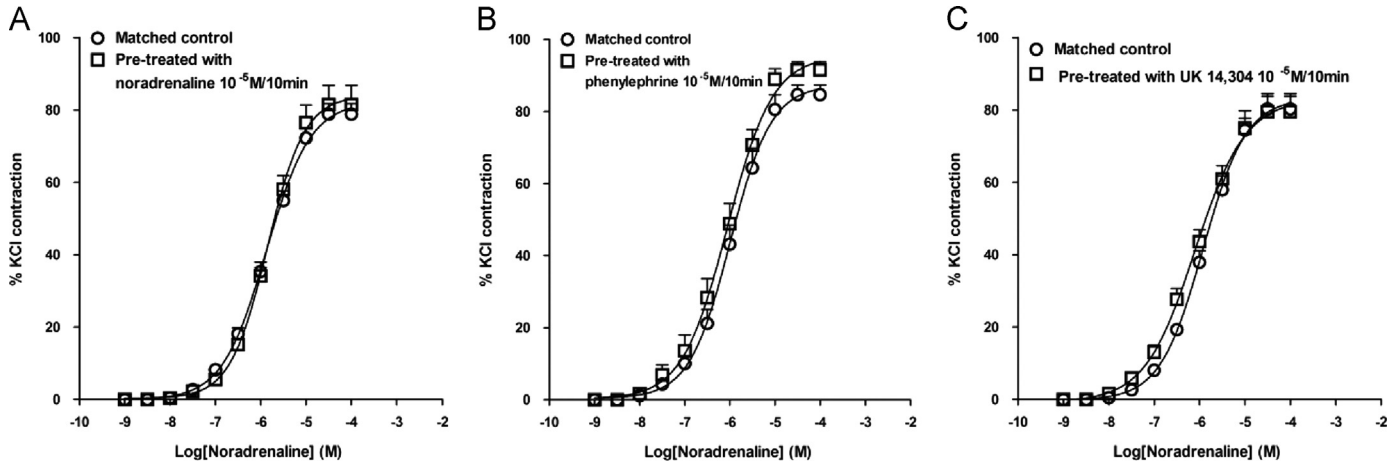

Fig. 4. Non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline after pretreatment with noradrenaline (A), phenylephrine (B) and UK 14,304 (C) at 10^{-5} M for 10 min compared with matched control (untreated tissue with agonists). Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5 independent experiments performed with tissues from different rats.

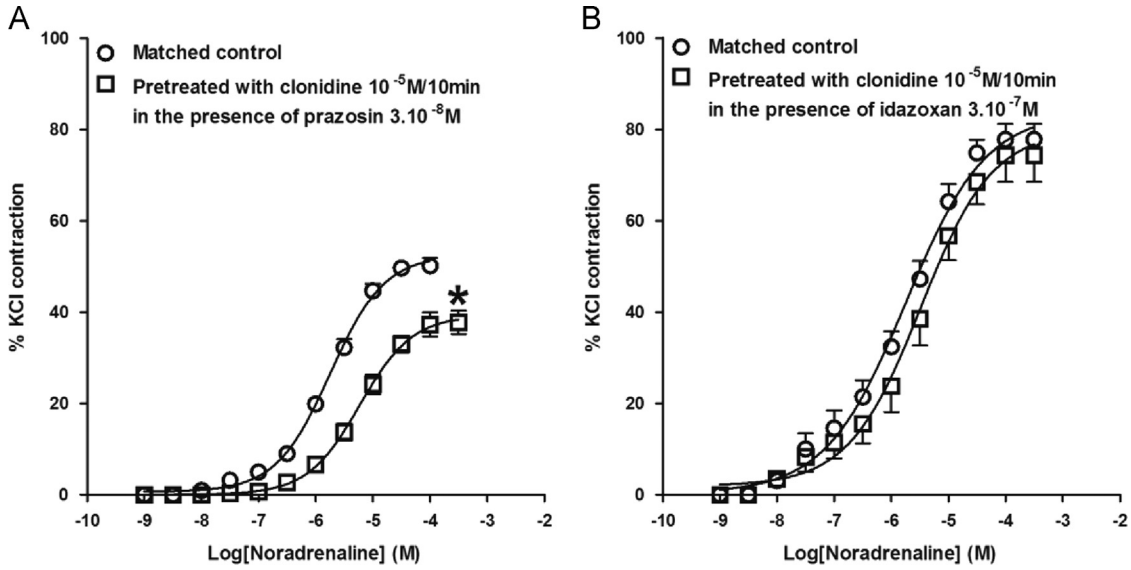

Fig. 5. Non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline after pretreatment with clonidine performed in the presence of prazosin (A) or idazoxan (B). Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5 independent experiments performed with tissues from different rats. *$P < 0.05$ in relation to the E_{max} of the matched control (untreated tissue with clonidine, but incubated with antagonists).

the second curve for clonidine was only rightward shifted by prazosin 10^{-8} M (by about 5.4-fold; $pK_B = 8.64 \pm 0.08$; $N = 9$) (Fig. 7C) while idazoxan 10^{-8} M only increased the E_{max} values for this agonist by about 48% (Fig. 7D).

3.8. Effects of the pretreatment with phenoxybenzamine in the noradrenaline-induced contractions

There is not information about the presence of functional postsynaptic α_2-adrenoceptors in the rat testicular capsule. The involvement of α_2-adrenoceptors in the contractions induced by noradrenaline was studied by the pretreatment with phenoxybenzamine 3×10^{-6} M plus idazoxan 10^{-6} M (P/I) for 30 min. After carefully wash out, a subsequent noradrenaline curve was constructed. The P/I treatment was able to rightward shift the noradrenaline curves by about 5-fold and diminish the E_{max} in 68%. Thus, the residual contraction was competitively antagonized by idazoxan at 3×10^{-7} M ($pK_B = 7.8 \pm 0.05$; $N = 5$). Prazosin was also able to rightward shift the residual contraction after phenoxybenzamine treatment, but only at 10^{-5} M, presenting pK_B values of 5.1 ± 0.11 ($N = 5$) (Fig. 8).

4. Discussion

In this study, we reported that the rat testicular capsule contracts in response to clonidine, but this response is decreased with consecutive curves for this agonist. Moreover, the rat TC pretreatment with clonidine also diminished the potency of the noradrenaline-induced contractions. The probable involvement of α_2-adrenoceptors in these effects was discussed below.

In addition, we have shown that the rat testicular capsule is able to contract in response to non-selective adrenergic agonists such as noradrenaline or adrenaline, as reported by previous presentations (Davis and Horowitz, 1978, 1979; Davis and Langford, 1969a, 1969b;

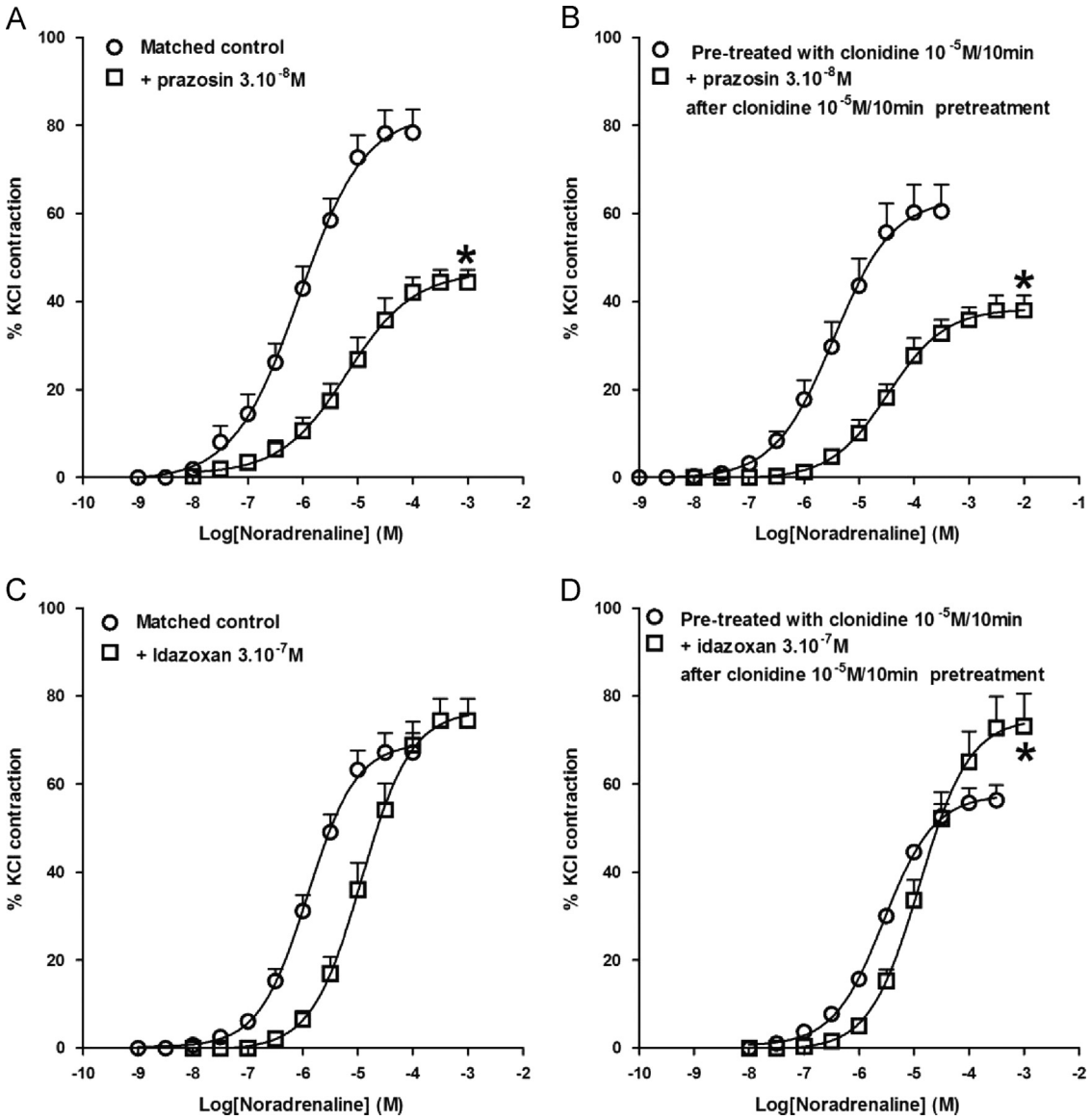

Fig. 6. The panels A and C present the non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline in the absence (matched control) or the presence of prazosin (A) or idazoxan (C). The panels B and D show the non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline after pretreatment clonidine and in the absence or presence of prazosin (B) or idazoxan (D). Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5 independent experiments performed with tissues from different rats. *$P < 0.05$ in relation to the E_{max} of the matched control (A and D) (untreated tissue with clonidine, but incubated with antagonists) or clonidine treated tissues without subsequent incubation of antagonists (B and C).

Jurkiewicz et al., 2006). However, the effects of clonidine, α_2-adrenoceptor agonist, were still not completely evaluated.

Clonidine is considered a selective α_2-adrenoceptors agonist with a partial agonist effect on α_1-adrenoceptors (Timmermans and van Zwieten, 1981). The activation of pre-synaptic α_2-adrenoceptors by clonidine is able to induce a decrease of sympathetic activity whereas the interaction of postsynaptic α_2-adrenoceptors promotes contraction of smooth muscles, such as saphenous veins, arterioles or venules from human or rodents (Civantos Calzada, Aleixandre de, 2001; Molin and Bendhack, 2004). However, postsynaptic α_1-adrenoceptors are also related to the contractile effects of clonidine in aorta (Molin and Bendhack, 2004), anococcygeus muscle (Uzbay and Onur, 1993) and vas deferens (Takeuchi et al., 1987) from rodents.

The contractile profile of clonidine in rat testicular capsule was determined by pharmacological parameters (pD_2 and E_{max}) and compared with other adrenergic agonist. Fig. 1 shows a high potency (pD_2) for clonidine, adrenaline and UK 14,304 which present higher affinity for α_2-adrenoceptors than α_1-adrenoceptors (Bylund et al., 1992; Goldberg and Robertson, 1984), whereas phenylephrine and methoxamine, which are considered as selective agonists for α_1-adrenoceptors, presented low potency (90 and 2500-fold, respectively, lower than clonidine or adrenaline) (Minneman et al., 1994).

In addition, clonidine produced a much smaller contractile response (E_{max}) compared with adrenaline and noradrenaline, and therefore, this drug could be classified as partial agonist. Accordingly, it has been described that clonidine acts as partial agonist of α_1- and α_2-adrenoceptors with α_1/α_2 receptor selectivity of approximately 1:200 (Zhao et al., 2008).

The repeated exposure to clonidine in rat testicular capsule led to diminished responses (desensitization, as previously defined by Neubig et al., 2003) as reflected by the decreased values of pD_2 and E_{max} with consecutive curves (Fig. 2A, Table 1). However, repeated

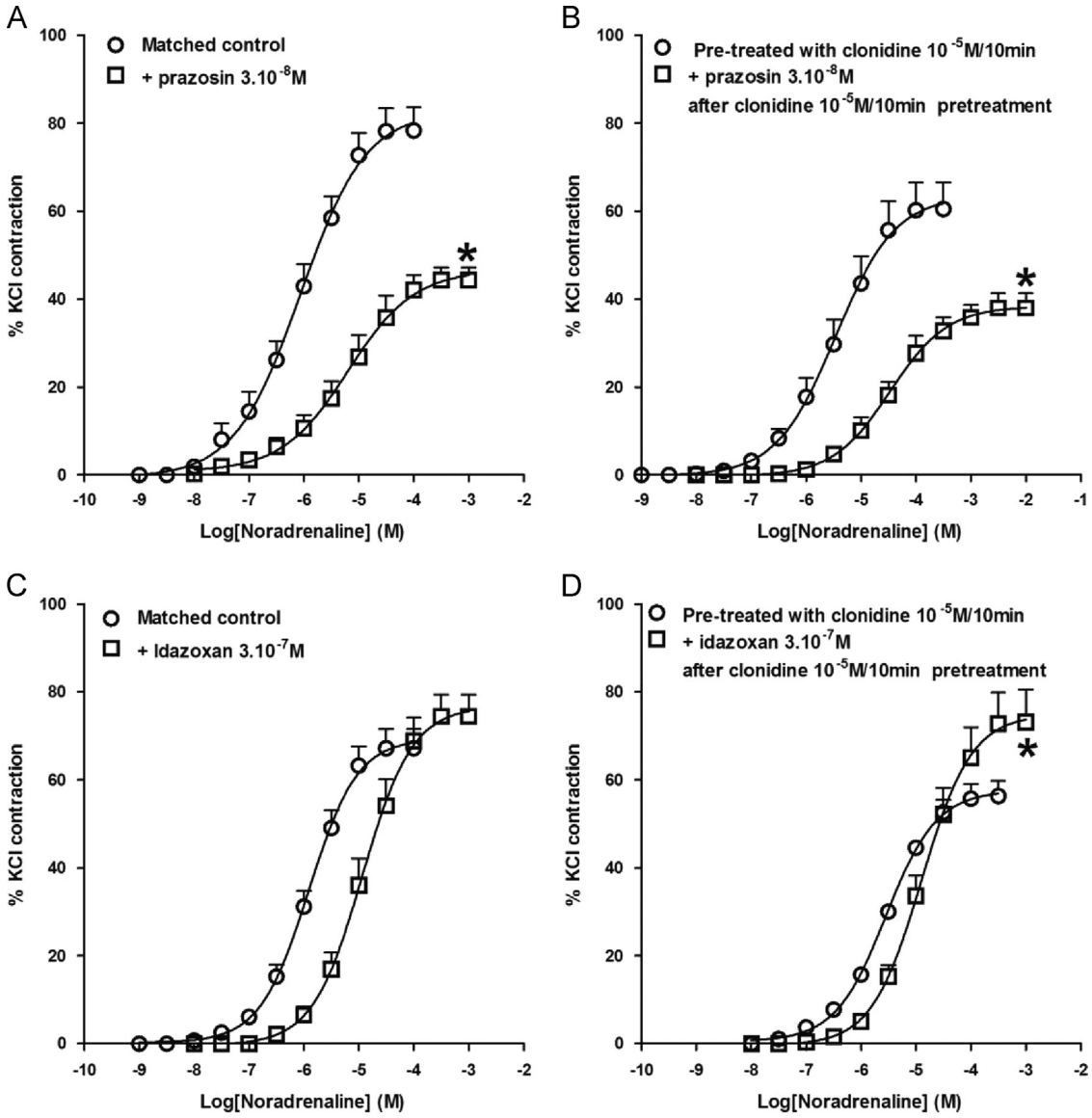

Fig. 7. Non-linear regression of the consecutive contractile responses obtained from cumulative concentration–response curves for clonidine in the absence (matched control) or presence of prazosin and idazoxan. The panels show the effects of prazosin or idazoxan in the first (A and B) or second curves (C and D) for clonidine. Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5–10 independent experiments performed with tissues from different rats. *$P < 0.05$ in relation to the E_{max} of the matched control (in the absence of antagonists).

exposure of testicular capsule with noradrenaline (α-adrenoceptor agonist), adrenaline (α-adrenoceptor agonist), phenylephrine (selective α_1-adrenoceptor agonist) or methoxamine (selective α_1-adrenoceptor agonist) presented reproducible responses, except the E_{max} values for noradrenaline and adrenaline which were slightly increased at the second curve (Fig. 2, Table 1). In addition, noradrenaline only presented diminished responses (pD_2 and E_{max}) when previously exposed to clonidine (Fig. 3), but not to noradrenaline, phenylephrine or UK 14,304 (selective α_2-adrenoceptor agonist) (Fig. 4). The same effects of clonidine were observed in rat spleen and distal portion of epididymis, both presenting populations of postsynaptic α_1- and α_2-adrenoceptors (Haynes and Hill, 1996; Kenakin and Novak, 1988), but not in vas deferens (present postsynaptic α_1-adrenoceptors (Campos et al., 2003; von Asmuth et al., 1991) (Silva-Junior, unpublished observations).

The noradrenaline curves conducted in the presence of uptake system inhibitors and β-adrenoceptors antagonist were potentiated by about 10-fold (pD_2) and the E_{max} values almost doubled, comparing the pharmacological parameters from this experiment (Section 3.2) to those shown in Table 1 (noradrenaline curves performed in the absence of uptake system inhibitors and β-adrenoceptors antagonist). Further, we also found that the consecutive curves for noradrenaline in the presence of these blockers presented reproducible responses (Section 3.2). However, it is important to mention that the presence of the uptake blockers and β-adrenoceptors antagonist could also affect the pharmacological parameters of the other adrenoceptors agonists used in this study as well as their ability to induce desensitization of the rat testicular capsule contraction.

Several studies indicate that UK 14,304 induces desensitization of α_2-adrenoceptor in cultured cells (Boehm et al., 1995; Gasic and Green, 1995). However, the inability of UK 14,304 to induce desensitization of noradrenaline curves in our work might be related to: (a) the short period of the incubation (10 min) used; (b) the subtype of α_2-adrenoceptors presents in the rat testicular capsule; or (c) specific mechanisms related to the tissue studied.

The noradrenaline responses showed a decreased E_{max} values in the tissues pre-exposed with clonidine (Fig. 3). We speculate

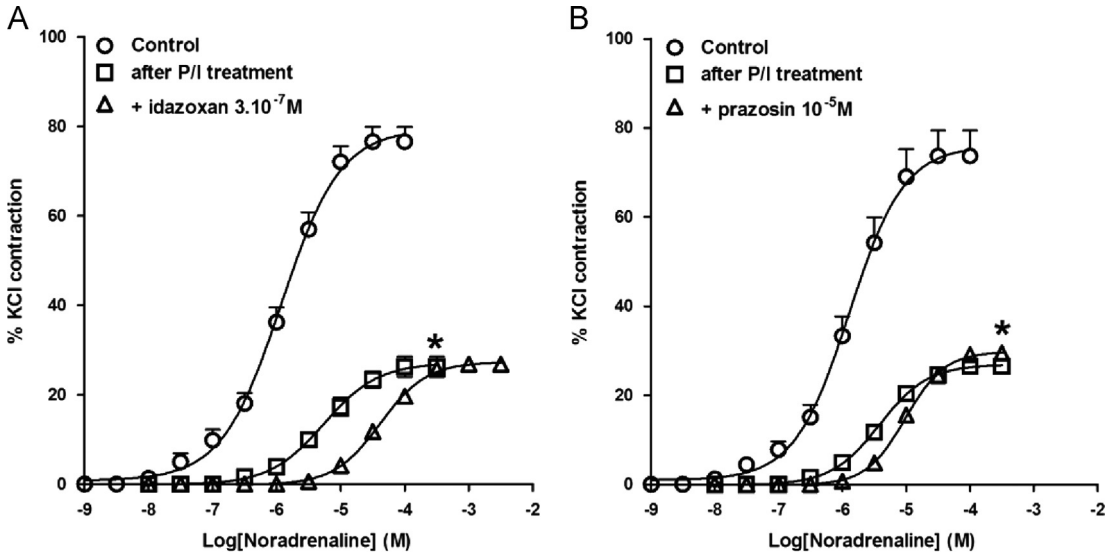

Fig. 8. Non-linear regression of the contractile responses obtained from cumulative concentration–response curves for noradrenaline before and after the pretreatment with phenoxybenzamine plus idazoxan (P/I) (idazoxan was used to prevent the collateral irreversible blockade of α_2-adrenoceptors exerted by phenoxybenzamine) (A and B). The panels A and B show the effect of idazoxan (A) or prazosin (B) in the residual contraction after P/I treatment. Each point was expressed as a percentage of the maximum contractile response induced by KCl 80 mM (% KCl contraction). Values are means ± S.E.M. of 5 independent experiments performed with tissues from different rats. *$P < 0.05$ in relation to the E_{max} of the control curve.

that clonidine at high concentrations could activate other α_2-adrenoceptor signaling pathway which would avoid the maximum development of tension for noradrenaline, such as stimulation of adenylyl cyclase or nitric oxide generation (Eason et al., 1992; Zhao et al., 2008). However, this hypothesis needs further investigations because the activation of these additional signaling pathways has only been observed to date in heterologous systems where α_2-adrenoceptors are expressed at relatively high density.

The reduction of noradrenaline potency induced by the pretreatment with clonidine in rat testicular capsule was only prevented by idazoxan, indicating that these effects were related with the activation of α_2-adrenoceptors. Additionally, the rightward shift by idazoxan in the noradrenaline contractions was drastically reduced if the tissues were pre-exposed to clonidine, reflecting a possible decrease in the functional α_2-adrenoceptors in this tissue. The desensitization of α_2-adrenoceptors was reported by several studies using cell culture system and most of them used noradrenaline or adrenaline for 30 min as a tool to induce desensitization (Daunt et al., 1997; Eason and Liggett, 1993; Liang et al., 1998; Liggett et al., 1992). In our study, we demonstrated that clonidine was able to induce desensitization at a short time and in a functional model.

Notwithstanding its role as a clinical agent candidate, our results indicate a complex participation of α-adrenergic receptor in the clonidine-induced contractions in rat testicular capsule. The first curve for clonidine appears to be mediated by α_1- and α_2-adrenoceptors while the second curve seems to be mediated by α_1-adrenoceptors. The pK_B values of idazoxan against the first curve for clonidine (8.12) were compatible with those reported in human platelet α_2-adrenoceptors (8.04) (Connaughton and Docherty, 1990) and pre-synaptic α_2-adrenoceptors in rat vas deferens (8.70) (Doxey et al., 1984). Thus, prazosin pK_B values against the second curve for clonidine (pK_B 8.64) are also similar for α_1-adrenoceptors in rat anococcygeus muscle (pK_B 8.43) (Adenekan and Tayo, 1982) and small mesenteric vein (pK_B 8.5) (Stam et al., 1999). Therefore, the differential activation of these receptors in the consecutive curves for clonidine may be due to a rapid desensitization of α_2-adrenoceptors. Additionally, we found that the E_{max} for noradrenaline after the pretreatment with clonidine (Fig. 6D) and the second curve for clonidine (Fig. 7D) were increased in the presence of idazoxan. The exact mechanisms related to these effects are unknown and need further investigation.

The presence of functional α_2-adrenoceptors was still not reported in rat testicular capsule. Previous study from our laboratory indicated that noradrenaline-induced contraction in isolated rat testicular capsule was mainly mediated by α_1-adrenoceptors (Jurkiewicz et al., 2006). However, the Schild slopes (different from one unity) for α-adrenoceptor antagonists (Jurkiewicz et al., 2006) and the resistant responses after phenoxybenzamine pretreatment (Fig. 8) strongly indicate the participation of other α-adrenoceptor in the noradrenaline-induced contractions. In fact, the residual noradrenaline contraction of rat testicular capsule after irreversible blockade of α_1-adrenoceptors induced by phenoxybenzamine was antagonized by idazoxan, showing pK_B values (7.8) compatible with those reported for its interaction with α_2-adrenoceptors (Kenakin and Novak, 1988; MacLennan et al., 1997). In addition, the low pK_B values for prazosin (5.1) against noradrenaline curves after phenoxybenzamine pretreatment is consistent to those reported for α_2-adrenoceptors induced contraction in smooth muscle (MacLennan et al., 1997). Altogether, these results might indicate the participation of functional α_1- and α_2-adrenoceptors in the noradrenaline induced contraction in rat testicular capsule.

Further evidence for distinct populations of α_1- and α_2-adrenoceptor in rat testicular capsule is also provided by (1) a non-competitive effect of prazosin against noradrenaline and clonidine curves (first curve – Figs. 6A, and 7A), (2) a competitive effect of this antagonist in the second curve for clonidine (Fig. 7C) and (3) unaltered E_{max} values of noradrenaline curves in the presence of prazosin after pretreatment with phenoxybenzamine (Fig. 8B). Similar results are described by Daly et al. (1988) and Ohlstein et al. (1989) which showed that prazosin produced a nonparallel rightward shift in the noradrenaline concentration–response curves with a marked depression in the maximal response, indicating noncompetitive antagonism as consequence of two distinctive adrenoceptors (α_1- and α_2-adrenoceptors) mediating the same contractile response (Daly et al., 1988; Ohlstein et al., 1989).

It is well established that the motor activity of testicular capsule is essential for the correct transport of sperm from seminiferous tubule to epididymis, and therefore, playing a significant role in the animal fertility. The clinical use of clonidine is related to male fertility disorders mainly due to its action in the central nervous system (Beeley, 1984; Clark et al., 1985; Srilatha et al., 1999; Weiss, 1991).

However, this is the first report that shows an impairment of noradrenaline-elicited contractions induced by clonidine in rat testicular capsule which presumably could affect the correct transport of non-motile spermatozoa from seminiferous tubule out of the testis.

5. Conclusion

In conclusion, our data indicate that clonidine induces contraction in rat testicular capsule and the involvement of functional α_2-adrenoceptors may be considered. Furthermore, clonidine may promote desensitization of functional α_2-adrenoceptors which could participate in the noradrenaline-induced contractions. The subtypes of α_2-adrenoceptor as well as the signaling pathway related to these effects remain open to investigations.

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