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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