

Atypical β -adrenoceptor Subtypes Mediate Relaxations of Rabbit Corpus Cavernosum

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ABBREVIATIONS: ACh, acetylcholine; BRL 37344, (\pm)-4-[2-[(2-(3-Chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid; CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol methanesulfonate); GTN, glyceryl trinitrate; ICI 118,551, (\pm)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride; L-NAME, N^o-nitro-L-arginine methyl ester; NO, nitric oxide; ODQ, 1H-[1,2,4] oxadiazolo [4,3,-a]quinoxalin-1-one; PDE, phosphodiesterase; RbCC, rabbit corpus cavernosum; SQ 22,536, 9-(2-tetrahydrofuryl) adenine; TA 2005, 8-hydroxy-5-[(1R)-1-hydroxy-2-[N-[(1R)-2-(p-methoxy-phenyl)-1-methylethyl]amino]ethyl]carbostyryl); TTX, tetrodotoxin.

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ABSTRACT

This study was performed to characterize the β -adrenoceptor population in rabbit isolated corpus cavernosum (RbCC) by using non-selective and selective β -adrenoceptor agonists and antagonists in functional assays. Metaproterenol, ritodrine, fenoterol and TA 2005 (3-100 nmol each) dose-dependently relaxed the RbCC preparations. These relaxations were markedly reduced by N^o-nitro-L-arginine methyl ester (L-NAME, 10 μ M) and ODQ (10 μ M), whereas the adenylyl cyclase inhibitor SQ 22,536 (10 μ M) had no effect. In contrast, neither L-NAME nor ODQ affected the isoproterenol-induced RbCC relaxations, but SQ 22,536 abolished this response. Sildenafil (1 μ M) significantly potentiated the relaxations induced by β_2 agonists without affecting the isoproterenol-evoked relaxations. Rolipram (10 μ M) enhanced the relaxations elicited by isoproterenol but had no effect on those induced by the selective β_2 agonists. Propranolol and ICI 118,551 determined a rightward shift in the concentration-response curves to isoproterenol in a non-competitive manner with a reduction of maximum response at the highest antagonist concentration, with the slope values significantly different from unity. Propranolol and ICI 118,551 had no effect on the relaxations elicited by fenoterol, TA 2005, metaproterenol and ritodrine. Atenolol and CGP 20712A (0.1-10 μ M) failed to affect the relaxations induced by all tested β -adrenoceptor agonists. Our study revealed the existence of two atypical β -adrenoceptors in the rabbit erectile tissue. Isoproterenol relaxes the rabbit cavernosal tissue by activating atypical β -adrenoceptors coupled to adenylyl cyclase pathway whereas the selective β_2 -adrenoceptor agonists relax the RbCC tissue through another atypical β -adrenoceptor subtype coupled to NO release from the sinusoidal endothelium.

The erectile tissue is contained within the corpora cavernosa and consists of endothelium-lined sinusoidal spaces surrounded by smooth muscle bundles. Penile erection, which follows arterial and corpus cavernosum smooth muscle relaxation, is regulated by a sequence of co-ordinated physiological, neurological and vascular events (Lue, 2000). The pattern of contraction and relaxation of penile cavernosal smooth muscle is complex and regulated by sympathetic, parasympathetic, non-adrenergic non-cholinergic fibres and by endothelium-derived vasoactive substances that diffuse to the underlying muscle and influence smooth muscle tone (Andersson and Wagner, 1995). Activation of adrenergic receptors in corpus cavernosum produces either contractile response mediated by α -adrenoceptors (Diederichs et al., 1990; Costa et al., 1993) or relaxant responses mediated by β -adrenoceptors (Carati et al., 1985; Dhabuwala et al., 1985; Hedlund and Andersson, 1985; Recio et al., 1997). The participation and characterization of α_1 -adrenoceptors by using selective agonists and antagonists are well established (Traish et al., 1999). Activation of α -adrenoceptors is involved in maintenance of corpus cavernosum tone in the flaccid state and suppression of erectile activity (Andersson et al., 2000). The relaxant response mediated by β -adrenoceptors in corpus cavernosum is poorly studied and the β -adrenoceptor subtypes involved in this response are still a matter of controversy. An early study showed that both β -adrenoceptor agonists, isoproterenol and salbutamol, cause relaxation of human cavernosal preparations that is blocked by non-selective β -adrenoceptor antagonist (propranolol), but not by β_1 - (practolol) or β_2 - (butoxamine) receptor antagonists, thus suggesting the existence of atypical β -adrenoceptors in this tissue (Adaikan and Karim, 1981). Other studies suggested that β -adrenoceptors in human (Dhabuwala et al., 1985; Hedlund and Andersson, 1985; Cirino et al., 2003) and canine (Carati et al., 1985) corpus cavernosum are of the β_2 or β_3 -subtypes. On the other hand, a mixed β_1 - and β_2 -adrenoceptor population predominantly mediates

the corporeal relaxations in the horse (Recio et al., 1997). Therefore, the purpose of the present study was to characterize the population of β -adrenoceptors that mediate the relaxation of rabbit cavernosal tissue by using selective and non-selective β -agonists and antagonists, in both bioassay cascade and organ bath experiments.

Materials and Methods

Isolation and Preparation of Rabbit Corpus Cavernosum (RbCC). Male New Zealand white rabbits (2.5-3.0 kg) were anaesthetised with pentobarbital sodium (Sagatal[®], 30-40 mg/kg, i.v.) and exsanguinated via the carotid artery. Following penectomy, the RbCC was rapidly removed and immersed in Krebs solution of the following composition (mM): NaCl, 118; NaHCO₃, 25; glucose, 5.6; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄ 7H₂O, 1.17 and CaCl₂ 2H₂O, 2.5). Tissues were dissected and cleared of the tunica albuginea and surrounding tissues. All procedures were designed in accordance with the guidelines for animal care of the State University of Campinas (UNICAMP).

Bioassay Cascade. Strips of RbCC were superfused in a cascade system with warmed (37°C) and oxygenated (95%O₂ + 5%CO₂) Krebs solution at a flow rate of 5 ml min⁻¹. The tissue responses (tension of 25 mN) were detected with auxotonic levers attached to Harvard heart/smooth muscle isotonic transducers and displayed on a Watanabe multichannel pen recorder (model WTR 381). After a 60-90 min period of equilibration, RbCC strips were precontracted with noradrenaline (3 µM) in order to increase the basal tone. The tissues were continuously infused with indomethacin (5.6 µM) and 17-β-estradiol (5 µM) to inhibit the generation of prostanoids and extraneuronal uptake for catecholamines, respectively.

β-Adrenoceptor agonists (isoproterenol, metaproterenol, ritodrine, fenoterol, TA 2005, salbutamol, salmeterol, terbutaline, procaterol and BRL 37344) and other substances (glyceryl trinitrate and acetylcholine) were administered as single bolus injections (10-100 µl). N^ω-Nitro-L-arginine methyl ester (L-NAME), L-arginine, 1H-[1,2,4] oxadiazolo [4,3,-a]quinoxalin-1-one (ODQ), 9-(2-tetrahydrofuryl) adenine (SQ 22,536), sildenafil, rolipram, propranolol, atenolol, CGP 20712A, butoxamine, ICI 118,551 and tetrodotoxin were infused over RbCC tissues 25 min

before and during bolus injection of the agents above-mentioned. The relaxations induced by β -adrenoceptor agonists and other agents were expressed relative to the sub-maximal relaxation induced by glyceryl trinitrate (GTN, 1.3 nmol), which was taken as 100%.

Organ Bath. Strips of RbCC were mounted in 10-ml organ baths containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O₂ + 5% CO₂, pH 7.4. The strips were connected to force-displacement transducers, and a tension of 10 mN was applied and adjusted until equilibration was achieved. Changes in isometric force were measured using Ugo Basile transducers (Varese, Italy) and recorded in a MacLab™ data acquisition system (software Chart, version 4.0, AD Instruments, MA). After equilibration time (60 min), the RbCC strips were precontracted with phenylephrine (10 μ M) in order to increase the basal tone. Indomethacin (5.6 μ M) and 17- β -estradiol (5 μ M) were added to the bath medium to inhibit the generation of prostanoids and extraneuronal uptake for catecholamines, respectively.

The relaxations in response to each β -agonist (isoproterenol, ritodrine, TA 2005, fenoterol and metaproterenol) were calculated as percentages of the maximal changes from the steady-state contraction produced by phenylephrine in each tissue. The EC₅₀ value for each agonist was determined as the molar concentration to produce 50% of the maximal relaxation elicited by the agonist in phenylephrine-contracted tissues. All concentration-response data were evaluated for a fit to a logistics function in the form:

$$E = E_{\max} / ((1 + (10^c / 10^x)^n) + \Phi)$$

where E is the effect of above basal; E_{max} is the maximum response produced by the agonist; c is the logarithm of the EC₅₀, the concentration of agonist that produces half-maximal response; x is the logarithm of the concentration of agonist; the exponential term, n, is a curve fitting parameter that defines the slope of the concentration-response line, and Φ is the response observed in the

absence of added agonist. Nonlinear regression analysis to determine the parameters E_{\max} , $\log EC_{50}$ and n were done using GraphPad Prism (GraphPad Software, San Diego, CA) with the constraint that $\Phi = \text{zero}$. The responses for each agonist are showed as the mean and S.E. of pEC_{50} .

Schild analysis. In the experiments in which antagonists were used to characterise the functional population of β -adrenoceptors, concentration-response curves to β -adrenoceptor agonists were obtained in absence or in presence of increasing concentrations (0.1 to 10 μM) of non-selective (propranolol), β_1 -selective (atenolol, CGP 20712A) or β_2 -selective (ICI 118,551) adrenoceptor antagonists. The equilibration time for all used antagonists was 30 min. EC_{50} values were used to calculate the concentration ratio (CR), that is given by $[A']/[A]$, where $[A']$ is the EC_{50} value in the presence of the antagonist and $[A]$ is the EC_{50} in the absence of the antagonist.

The slopes values (n) were determined by plotting of $\log (CR - 1)$ versus \log molar concentration of antagonist $[B]$ by the following equation (Arunlakshana & Schild, 1959):

$$\text{Log} (CR - 1) = n \log [B] - \log K_B$$

Drugs. Acetylcholine, L-arginine, atenolol, butoxamine, 17- β -estradiol, fenoterol, indomethacin, (-)-isoproterenol, metaproterenol, N^{O} -nitro-L-arginine methyl ester (L-NAME), (-)-noradrenaline, 1H-[1,2,4] oxadiazolo [4,3,-a]quinoxalin-1-one (ODQ), (-)-phenylephrine, procaterol, propranolol, ritodrine, rolipram, salbutamol, salmeterol, terbutaline, 9-(2-tetrahydrofuryl) adenine (SQ 22,536) and tetrodotoxin were purchased from Sigma Chemical Co. (St. Louis, MO). CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol methanesulfonate) was obtained from Ciba Geigy (Basle, Switzerland). (\pm)-4-[2-[(2-(3-Chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid (BRL 37344) and (\pm)-1-[2,3-(dihydro-7-methyl-

1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI 118,551) were acquired from Research Biochemicals International (Nattick, USA). Glyceryl trinitrate (ampoules containing 1 mg ml⁻¹ in isotonic saline) and pentobarbital sodium (Sagatal[®]) were obtained from Liplha Pharmaceuticals (London, UK) and May & Baker (Dagenham, Essex, UK), respectively. Sildenafil citrate was obtained from Laboratórios Cristália (Itapira, SP, Brazil). β -Adrenoceptor agonists and test agents were stored in stock solution at -20°C and then diluted with deionized water.

Statistical Analysis. The program InStat (GraphPad Software) was used for statistical analysis. Data are expressed as the mean \pm S.E. of *n* experiments and were evaluated by non-linear regression analysis to estimate the maximum response (E_{\max}) and potency (pEC_{50}). Where appropriate, one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons post hoc test was performed. $P < 0.05$ was accepted as significant.

Results

Involvement of Nitric Oxide (NO) in the RbCC Relaxations Induced by β_2 -Adrenoceptor Agonists. The non-selective β -adrenoceptor agonist isoproterenol (3-100 nmol) caused dose-dependent RbCC relaxations (n=9; Fig. 1). Similar responses were observed for the β_2 -adrenoceptor agonists metaproterenol (3-100 nmol), ritodrine (3-100 nmol), fenoterol (3-100 nmol) and TA 2005 (3-100 nmol), whereas terbutaline, salbutamol, salmeterol and procaterol (3-100 nmol; n = 7 each) slightly relaxed the corporeal strips (about of 20%, data not shown). The selective β_3 -adrenoceptor agonist BRL 37344 (up to 150 nmol) had no effect on the RbCC tone (n = 5; data not shown).

Figure 2 shows that infusion of the NO synthesis inhibitor L-NAME (10 μ M; n=9) increased the basal tone of the preparations and markedly reduced the relaxation evoked by ACh (0.6 nmol). Similarly, L-NAME significantly reduced ($P < 0.01$) the RbCC relaxations induced by metaproterenol, ritodrine, fenoterol and TA 2005 (100 nmol each) without affecting that elicited by GTN (Table 1). The relaxation elicited by isoproterenol (100 nmol) was not affected during L-NAME infusion (Table 1). The subsequent infusion of L-arginine (300 μ M; n = 9) partially reversed the increased tone and significantly restored ($P < 0.01$) the relaxations induced by ACh, metaproterenol, ritodrine, fenoterol and TA 2005 (Table 1, Fig. 2).

The infusion of the selective inhibitor of NO-stimulated soluble guanylyl cyclase activity ODQ (10 μ M; n = 5) caused a marked increase in cavernosal smooth muscle tone and nearly abolished the RbCC relaxation induced by ACh (Table 2). Likewise, the relaxations caused by metaproterenol (30 nmol), ritodrine (30 nmol), fenoterol (30 nmol) and TA 2005 (30 nmol) were virtually abolished in the presence of ODQ (Table 2). The relaxant response elicited by

isoproterenol (30 nmol) remained unaffected following treatment with ODQ (Table 2). The GTN (1.3 nmol)-induced RbCC relaxation was significantly reduced by ODQ ($97 \pm 3\%$ inhibition).

Effect of Inhibitors of Adenylyl Cyclase and Phosphodiesterase Types 4 and 5. The infusion of the adenylyl cyclase inhibitor SQ 22,536 (10 μ M; $n = 4$) did not significantly alter the RbCC tone and virtually abolished the relaxation induced by isoproterenol (30 nmol; Table 2). The RbCC relaxations evoked by metaproterenol, ritodrine, fenoterol and TA 2005 (30 nmol each) were not significantly affected by SQ 22,536. Similarly, the GTN- and ACh-induced relaxations remained unaltered by SQ 22,536.

The relaxations induced by ritodrine, metaproterenol, fenoterol and TA 2005 were significantly potentiated by sildenafil (1 μ M, PDE5 inhibitor; $n = 6$), but unaffected by rolipram (10 μ M, PDE4 inhibitor; $n = 5$). Conversely, isoproterenol-induced relaxations were not altered by sildenafil infusion, but significantly potentiated by rolipram (Fig. 3). In addition, sildenafil, but not rolipram, enhanced relaxations elicited by ACh and GTN (data not shown). At the concentrations used above, sildenafil and rolipram significantly decreased the RbCC tone in a similar fashion (not shown).

Effect of Na⁺ Channel Blockade on the RbCC Relaxation Induced by β -Agonists. The infusion of the Na⁺ channel blocker tetrodotoxin (TTX, 1 μ M; $n = 6$) neither affected the tone of the preparations nor the relaxation induced by ACh (0.6 nmol, $92 \pm 20\%$ before and $93 \pm 22\%$ during TTX infusion). In addition, TTX did not significantly affect the relaxations induced by isoproterenol (100 nmol, $51 \pm 9\%$ before and $46 \pm 7\%$ during TTX infusion), metaproterenol (100 nmol, $40 \pm 8\%$ before and $33 \pm 6\%$ during TTX infusion), ritodrine (30 nmol, $64 \pm 10\%$ before and $58 \pm 9\%$ during TTX infusion), fenoterol (30 nmol, $70 \pm 16\%$ before and $61 \pm 12\%$

during TTX infusion) and TA 2005 (30 nmol, $83 \pm 19\%$ before and $71 \pm 15\%$ during TTX infusion).

Effects of β -Adrenoceptor Antagonists. The infusion of propranolol (1 μ M, non-selective β -adrenoceptor antagonist), ICI 118,551 (1 μ M, selective β_2 -adrenoceptor antagonist) or butoxamine (3 μ M, selective β_2 -adrenoceptor antagonist) significantly inhibited the isoproterenol- (100 nmol) induced relaxations, but failed to affect those evoked by metaproterenol, ritodrine, fenoterol and TA 2005 (100 nmol each; Table 3). The infusion of atenolol (1 μ M, selective β_1 -adrenoceptor antagonist) had no significant effect on the relaxations induced by acetylcholine, isoproterenol, metaproterenol, ritodrine, fenoterol and TA 2005 (Table 3). Similar results were observed with CGP 20712A (1 μ M, selective β_1 -adrenoceptor antagonist; not shown). All of these β -adrenoceptor antagonists had no effect on the relaxation induced by GTN and ACh.

Organ Bath Experiments: Characterisation of β -Adrenoceptor Population Mediating Relaxation in RbCC. Cumulative concentration-response curves (0.01-100 μ M) were constructed for each β -adrenoceptor agonist in the RbCC and the rank order of potencies (pEC_{50}) was: isoproterenol (5.78 ± 0.02) > TA 2005 (4.38 ± 0.02) = ritodrine (4.30 ± 0.09) = fenoterol (4.19 ± 0.05) > metaproterenol (3.94 ± 0.09). The maximal responses (E_{max}), calculated as percentages of phenylephrine-induced contraction, were $14.3 \pm 0.5\%$, $24.1 \pm 0.9\%$, $43.4 \pm 2.7\%$, $46.7 \pm 2.4\%$ and $56.0 \pm 3.5\%$ relaxation for metaproterenol, isoproterenol, fenoterol, ritodrine and TA 2005, respectively ($n = 4$).

Propranolol and ICI 118,551 (0.1-10 μ M; $n = 4$ each) caused a rightward shift in the concentration-response curves to isoproterenol with a decrease of maximal responses when the highest concentration (10 μ M) of both antagonists was used (Fig. 4). The plot of Schild

regression revealed slope values statistically different from unity for both propranolol (0.73 ± 0.04) and ICI 118,551 (0.58 ± 0.09). On the other hand, propranolol and ICI 118,551 (0.1-10 μM) did not affect the concentration-response curves to metaproterenol, ritodrine, TA 2005 and fenoterol in the cavernosal tissues (Table 4; $n = 4$). Atenolol (0.1-10 μM ; $n = 4$) had no effect in the concentration-response curves to isoproterenol and for all used β_2 -adrenoceptor agonists (Table 4). Similarly, CGP 20712A (0.1-10 μM ; $n = 4$) had no effect in the concentration-response curves to isoproterenol and for all used β_2 -adrenoceptor agonists (not shown).

Discussion

Our findings show that the NO-cGMP pathway is clearly involved in the RbCC relaxations induced by the selective β_2 -adrenoceptor agonists metaproterenol, ritodrine, fenoterol and TA 2005 whereas the cAMP pathway mediates the isoproterenol-induced relaxations. Schild analysis showed that propranolol and ICI 118,551 shift the concentration-response curves to isoproterenol in a non-competitive manner with slope values significantly different from unity. In contrast, all the classical antagonists tested (propranolol, ICI 118,551, butoxamine and atenolol) failed to antagonize the relaxations induced by the selective β_2 -adrenoceptors agonists. Collectively, these findings suggest the existence of two atypical β -adrenoceptor subtypes that mediate the relaxant response in RbCC.

It is well known that a great diversity in the potency in vascular tissue exists that depends on animal species, vessel caliber, innervation, receptor density, and second messenger pathway (Guimaraes and Moura, 2001). In our study, cumulative concentration-response curves for β -agonists in RbCC tissue showed a rank order of potencies (pEC_{50}) of isoproterenol > TA 2005 = ritodrine = fenoterol > metaproterenol, being the isoproterenol potency about of 25- to 70-fold higher compared with the selective β_2 -agonists. The pD_2 values of isoproterenol are similar to those obtained in other vascular tissues such as aorta, pulmonary and carotid arteries (Oriowo et al., 1995; Tagaya et al., 1999). On the other hand, the low pD_2 values observed for the selective β_2 -agonists (about of 4.0) suggest that RbCC may have a small density receptor population or a low efficacy of signal transduction coupling to these receptor population.

The characterization of β -adrenoceptor subtypes and the cellular transduction mechanisms by which they mediate the vasodilator response in blood vessels is a controversial matter. Our results showed that RbCC relaxations mediated by β_2 -adrenoceptor agonists metaproterenol,

ritodrine, fenoterol and TA 2005 were markedly inhibited by the non-selective NO synthesis inhibitor L-NAME, an effect completely reversed by L-arginine infusion. Similarly, the selective inhibitor of NO-stimulated soluble guanylyl cyclase ODQ nearly abolished these RbCC relaxations, strongly indicating that activation of β -adrenoceptors by β_2 -selective agonists cause RbCC relaxations through the NO-cGMP pathway. Previous studies have reported that relaxation mediated by classical β -adrenoceptors (β_1 and β_2) is endothelium-dependent in the rat mesenteric (Graves and Poston, 1993; Blankesteyn and Thien, 1993), basilar (Hempelmann and Ziegler, 1993) and pulmonary arteries (Priest et al., 1997). As opposed to β_2 -adrenoceptor agonists, the isoproterenol-induced RbCC relaxation was unaffected by L-NAME and ODQ, showing that this agonist evokes relaxation by NO-independent mechanisms. Similar findings were seen in the coronary artery where NO is not directly involved in the relaxing responses (Béa et al., 1994).

Cyclic nucleotide PDEs are enzymes responsible for the cleavage of cyclic nucleotide phosphodiester bond with production of the inactive metabolites 5'-GMP and 5'-AMP. Therefore, agents that inhibit cyclic nucleotide hydrolysis may increase the cGMP/cAMP signal and could be expected to enhance relaxation of the corporeal smooth muscle. Of the PDE isozyme families, PDE5, PDE6 and PDE9 are specific for cGMP whereas PDE4, PDE7 and PDE8 isoforms are specific for cAMP. The PDE1, PDE2, PDE3 and PDE10 hydrolyse both cGMP and cAMP (Beavo, 1995; Corbin and Francis, 1999). Sildenafil, a potent and selective PDE5 inhibitor, enhances NO-mediated relaxation in rabbit (Jeremy et al., 1997; Chuang et al., 1998) and human (Ballard et al., 1998; Moreland et al., 1998) corpus cavernosum. Furthermore, sildenafil also potentiates non-adrenergic non-cholinergic neurotransmission in bovine penile small arteries (Simonsen et al., 2001). *In vivo* experiments demonstrated that this inhibitor enhances the intracavernosal pressure caused by NO released from nitrergic fibres following

pelvic nerve stimulation in dogs (Carter et al., 1998). Accordingly, our results show that RbCC relaxations induced by metaproterenol, ritodrine, fenoterol and TA 2005 were significantly potentiated by sildenafil whereas the adenylyl cyclase inhibitor SQ 22,536 had no effect in these responses. This further corroborates our findings that activation of β -adrenoceptors by selective β_2 -agonists is mediated through a NO-cGMP pathway. The failure of sildenafil to affect the isoproterenol-induced relaxation shows that this agonist might act through an alternative pathway to generate the cellular response. This hypothesis is confirmed by using the selective PDE4 inhibitor rolipram and by the adenylyl cyclase inhibitor SQ 22,536, the former of which enhanced the relaxation evoked by isoproterenol and the latter inhibited this response. Both inhibitors had no effect in the RbCC relaxations induced by the selective β_2 -agonists. Taken together, our results reveal that stimulation of β -adrenoceptor populations in RbCC can activate distinct cellular transductional pathways involving either adenylyl cyclase stimulation with subsequent cAMP generation or the NO/cGMP pathway.

Studies using selective agonists and antagonists revealed the existence of at least three subtypes of β -adrenoceptors, namely β_1 , β_2 and β_3 (Lands et al., 1967; Emorine et al., 1989). The β -adrenoceptor population that predominantly mediates the relaxation of vascular smooth muscle is the β_2 -adrenoceptor subtype. However, in some arterial beds, the β_1 -adrenoceptors can also produce vasodilation (Ferro et al., 1993). Our present findings clearly exclude the participation of β_1 - and β_3 -adrenoceptor subtypes in the relaxing responses of RbCC since atenolol and CGP 20712A, selective β_1 -adrenoceptor antagonists, had not effect in the concentration-response curves to isoproterenol, and the selective β_3 -adrenoceptor agonist BRL 37344 failed to evoke appreciable relaxant responses in this preparation. In organ bath experiments, isoproterenol-induced relaxation was antagonised by propranolol and ICI 118,551 in a non-competitive manner

with a slope less than unity which is consistent with the concept that isoproterenol is interacting with two subtypes of β -adrenoceptors, the classical β_2 -adrenoceptor and an atypical β -adrenoceptor. Unexpectedly, the relaxant responses evoked by the selective β_2 -adrenoceptor agonists were not blocked by all four antagonists used (propranolol, atenolol, ICI 118,551, and butoxamine) indicating that the β -adrenoceptor subtype mediating this response is not the classical β_2 -adrenoceptor. The interpretation of these findings is complex since one would expect at least a partial blockade with propranolol, ICI 118,551 and butoxamine taking into consideration that data with isoproterenol indicate the existence of classical β_2 -adrenoceptors. Nevertheless, our findings suggest the existence of two atypical β -adrenoceptor subtypes mediating the relaxations in RbCC. In fact, the presence of an atypical β -adrenoceptor (β_4 ?) subtype has been demonstrated in vascular (Oriowo, 1994; Shafiei and Mahmoudian, 1999; Tagaya et al., 1999) and non-vascular smooth muscle (De Ponti et al., 1999; Horinouchi and Koike, 2001). It is thus conceivable to believe that a heterogeneous population of atypical β -adrenoceptor subtypes mediates the relaxant responses in RbCC. To test possible interference of presynaptic receptors and neurotransmitter release (Majewski, 1983) in the actions of all β -adrenoceptor agonists in RbCC, some experiments were carried out in presence of the Na^+ channel blocker tetrodotoxin. The failure of tetrodotoxin to affect the relaxing response shows that the actions of all used β -adrenoceptor agonists occur at the postjunctional level in this preparation.

In conclusion, our findings show that isoproterenol relaxes the rabbit cavernosal tissue by activating atypical β -adrenoceptors at postjunctional level coupled to adenylyl cyclase activation while the RbCC relaxations induced by metaproterenol, ritodrine, fenoterol and TA 2005 are mediated by another atypical β -adrenoceptor subtype through NO release from the sinusoidal

endothelium. We therefore present evidences supporting the existence of two novel β -adrenoceptor population in the rabbit erectile tissue.

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TABLE 1.

Effect of L-NAME (10 μ M) and L-arginine (L-Arg, 300 μ M) in the rabbit corpus cavernosum (RbCC) relaxations induced by acetylcholine (ACh, 0.6 nmol), metaproterenol (MET, 100 nmol), isoproterenol (ISO, 100 nmol), ritodrine (RIT, 100 nmol), fenoterol (FEN, 100 nmol) and TA 2005 (TA, 100 nmol). The RbCC relaxations induced by MET, ISO, RIT, FEN and TA were expressed (mean \pm S.E, $n = 9$) relative to the sub-maximal relaxation induced by glyceryl trinitrate which was taken as 100%.

RbCC Relaxation (%)			
	Control	L-NAME	L-Arg
ACh	96 \pm 7	9 \pm 3*	55 \pm 4
MET	71 \pm 8	8 \pm 5*	63 \pm 10**
RIT	106 \pm 11	42 \pm 9*	101 \pm 9**
FEN	99 \pm 13	17 \pm 6*	79 \pm 10**
TA	97 \pm 5	36 \pm 6*	85 \pm 8**
ISO	77 \pm 12	73 \pm 8	70 \pm 10

* $P < 0.01$ compared to control values of relaxation; ** $P < 0.01$ compared to values in presence of L-NAME.

TABLE 2.

Effects of the soluble guanylyl cyclase inhibitor ODQ (10 μ M, n=5) and adenylyl cyclase inhibitor SQ 22,536 (10 μ M; n=4) in the rabbit corpus cavernosum (RbCC) relaxations evoked by acetylcholine (ACh, 0.6 nmol), metaproterenol (MET, 30 nmol), isoproterenol (ISO, 30 nmol), ritodrine (RIT, 30 nmol), fenoterol (FEN, 30 nmol) and TA 2005 (TA, 30 nmol). The RbCC relaxations induced by MET, ISO, RIT, FEN and TA were expressed (mean \pm S.E) relative to the sub-maximal relaxation induced by glyceryl trinitrate, which was taken as 100%.

	RbCC Relaxation (%)			
	ODQ		SQ 22,536	
	Control	Treated	Control	Treated
ACh	86 \pm 5	4 \pm 4*	51 \pm 9	50 \pm 11
MET	83 \pm 8	10 \pm 5*	85 \pm 12	80 \pm 11
RIT	78 \pm 6	8 \pm 3*	51 \pm 10	47 \pm 7
FEN	65 \pm 9	10 \pm 3*	61 \pm 6	55 \pm 8
TA	67 \pm 8	10 \pm 4*	44 \pm 6	36 \pm 7
ISO	73 \pm 14	80 \pm 17	35 \pm 3	3 \pm 1*

* $P < 0.01$ compared to the respective control.

TABLE 3.

Effects of propranolol (1 μ M; n=6), atenolol (1 μ M; n=5), ICI 118,551 (1 μ M; n=6) and butoxamine (3 μ M; n=6) on the rabbit corpus cavernosum (RbCC) relaxations evoked by acetylcholine (ACh, 0.6 nmol), metaproterenol (MET, 100 nmol), isoproterenol (ISO, 100 nmol), ritodrine (RIT, 100 nmol), fenoterol (FEN, 100 nmol) and TA 2005 (TA, 100 nmol). The RbCC relaxations induced by MET, ISO, RIT, FEN and TA were expressed (mean \pm S.E.) relative to the sub-maximal relaxation induced by glyceryl trinitrate, which was taken as 100%.

	RbCC Relaxation (%)							
	Propranolol		Atenolol		ICI 118,551		Butoxamine	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
ACh	88 \pm 25	90 \pm 28	112 \pm 15	105 \pm 18	72 \pm 10	69 \pm 8	91 \pm 16	73 \pm 12
MET	52 \pm 11	48 \pm 9	73 \pm 7	76 \pm 8	50 \pm 8	56 \pm 10	34 \pm 4	32 \pm 6
RIT	66 \pm 10	61 \pm 8	107 \pm 10	112 \pm 13	84 \pm 11	93 \pm 15	66 \pm 6	71 \pm 9
TA	85 \pm 13	71 \pm 10	87 \pm 11	90 \pm 14	91 \pm 13	86 \pm 9	108 \pm 18	96 \pm 13
FEN	70 \pm 8	62 \pm 7	93 \pm 9	99 \pm 12	75 \pm 7	87 \pm 10	57 \pm 9	65 \pm 10
ISO	45 \pm 5	6 \pm 3*	70 \pm 8	65 \pm 11	61 \pm 12	13 \pm 4*	36 \pm 5	9 \pm 4*

* $P < 0.01$ compared to the respective control.

TABLE 4.

Potency (pEC_{50}) and maximal responses (E_{max}) of cumulative concentration-response curves (0.01-100 μ M) to β_2 -adrenoceptor agonists in rabbit corpus cavernosum (RbCC) in absence or presence of propranolol (PRO, 0.1-10 μ M), atenolol (ATE, 0.1-10 μ M) or ICI 118,551 (ICI, 0.1-10 μ M). The RbCC relaxations induced by metaproterenol (MET), ritodrine (RIT), fenoterol (FEN) and TA 2005 (TA) were expressed (mean \pm S.E.; $n = 4$) relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%.

Antagonist	FEN		TA		RIT		MET	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}
PRO control	4.0 \pm 0.1	34 \pm 4	4.4 \pm 0.1	70 \pm 9	4.2 \pm 0.1	46 \pm 4	4.0 \pm 0.1	12 \pm 5
0.1 μ M	4.0 \pm 0.1	34 \pm 3	4.3 \pm 0.1	59 \pm 7	4.1 \pm 0.1	50 \pm 4	3.8 \pm 0.3	12 \pm 4
1 μ M	4.2 \pm 0.1	37 \pm 4	4.2 \pm 0.1	61 \pm 7	4.0 \pm 0.1	50 \pm 3	3.8 \pm 0.2	13 \pm 2
10 μ M	4.2 \pm 0.1	35 \pm 3	4.2 \pm 0.1	60 \pm 6	4.1 \pm 0.2	47 \pm 3	3.7 \pm 0.1	11 \pm 2
ATE control	3.9 \pm 0.1	28 \pm 2	4.5 \pm 0.1	60 \pm 7	3.7 \pm 0.1	50 \pm 5	4.1 \pm 0.4	10 \pm 1
0.1 μ M	4.2 \pm 0.1	30 \pm 1	4.4 \pm 0.1	59 \pm 2	3.6 \pm 0.1	55 \pm 2	4.1 \pm 0.1	8 \pm 4
1 μ M	4.2 \pm 0.1	30 \pm 2	4.2 \pm 0.1	53 \pm 4	3.5 \pm 0.1	52 \pm 2	3.9 \pm 0.2	10 \pm 5
10 μ M	4.3 \pm 0.2	30 \pm 3	4.4 \pm 0.1	51 \pm 2	3.7 \pm 0.1	49 \pm 2	3.8 \pm 0.2	9 \pm 4
ICI control	4.1 \pm 0.1	51 \pm 2	4.6 \pm 0.1	65 \pm 6	3.3 \pm 0.3	45 \pm 1	4.2 \pm 0.1	13 \pm 2
0.1 μ M	4.1 \pm 0.1	57 \pm 3	4.5 \pm 0.1	60 \pm 7	3.8 \pm 0.1	50 \pm 2	4.0 \pm 0.3	11 \pm 1
1 μ M	3.8 \pm 0.1	53 \pm 2	4.4 \pm 0.1	56 \pm 4	3.6 \pm 0.2	43 \pm 2	4.0 \pm 0.2	10 \pm 1
10 μ M	3.7 \pm 0.2	46 \pm 2	4.3 \pm 0.1	51 \pm 3	3.7 \pm 0.1	39 \pm 1	4.0 \pm 0.4	9 \pm 1

Fig. 1. Dose-response curves to isoproterenol, fenoterol, ritodrine, metaproterenol, and TA 2005 in rabbit corpus cavernosum. Each point is the mean \pm S.E. of relaxation of n experiments, calculated as a percentage of the sub-maximal relaxation induced by glyceryl trinitrate (GTN, 1.3 nmol).

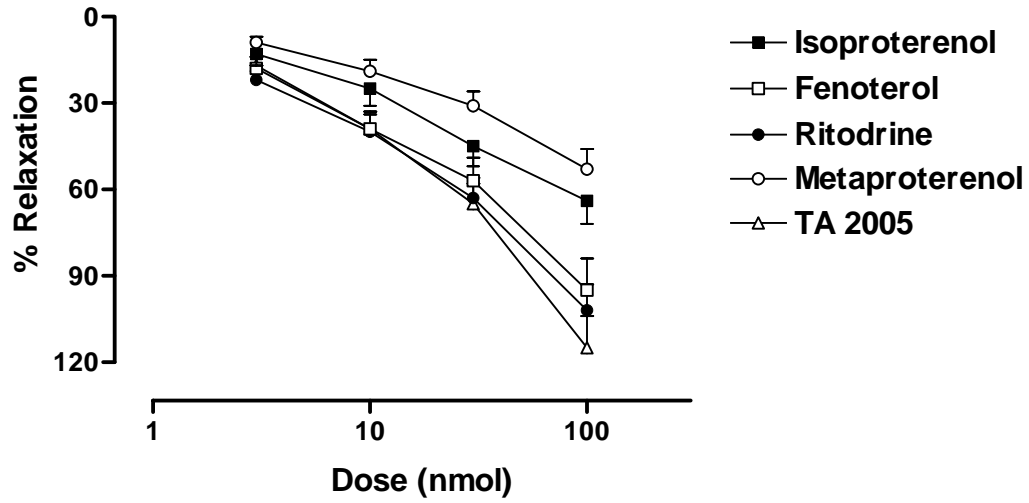
Fig. 2. Effects of L-NAME (10 μ M) and L-arginine (300 μ M) on rabbit corpus cavernosum (RbCC) strips. The infusion of L-NAME increased the RbCC tone and markedly reduced the relaxations induced by acetylcholine (ACh, 0.6 nmol), metaproterenol (MET, 100 nmol), ritodrine (RIT, 100 nmol), fenoterol (FEN, 100 nmol) and TA 2005 (TA, 100 nmol). The relaxations induced by either glyceryl trinitrate (GTN, 1.3 nmol) or isoproterenol (ISO, 100 nmol) were not significantly affected by L-NAME infusion. Subsequent infusion of L-arginine reversed the increased cavernosal tone and also significantly restored the relaxations induced by ACh and β_2 -agonists. This is a representative tracing of 9 experiments.

Fig. 3. Effects of sildenafil (1 μ M; n=6) and rolipram (10 μ M; n=5) on the rabbit corpus cavernosum (RbCC) relaxations induced by isoproterenol (ISO, 3-10 nmol), ritodrine (RIT, 3-10 nmol), metaproterenol (MET, 3-10 nmol), fenoterol (FEN, 3-10 nmol) and TA 2005 (TA, 3-10 nmol). Experimental values were calculated as a percentage of the sub-maximal relaxation induced by glyceryl trinitrate (GTN, 1.3 nmol). Data represent the mean \pm S.E. of *n* experiments.

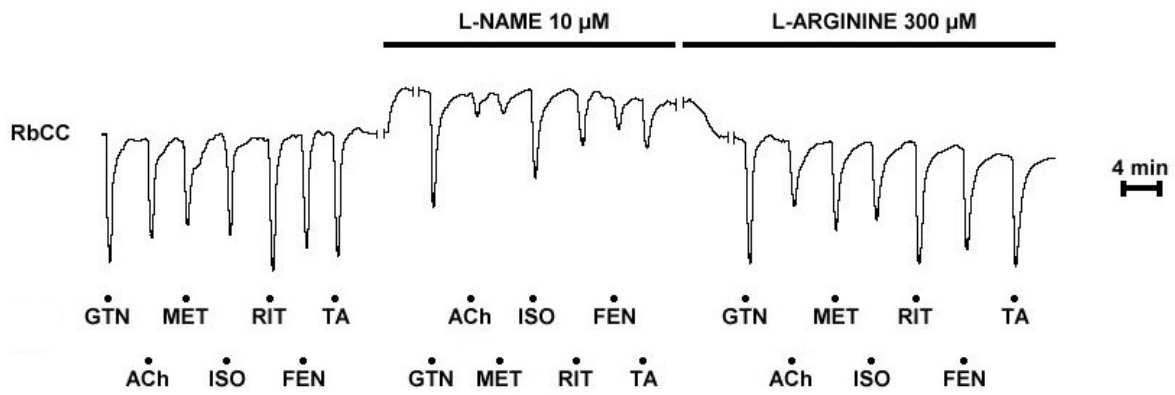
* $P < 0.05$ and ** $P < 0.01$ compared to the respective controls.

Fig. 4. Concentration-response curves to isoproterenol (0.01 – 100 μM) in absence (●) or in presence of propranolol (panel A), atenolol (panel B) and ICI 118,551 (panel C) at concentration of 0.1 μM (○), 1 μM (■) and 10 μM (□) in rabbit corpus cavernosum (RbCC) preparations. Data represent the mean \pm S.E. of relaxation of 4 experiments, calculated as a percentage of tone induced by phenylephrine.

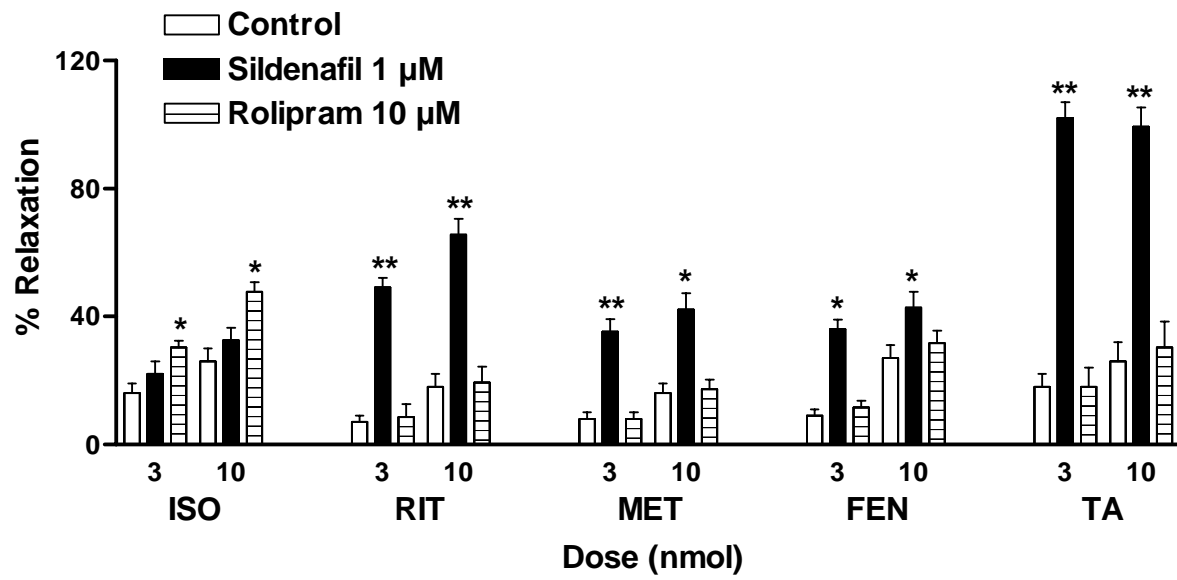
TEIXEIRA ET AL. – figure 1



TEIXEIRA ET AL. – figure 2



TEIXEIRA ET AL. – figure 3



TEIXEIRA ET AL. – figure 4

