Atypical β-Adrenoceptor Subtypes Mediate Relaxations of Rabbit Corpus Cavernosum

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ABSTRACT

This study was performed to characterize the β-adrenoceptor population in rabbit isolated corpus cavernosum (RbCC) by using nonselective and selective β-adrenoceptor agonists and antagonists in functional assays. Metaproterenol, ritodrine, fenoterol, and 8-hydroxy-5-[(1R)-1-hydroxy-2-[N-[1R)-2-(p-methoxyphenyl)-1-methylethyl]amino]ethyl]carbostyril (TA 2005) (3–100 nmol each) dose dependently relaxed the RbCC preparations. These relaxations were markedly reduced by Nω-nitro-L-arginine methyl ester (L-NAME; 10 μM) and 1H-[1,2,4]-oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) (10 μM), whereas the adenylyl cyclase inhibitor SQ 22,536 [9-(2-tetrahydrofuryl) adenine] (10 μM) had no effect. In contrast, neither L-NAME nor ODQ affected the isoproterenol-induced relaxations, but SQ 22,536 abolished this response. Sildenafil (1 μM) significantly potentiated the relaxations induced by β2-agonists without affecting the isoproterenol-evoked relaxations. Rolipram (10 μM) enhanced the relaxations elicited by isoproterenol but had no effect on those induced by the selective β2 agonists. Propranolol and (-)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yi)oxy]-3-[1-methyl(di)amino]-2-butanol hydrochloride (ICI 118,551) determined a rightward shift in the concentration-response curves to isoproterenol in a noncompetitive manner with a reduction of maximum response at the highest antagonist concentration, with the slope values significantly different from unity. Propranolol and with ICI 118,551 had no effect on the relaxations elicited by fenoterol, TA 2005, metaproterenol, and ritodrine. Atenolol and 1-[2-[(3-carbamoyl-4-hydroxy)phenoxyl]ethyl]amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol methanesulfonate (CGP 20712A) (0.1–10 μM) 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 β2-adrenoceptor agonists relax the RbCC tissue through another atypical β-adrenoceptor subtype coupled to nitric oxide 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 coordinated 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, and nonadrenergic noncholinergic fibers, 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 α1-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-adreno-

ABBREVIATIONS: RbCC, rabbit corpus cavernosum; TA 2005, 8-hydroxy-5-[(1R)-1-hydroxy-2-[N-[1R)-2-(p-methoxyphenyl)-1-methylethyl]amino]ethyl]carbostyril; BRL 37344, (-)-4-[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxyacetic acid; L-NAME, Nω-nitro-L-arginine methyl ester; ODQ, 1H-[1,2,4]-oxadiazolo-[4,3-a]quinoxalin-1-one; SQ 22,536, 9-(2-tetrahydrofuryl) adenine; CGP 20712A (1-[2-[(3-carbamoyl-4-hydroxy)phenoxyl]ethyl]amino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol methanesulfonate); ICI 118,551, (-)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yi)oxy]-3-[1-methyl(di)amino]-2-butanol hydrochloride; GTN, glyceryl trinitrate; NO, nitric oxide; ACh, acetylcholine; TTX, tetrodotoxin; PDE, phosphodiesterase.
ceptors 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 β-adrenoceptor agonists isoproterenol and salbutamol cause relaxation of human cavernosal preparations that is blocked by nonselective β-adrenoceptor antagonist (pranopanol), but not by β₁- (practolol) or β₂- (butoxamine)-receptor antagonists, thus suggesting the existence of atypical β-adrenoceptors in this tissue (Adakan 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 β₂- or β₁-subtypes. On the other hand, a mixed β₁- and β₂-adrenoceptor population predominantly mediates the corporo real 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 nonselective 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 anesthetized with pentobarbital sodium (Sagatal, 30–40 mg/kg i.v.) and exsanguinated via the carotid artery. After penectomy, the RbCC was rapidly removed and immersed in Krebs' solution of the following composition: 118 mM NaCl, 25 mM NaHCO₃, 5.6 mM glucose, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.17 mM MgSO₄·7H₂O, 2.5 mM CaCl₂·2H₂O. 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.

**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 isometric transducers and displayed on a Watanabe multichannel pen recorder (model WTR 381). After a 60- to 90-min period of equilibration, RbCC strips were precontracted with noradrenaline (3 μM) 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 (-)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid (BRL 37344)] and other substances (glyceryl trinitrate, L-NAME, and Nω-nitro-L-arginine methyl ester (L-NAME), l-arginine, 1H-[1,2,4]oxidiazolo[4,3-a]quinazolin-1-one (ODQ), L-NAME, and Nω-nitro-L-arginine methyl ester (L-NAME), l-arginine, 1H-[1,2,4]oxidiazolo[4,3-a]quinazolin-1-one (ODQ), 9-(2-tetrahydrofuryl) adenine (SQ 22,536), sildenafil, rolipram, propranol, atenolol, 1-(2-[(3-carbamoyl-4-hydroxy)phenoxy]ethylnamino)-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-2-propanol methanesulfonate (CGP 20712A), atenolol, 1-(2-[(3-carbamoyl-4-hydroxy)phenoxy]ethylnamino)-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-2-propanol [CGP 20712A], atenolol, 1-(2-[(3-carbamoyl-4-hydroxy)phenoxy]ethylnamino)-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-2-propanol methanesulfonate (CGP 20712A), butoxamine, (-)-1-[(2,3-dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[1-methylthyl]aminol]-2-butanol hydrochloride (ICI 118,551), and tetrodotoxin were purchased from Sigma-Aldrich.

**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 and 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 transducers (Ugo Basile, Varese, Italy) and recorded in a MacLab data acquisition system (software Chart, version 4.0; AD Instruments, Colorado Springs, CO). After equilibration time (60 min), the RBCC strips were precontracted with phenylephrine (10 μM) to increase the basal tone. Indomethacin (5.6 μM) and 17-β-estradiol (5 μM) 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:

\[
E = E_{\text{max}}/(1 + (10^{[\log EC_{50} - \log A]}/[A]) + \Phi)
\]

where \(E\) is the effect of above basal; \(E_{\text{max}}\) 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 \(\Phi\) is the response observed in the absence of added agonist. Nonlinear regression analysis to determine the parameters \(E_{\text{max}}, \log EC_{50}\) and \(n\) were done using GraphPad Prism (GraphPad Software Inc., San Diego, CA) with the constraint that \(\Phi = 0\). The responses for each agonist are shown as the mean and S.E. of pEC₅₀.

**Schild Analysis.** In the experiments in which antagonists were used to characterize the functional population of β-adrenoceptors, concentration-response curves to β-adrenoceptor agonists were obtained in absence or in presence of increasing concentrations (0.1–10 μM) of nonselective (propranolol), β₁-selective (atenolol and CGP 20712A), or β₂-selective (ICI 118,551) adrenoceptor antagonists. The equilibration time for all used antagonists was 30 min. EC₅₀ values were used to calculate the concentration ratio (CR), that is given by \([A]/[A]\), where \([A]\) is the EC₅₀ value in the presence of the antagonist and \([A]\) is the EC₅₀ 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 and Schild, 1959):

\[\log(CR-1) = n\log[B] - \log K_B\]

**Drugs.** Acetylcholine, l-arginine, atenolol, butoxamine, 17-β-estradiol, fenoterol, indomethacin, (-)-isoproterenol, metaproterenol, l-NAME, (-)-noradrenaline, ODQ, (-)-phenylephrine, procaterol, propranolol, ritodrine, rolipram, salbutamol, salmeterol, terbutaline, SQ 22,536, and tetrodotoxin were purchased from Sigma-Aldrich.

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**Fig. 1.** Dose-response curves to isoproterenol, fenoterol, ritodrine, metaprotenerol, and TA 2005 in rabbit corpus cavernosum. Each point is the mean ± S.E. of relaxation of n experiments, calculated as a percentage of the submaximal relaxation induced by GTN (1.3 nmol).
Results

Involvement of Nitrergic Oxide (NO) in the RbCC Relaxations Induced by \( \beta \)-Adrenoceptor Agonists. The nonselective \( \beta \)-adrenoceptor agonist isoproterenol (3–100 nmol) caused dose-dependent RbCC relaxations (\( n = 9 \); Fig. 1). Similar responses were observed for the \( \beta \)-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 \( \beta \)-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 \( \mu \)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 methaprotenerol, ritodrine, fenoterol, and TA 2005 (100 nmol each) without affecting that elicited by GTN (Table 1). The relaxation elicited by isoprotenerol (100 nmol) was not affected during L-NAME infusion (Table 1). The subsequent infusion of L-arginine (300 \( \mu \)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 \( \mu \)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 metaprotenerol (30 nmol), ritodrine (30 nmol), fenoterol (30 nmol), and TA 2005 (30 nmol) were virtually abolished in the presence of ODQ (Table 2). The GTN (1.3 nmol)-induced RbCC relaxation was significantly reduced by ODQ (97 \( \pm \)3\% inhibition).

Table 1

<table>
<thead>
<tr>
<th>Control</th>
<th>L-NAME</th>
<th>L-Arg</th>
</tr>
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<tbody>
<tr>
<td>ACh</td>
<td>96 ( \pm ) 7</td>
<td>9 ( \pm ) 3*</td>
</tr>
<tr>
<td>MET</td>
<td>71 ( \pm ) 8</td>
<td>8 ( \pm ) 5*</td>
</tr>
<tr>
<td>RIT</td>
<td>106 ( \pm ) 11</td>
<td>42 ( \pm ) 9*</td>
</tr>
<tr>
<td>FEN</td>
<td>99 ( \pm ) 13</td>
<td>17 ( \pm ) 6*</td>
</tr>
<tr>
<td>TA</td>
<td>97 ( \pm ) 5</td>
<td>36 ( \pm ) 6*</td>
</tr>
<tr>
<td>ISO</td>
<td>77 ( \pm ) 12</td>
<td>73 ( \pm ) 8</td>
</tr>
</tbody>
</table>

* \( P < 0.01 \) compared with control values of relaxation; **\( P < 0.01 \) compared with values in presence of L-NAME.

Table 2

<table>
<thead>
<tr>
<th>ODQ</th>
<th>SQ 22,536</th>
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<tbody>
<tr>
<td>ACh</td>
<td>86 ( \pm ) 5</td>
</tr>
<tr>
<td>MET</td>
<td>83 ( \pm ) 8</td>
</tr>
<tr>
<td>RIT</td>
<td>78 ( \pm ) 6</td>
</tr>
<tr>
<td>FEN</td>
<td>65 ( \pm ) 9</td>
</tr>
<tr>
<td>TA</td>
<td>67 ( \pm ) 8</td>
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<tr>
<td>FEN</td>
<td>65 ( \pm ) 9</td>
</tr>
<tr>
<td>TA</td>
<td>67 ( \pm ) 8</td>
</tr>
</tbody>
</table>

* \( P < 0.01 \) compared with the respective control.
TABLE 3

<table>
<thead>
<tr>
<th>Propranolol</th>
<th>Atenolol</th>
<th>ICI 118,551</th>
<th>Butoxamine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>Treated</strong></td>
<td><strong>Control</strong></td>
<td><strong>Treated</strong></td>
</tr>
<tr>
<td>ACh</td>
<td>88 ± 25</td>
<td>90 ± 28</td>
<td><strong>112 ± 15</strong></td>
</tr>
<tr>
<td>MET</td>
<td>52 ± 11</td>
<td>48 ± 9</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>RIT</td>
<td>66 ± 10</td>
<td>61 ± 8</td>
<td><strong>107 ± 10</strong></td>
</tr>
<tr>
<td>TA</td>
<td>85 ± 13</td>
<td>71 ± 10</td>
<td>87 ± 11</td>
</tr>
<tr>
<td>FEN</td>
<td>70 ± 8</td>
<td>62 ± 7</td>
<td><strong>93 ± 9</strong></td>
</tr>
<tr>
<td>ISO</td>
<td>45 ± 5</td>
<td>6 ± 3</td>
<td><strong>70 ± 8</strong></td>
</tr>
</tbody>
</table>

* P < 0.01 compared with the respective controls.

Effects of β-Adrenoceptor Antagonists. The infusion of propranolol (1 μM, nonselective β-adrenoceptor antagonist), ICI 118,551 (1 μM, selective β2-adrenoceptor antagonist), or butoxamine (3 μM, selective β2-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 μM, selective β1-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 μM, selective β1-adrenoceptor antagonist; data not shown). All of these β-adrenoceptor antagonists had no effect on the relaxation induced by GTN and ACh.

Organ Bath Experiments: Characterization of β-Adrenoceptor Population Mediating Relaxation in RbCC. Cumulative concentration-response curves (0.01–100 μM) were constructed for each β-adrenoceptor agonist in the RbCC and the rank order of potencies (pEC50) 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 (Emax) calculated as percentages of phenylephrine-induced contraction, were 14.3 ± 0.5, 24.1 ± 0.9, 43.4 ± 2.7, 46.7 ± 2.4, and 56.0 ± 3.5% for metaproterenol, isoproterenol, fenoterol, ritodrine, and TA 2005, respectively (n = 4).

Propranolol and ICI 118,551 (0.1–10 μM; n = 4 each) caused a rightward shift in the concentration-response

![Fig. 3. Effects of sildenafil (1 μM; n = 6) and rolipram (10 μM; n = 5) on the RbCC relaxations induced by isoproterenol (ISO; 3–10 nmol), metaproterenol (MET; 3–10 nmol), isoproterenol (ISO; 100 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 submaximal relaxation induced by GTN (1.3 nmol). Data represent the mean ± S.E. of n experiments. *, P < 0.05 and **, P < 0.01 compared with the respective controls.](image-url)
curves to isoproterenol with a decrease of maximal responses when the highest concentration (10 μM) of both antagonists was used (Fig. 4). The plot of Schönd 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 β₂-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 β₂-adrenoceptor agonists (data not shown).

Discussion

Our findings show that the NO-cGMP pathway is clearly involved in the RbCC relaxations induced by the selective β₂-adrenoceptor agonists metaproterenol, ritodrine, fenoterol, and TA 2005, whereas the cAMP pathway mediates the isoproterenol-induced relaxations. Schönd analysis showed that propranolol and ICI 118,551 shift the concentration-response curves to isoproterenol in a noncompetitive 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 β₂-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-adrenoceptors in RbCC tissue showed a rank order of potencies (pEC₅₀) of isoproterenol > TA 2005 = ritodrine = fenoterol > metaproterenol, being the isoproterenol potency about of 25- to 70-fold higher compared with the selective β₂-adrenoceptors. The pD₂ 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 pD₂ values observed for the selective β₂-adrenoceptors (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 populations.

The characterization of β-adrenoceptor subtypes and the cellular transduction mechanisms by which they mediate the vasodilatator response in blood vessels is a controversial matter. Our results showed that RbCC relaxations mediated by β₂-adrenoceptor agonists metaproterenol, ritodrine, fenoterol, and TA 2005 were markedly inhibited by the nonselective 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 β₂-selective agonists cause RbCC relaxations through the NO-cGMP pathway. Previous studies have reported that relaxation mediated by classical β-adrenoceptors (β₁ and β₂) is endothelium-dependent in the rat mesenteric (Graves and Poston, 1993; Blankesteijn and Thien, 1993), basilar (Hempelmann and Ziegler, 1993), and pulmonary arteries (Priest et al., 1997). As opposed to β₂-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 pro-

Fig. 4. Concentration-response curves to isoproterenol (0.01–100 μM) in absence (○) or in presence of propranolol (A), atenolol (B) and ICI 118,551 (C) at concentration of 0.1 μM (○), 1 μM (■), and 10 μM (□) in RbCC preparations. Data represent the mean ± S.E. of relaxation of four experiments, calculated as a percentage of tone induced by phenylephrine.
duction 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 isozymes are specific for cAMP. The PDE1, PDE2, PDE3, and PDE10 hydrolyze 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 nonadrenergic noncholinergic neurotransmission in bovine penile small arteries (Simsen et al., 2001). In vivo experiments demonstrated that this inhibitor enhances the intracavernosal pressure caused by NO released from nitric fibers after 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 β2-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 β2-agonists. 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, β1, β2, and β3 (Lands et al., 1967; Emorine et al., 1989). The β-adrenoceptor population that predominantly mediates the relaxation of vascular smooth muscle is the β2-adrenoceptor subtype. However, in some arterial beds, the β1-adrenoceptors can also produce vasodilation (Ferro et al., 1993). Our present findings clearly exclude the participation of β1- and β2-adrenoceptor subtypes in the relaxing responses of RbCC because atenolol and CGP 20712A, selective β1-adrenoceptor antagonists, had not effect in the concentration-response curves to isoproterenol, and the selective β2-adrenoceptor agonist BRL 37344 failed to evoke appreciable relaxant responses in this preparation. In organ bath experiments, isoproterenol-induced relaxation was antagonized by propranolol and ICI 118,551 in a noncompetitive 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 β2-adrenoceptor and an atypical β-adrenoceptor. Unexpectedly, the relaxant responses evoked by the selective β2-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 β2-adrenoceptor. The interpretation of these findings is complex because 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 β2-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 (β2·?) subtype has been demonstrated in vascular (Oriowo, 1994; Shafiei and Mahmoudian, 1999; Tagaya et al., 1999) and nonvascular 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, whereas 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 evidence supporting the existence of two novel β-adrenoceptor population in the rabbit erectile tissue.

References

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