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-methoxy-phenyl)-1-methylethyl]amino][ethyl]carbostyril (TA 2005) (3–100 nmol each) dose dependently relaxed the RbCC preparations. These relaxations were markedly reduced by N^+-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 RbCC relaxations, but SQ 22,536 abolished this response. Sildenafil (1 μM) significantly potentiated the relaxations induced by β_2-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 β_2 agonists. Propranolol and (-)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl]oxy]-3-[1-methylethyl]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] ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-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 β_2-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 cavernous 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 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-adreno-

ABBREVIATIONS: RbCC, rabbit corpus cavernosum; TA 2005, 8-hydroxy-5-[(1R)-1-hydroxy-2-[N-[(1R)-2-(p-methoxy-phenyl)-1-methylethyl]amino][ethyl]carbostyril; BRL 37344, (-)-4-[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxyacetic acid; l-NAME, N^+-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]ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-2-propanol methanesulfonate); ICI 118,551, (-)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl]oxy]-3-[1-methylethyl]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 (propranolol), but not by β1- (practolol) or β2- (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 β2- or β2-subtypes. On the other hand, a mixed β1- and β2-adrenoceptor population predominantly mediates the corporo-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 warm (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- 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 17β-estradiol (5 μM) to inhibit the generation of prostanooids and extra-neuronal uptake for catecholamines, respectively.

β-Adrenoceptor agonists (isoproterenol, metaproterenol, ritodrine, fenoterol, TA 2005, salbutamol, salmeterol, terbutaline, procaterol, and (±)-4-[2-(3-chlorophenyl)-2-hydroxyethylaminopropyl][phenoxacyetic acid (BRL 37344)] and other substances (glyceryl trinitrate and acetylcholine) were administered as single bolus injections (10–100 μl). N’-Nitro-l-arginine methyl ester (l-NNAME), 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, 1-[2-[(3-carbamoyl-4-hydroxy)phenoxy]ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxyl]-2-propanol methanesulfonate (CGP 20712A), butoxamine, (±)-1-[2-(3-diisocyanato-7-methyl-1H-inden-4-yl)oxy]-3-[1-methylthethylamino]-2-butanol hydrochloride (ICI 118,551), and tetrodotoxin were infused over RbCC tissues 25 min before and during bolus injection of the agents mentioned above. The relaxations induced by β-adrenoceptor agonists and other agents were expressed relative to the submaximal 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 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 17β-estra-diol (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:

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

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 \( n \) was done using GraphPad Prism (GraphPad Software Inc., San Diego, CA) with the constraint that \( \Phi = 0 \). The responses for each agonist are showed 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 \([A]/[A]_0 \), where \([A]_0 \) is the EC50 value in the absence 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, 17β-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 (St.
Infusion. Subsequent infusion of L-arginine reversed the increased cavernosal tone and also significantly restored the relaxations induced by ACh and β2-agonists. This is a representative tracing of nine experiments.

Statistical Analysis. The program InStat (GraphPad Software Inc.) was used for statistical analysis. Data are expressed as the mean ± S.E. of n experiments and were evaluated by nonlinear regression analysis to estimate the maximum response (Emax) and potency (pEC50). Where appropriate, one-way analysis of variance 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 β2-Adrenoceptor Agonists. The nonselective β-adrenoceptor agonist isoproterenol (3–100 nmol) caused dose-dependent RbCC relaxations (n = 9; Fig. 1). Similar responses were observed for the β2-adrenoceptor agonists metaproterenol (3–100 nmol), ritodrine (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 β2-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 μM; 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 μM; 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 μM; 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 GTN (1.3 nmol)-induced RbCC relaxation was significantly reduced by ODQ (97 ± 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 μM; n = 4) did not significantly alter the RbCC tone and virtually abolished the relaxations induced by ACh, metaproterenol, ritodrine, fenoterol, and TA 2005 (100 nmol each; Table 1). 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 GTN (1.3 nmol)-induced RbCC relaxation was significantly reduced by ODQ (97 ± 3% inhibition).

Table 1 shows that the infusion of the soluble guanylyl cyclase inhibitor ODQ (10 μM; n = 5) and adenylyl cyclase inhibitor SQ 22,536 (10 μM; n = 4) in RbCC relaxations evoked by 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) RbCC relaxations induced by MET, ISO, RIT, FEN, and TA were expressed (mean ± S.E.; n = 9) relative to the submaximal relaxation induced by glyceryl trinitrate, which was taken as 100%.

Table 2 shows that the infusion of the soluble guanylyl cyclase inhibitor ODQ (10 μM; n = 5) and adenylyl cyclase inhibitor SQ 22,536 (10 μM; n = 4) in RbCC relaxations evoked by 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) RbCC relaxations induced by MET, ISO, RIT, FEN, and TA were expressed (mean ± S.E.; n = 9) relative to the submaximal relaxation induced by glyceryl trinitrate, which was taken as 100%.

*P < 0.01 compared with control values of relaxation; **P < 0.01 compared with values in presence of L-NAME.
Effects of propranolol (1 μM; n = 6), atenolol (1 μM; n = 5), ICI 118,551 (1 μM; n = 6), and butoxamine (3 μM; n = 6) on RbCC relaxations evoked by 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) on RbCC relaxations induced by MET, ISO, RIT, FEN, and TA were expressed (mean ± S.E.) relative to 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.

Effects of Na+ Channel Blockade on the RbCC Relaxation Induced by β-Agonists. The infusion of the Na+ channel blocker tetrodotoxin (TTX, 1 μM; n = 6) neither affected the tone of the preparations nor the relaxation induced by ACh (0.6 nmol, 92 ± 20% before and 93 ± 22% during TTX infusion). In addition, TTX did not significantly affect the relaxations induced by isoproterenol (100 nmol, 51 ± 9% before and 46 ± 7% during TTX infusion), metaproterenol (100 nmol, 40 ± 8% before and 53 ± 6% during TTX infusion), ritodrine (30 nmol, 64 ± 10% before and 58 ± 9% during TTX infusion), fenoterol (30 nmol, 70 ± 16% before and 61 ± 12% during TTX infusion), and TA 2005 (30 nmol, 83 ± 19% before and 71 ± 15% during TTX infusion).

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 β1-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% relaxation 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
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 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 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, and 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 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 tissue. 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; Blanksteijn and Thien, 1993), basilar (Hempelmann and Ziegler, 1993), and pulmonary arteries (Priet 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-
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 corporal 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 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 (Silinskas et al., 2001). In vivo experiments demonstrated that this inhibitor enhances the intracavernosal pressure caused by NO released from nitricergic 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 mediated by type 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; Shafiee 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.

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**TABLE 4** Potency (pEC50) and maximal responses (E_max) of cumulative concentration-response curves (0.01–100 µM) to β1- and β2-adrenoceptor agonists in RbCC in absence or presence of propranolol (PRO; 0.1–10 µM), atenolol (ATE; 0.1–10 µM), or ICI 118,551 (ICI; 0.1–10 µM). RbCC relaxations induced by metaproterenol (MET), ritodrine (RIT), fenoterol (FEN), and TA 2005 (TA) were expressed (mean ± S.E.; n = 4) relative to the maximal change from the contraction produced by phenylephrine in each tissue, which was taken as 100%.
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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