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European Journal of Pharmacology 519 (2005) 146 – 153

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Effects of β-adrenoceptor antagonists in the neural nitric oxide release induced by electrical field stimulation and sodium channel activators in the rabbit corpus cavernosum

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Received 17 March 2005; received in revised form 1 July 2005; accepted 8 July 2005
Available online 8 August 2005

Abstract

β-Adrenoceptor antagonists may present receptor-independent mechanisms, such as blockade of voltage-gated sodium channels. This study aimed to investigate the effects of non-selective (propranolol), and selective β_1- (atenolol, metoprolol and betaxolol) and β_2-adrenoceptor (ICI 118,551) antagonists in the nitric oxide (NO)-mediated rabbit corpus cavernosum relaxations induced by either electrical field stimulation (EFS) or activators of voltage-gated sodium channels. The sodium channel blockers tetrodotoxin and saxitoxin abolished the relaxations induced by EFS or sodium channel activators of binding site-2 (aconitine and veratridine), site-3 (Ts3 toxin), site-4 (Ts1 toxin) and site-5 (brevetoxin-3). The β-adrenoceptor antagonists failed to affect the relaxations induced by EFS, aconitine and veratridine. Relaxations induced by Ts3 and Ts1 toxins, as well as brevetoxin-3, were markedly reduced by prior addition of propranolol, betaxolol and ICI 118,551. During the established relaxation induced by Ts3 toxin, propranolol failed to restore the basal tone. In conclusion, β-adrenoceptor antagonists may cause an allosteric inhibition at the binding site-3, -4 and -5 of voltage-gated sodium channels, leading to blockade of neural NO release.
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Keywords: β-Adrenoceptor antagonist; Nitrergic nerve; Nitric oxide; Sodium channel activator; *Tityus serrulatus* venom

1. Introduction

Nitric oxide (NO) has been recognized as the main transmitter released from sinusoidal endothelium and nitrergic nerves involved in initiating and maintaining erection (see Andersson, 2001). Stimulation of cavernous nerves in anaesthetized animals causes penile erection due to NO-mediated increases in intracavernosal pressure (Trigo-Rocha et al., 1993; Escrig et al., 1999; Burnett et al., 2002). Furthermore, in vitro studies reported that corpora cavernosa from humans and other animal species relax to transmural stimulation, and that the relaxations are sensitive to both NO synthesis inhibitors and voltage-gated sodium channel blockers (Ignarro et al., 1990; Knispel et al., 1992; Holmquist et al., 1992). Once release, NO acts to activate soluble guanylyl cyclase in cavernosal smooth muscle to increase cGMP levels, thus causing corpus cavernosum relaxation and penile erection.

Voltage-gated sodium channels are responsible for the initial inward current during the depolarization phase of action potential in excitable cells. They are transmembrane proteins consisting of a pore-forming α subunit of 260 kDa associated with auxiliary subunits, namely β_1, β_2 and β_3 (see Denac et al., 2000). Sodium channels are the molecular targets for several groups of neurotoxins known to interact with specific, allosterically coupled binding sites, which alter channel function by binding to specific receptor sites (see Denac et al., 2000), thus providing valuable tools to elucidate the mechanisms underlying nerve depolarization

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doi:10.1016/j.ejphar.2005.07.011

and NO release. Site-1 is thought to be located in the vestibule of the channel, and binding of water soluble toxins, such as tetrodotoxin and saxitoxin, directly leads to inhibition of ion conductance. Site-2 is located in the inner side of the transmembrane region, and is involved in the gating of the channel. Lipophilic toxins such as veratridine and aconitine bind at this site causing a persistent activation of the channel. Scorpion venom toxins also bind with high affinity to specific sites in the outer surface of the Na^+ channels. These toxins are divided into two subgroups according to their effect on voltage-gated sodium channel, causing either blockade of channel inactivation by binding to receptor site-3 (α-toxins, e.g. Ts3) or shifting the voltage dependence of Na^+ channel to more negative membrane potentials through binding to site-4 (β-toxins, e.g. Ts1). Receptor site-5 is also found in the extracellular region of the channel and binds the lipid-soluble polyether marine toxins brevetoxins and ciguatoxins, agents that cause repetitive neuronal firing, shift the voltage-dependence and block Na^+ channel inactivation.

Scorpion venoms are able to release NO from nitrergic terminals in corpus cavernosum from rabbits and humans, all these actions being blocked by the voltage-gated sodium channel blocker tetrodotoxin (Teixeira et al., 1998, 2001a,b). One of the scorpion toxins responsible for the neural NO release in cavernosal tissue was recently identified as Ts3 scorpion α-toxin, and fully characterized in both the rabbit and human corpus cavernosum (Teixeira et al., 2003, 2004). Furthermore, neurotoxins such as alkaloids (veratridine, aconitine, batrachotoxin), scorpion β-toxin (Ts1) and polyether marine toxin (brevetoxin-3) relax the rabbit corpus cavernosum as a consequence of nerve-mediated NO release and smooth muscle cGMP accumulation (de Oliveira et al., 2003).

It is well-established that β-adrenoceptor antagonists act primarily by blocking adrenoceptors in diverse target tissues, both in the central nervous system and the periphery. Besides, β-adrenoceptor antagonists such as propranolol (non-selective β-adrenoceptor antagonist) can block the voltage-gated sodium channel in different tissues via receptor-independent mechanisms, and this takes part of the so-called membrane-stabilizing activity of this class of drugs (Jaeger et al., 1979; Matthews and Baker, 1982). These mechanisms appear to potentially contribute, at least in part, to its antiarrhythimic therapeutic actions (Daugherty et al., 1986; Marano et al., 2002). More recently, the β_1-adrenoceptor antagonist betaxolol has been shown to reduce Na^+ influx in rat cortical synaptosomes (Chidlow et al., 2000). Given that β-adrenoceptor antagonists may inhibit voltage-gated sodium channel activity, we tested the hypothesis that β-adrenergic antagonists inhibit NO release in corpus cavernosum not by passive occupancy of pre-synaptic β-receptors, but by combination with a membrane gating system and alteration of depolarization-associated Na^+ influx. Therefore, the aim of this study was to examine the effects of non-selective (propranolol), β_1- (atenolol, metoprolol and betaxolol) and β_2-adrenoceptor antagonists (ICI 118,551) in the neural NO-mediated rabbit corpus cavernosum relaxation elicited by voltage-gated sodium channel activation, triggered by electrical field stimulation (EFS) or neurotoxins that activate Na^+ channels (aconitine, veratridine, brevetoxin-3, and the scorpion Ts1 and Ts3 toxins).

2. Materials and methods

2.1. Isolation and preparation of rabbit corpus cavernosum

The experimental protocols here employed have been approved by the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation (COBEA). Briefly, male New Zealand white rabbits (2.5–3.0 kg) were anaesthetized with pentobarbital sodium (Hypnol®, 30–40 mg/kg, i.v.) and exanguinated via the carotid artery. The penis was removed at the level of attachment of the corporeal body to the ischium and immersed in Krebs solution of the following composition (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4{\cdot}7H_2O$, 1.17 and $CaCl_2{\cdot}2H_2O$, 2.5. Once the tunica albuginea was fully exposed, a slit was made at its proximal end and extended distally. The corpus cavernosum was sharply dissected free from the tunica bilaterally. Two segments of cavernosal tissue ($10 \times 3 \times 2$ mm) from each rabbit were obtained and maintained in chilled Krebs solution until use.

2.2. Isometric force recording

Strips of rabbit corpus cavernosum were transferred to 10-ml organ baths containing Krebs solution at 37 °C continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The strips were tied at each end by means of cotton thread ligatures and vertically suspended between two metal hooks. The hook anchoring the upper end of the strips was connected to a force–displacement transducer that was attached to a moveable unit allowing precise adjustment of the preload tension. The resting force applied to the tissues was periodically adjusted to 10 mN until equilibration was achieved. Changes in isometric force were measured using Ugo Basile transducers (Varese, Italy) and recorded using a PowerLab 400™ data acquisition system (software Chart, version 4.0, AD Instruments, MA, USA). The cavernosal strips were allowed to equilibrate in the bathing medium for 60 min, during which the medium was replaced every 15 min. Corporeal smooth muscle was precontracted submaximally with phenylephrine (10 μM) in order to increase the basal tone. Indomethacin (5.6 μM) was added to the bathing medium to inhibit the generation of prostanoids.

2.3. Electrical field stimulation

Electrical field stimulation (EFS) was accomplished with the aid of two platinum ring electrodes. The gaps between the strips and the electrodes were wide enough to allow undisturbed contraction and relaxation, and yet sufficiently narrow to stimulate nerve terminals effectively. Electrical field stimulation was conducted at 20 V for 10 s at varying frequencies (2, 4, 8 and 16 Hz) in the form of square-wave pulses (0.5 ms pulse width), using a Grass S88 stimulator (Astro-Med Industrial Park, RI, USA). Electrical

stimuli were applied to precontracted strips, allowing preparations to recover completely from each relaxant response prior to the next applied stimulus.

2.4. Drugs and chemicals

Aconitine, atenolol, betaxolol, brevetoxin-3, (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride (ICI 118,551), indomethacin, lidocaine, metoprolol, phenylephrine, propranolol, saxitoxin, tetrodotoxin and veratridine were obtained from Sigma Chemical Co. (St. Louis, USA). Pentobarbital sodium (Hypnol®) was obtained from Cristália Produtos Químicos e Farmacêuticos (Itapira, São Paulo, Brazil). The toxins Ts1 and Ts3 were purified in house according to Arantes et al. (1989) and Teixeira et al. (2003), respectively.

2.5. Statistical analysis

The magnitude of relaxant responses was given as a percentage of the previously induced contraction, which was taken as 100%. Quantitative data were expressed as mean±S.E.M. of n experiments and the differences between two means were evaluated by a Student's two-tailed t-test for paired or unpaired observations, as appropriate. A probability of less than 0.05 was considered statistically significant. Analysis of variance (ANOVA) for repeated measurements was performed for the appropriate results and Bonferroni method was chosen as a post-test. A program package was used for the statistical analysis of all data (GraphPAD Instat, 1997, version 3.00, GraphPAD Software, USA).

3. Results

3.1. Lack of effect of β-adrenoceptor antagonists in the electrical field stimulation (EFS)-induced relaxations

Electrical field stimulation (EFS, 2–16 Hz) caused frequency-dependent, rapidly developing and transient relaxations of precontracted corporeal segments, which were abolished in presence of the voltage-gated sodium channel blockers tetrodotoxin and saxitoxin (0.1 μM each; $n=6$).

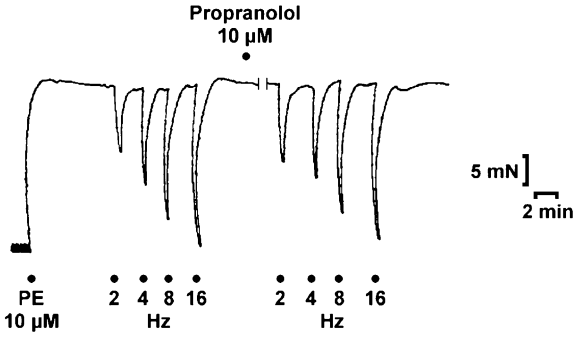

Fig. 1. Isometric force recording for electrical field stimulation (EFS, 2–16 Hz) in corporeal smooth muscle strips contracted by phenylephrine (PE, 10 μM). Addition of propranolol (10 μM) 20 min before the second frequency–response curve had no significant effect on the magnitude of electrically induced relaxations. This is a representative tracing of six experiments.

Table 1
Effect of the β-adrenoceptor antagonists propranolol, atenolol, metoprolol, betaxolol and ICI 118,551 on the relaxations of rabbit corpus cavernosum induced by electrical field stimulation (EFS, 2–16 Hz; $n=4–6$) in tissues precontracted with phenylephrine (10 μM)

	Rabbit corpus cavernosum relaxation (%)			
	2 Hz	4 Hz	8 Hz	16 Hz
Control	38±1	55±2	74±2	87±3
Propranolol (10 μM)	35±4	50±4	73±6	89±9
Atenolol (3 μM)	42±3	52±3	79±7	97±7
Metoprolol (10 μM)	44±4	58±3	70±3	79±1
Betaxolol (10 μM)	42±2	56±4	70±3	82±3
ICI 118,551 (3 μM)	44±2	61±3	73±3	82±4

The relaxations were expressed (mean±S.E.M.) relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%.

Addition of the non-selective β-adrenoceptor antagonist propranolol (10 μM; $n=6$) to the bathing medium did not affect the relaxations evoked by EFS at all frequencies tested (Fig. 1; Table 1). The selective β_1-adrenoceptor antagonists atenolol (3 μM; $n=5$), metoprolol (10 μM; $n=4$) and betaxolol (10 μM; $n=6$), along with the selective β_2-adrenoceptor antagonist ICI 118,551 (3 μM; $n=5$) also failed to affect the EFS-induced corpus cavernosum relaxations (Table 1). At the concentrations used, none of these β-adrenoceptor antagonists affected the basal tone of the phenylephrine (10 μM)-precontracted corpus cavernosum strips. In addition, prior incubation with propranolol (10 μM; $n=4$) for 30 min did not significantly prevent the inhibition caused by tetrodotoxin (0.1 μM) on EFS-induced relaxations at either 2 Hz (41±4% in the absence and 0% in the presence of propranolol and tetrodotoxin), 4 Hz (51±3% in the absence and 1±1% in the presence of propranolol and tetrodotoxin), 8 Hz (72±5% in the absence and 2±1% in the presence of propranolol and tetrodotoxin) or 16 Hz (87±7% in the absence and 4±2% in the presence of propranolol and tetrodotoxin).

3.2. Lack of effect of β-adrenoceptor antagonists in corpus cavernosum relaxations induced by binding site-2 ligands

The binding site-2 activators aconitine (30 μM) and veratridine (30 μM) produced long-lasting and non-tachyphylactic relaxations, which were fully antagonized by the Na^+

Table 2
Effect of the β-adrenoceptor antagonists propranolol, betaxolol and ICI 118,551 on the relaxations of rabbit corpus cavernosum induced by the binding site-2 activators aconitine (30 μM; $n=5–6$) and veratridine (30 μM; $n=4–6$) in tissues precontracted with phenylephrine (10 μM)

	Corpus cavernosum relaxation (%)			
	Aconitine		Veratridine	
	Control	Treated	Control	Treated
Propranolol (10 μM)	64±6	72±5	55±2	67±3
Betaxolol (10 μM)	65±6	69±4	49±4	56±5
ICI 118,551 (3 μM)	65±5	71±3	51±4	61±4

The relaxations were expressed (mean±S.E.M.) relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%.

channel blockers tetrodotoxin and saxitoxin (0.1 μM each). Addition of β-adrenoceptor antagonists (propranolol, betaxolol or ICI 118,551) to the bathing medium had no inhibitory effect on the relaxant responses evoked by either toxin (n=4–6; Table 2)

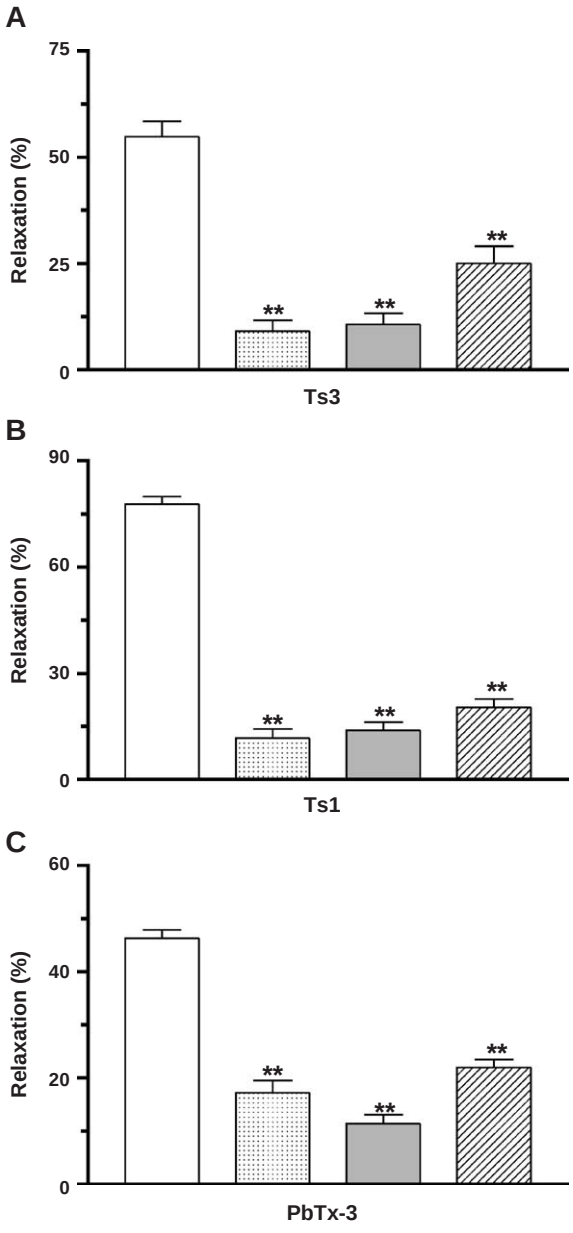

Fig. 2. Effects of non-selective (propranolol, 10 μM; n=4–5), β_1-(betaxolol, 10 μM; n=3–4) and β_2-selective (ICI 118,551, 3 μM; n=3–4) adrenoceptor antagonists on the relaxations induced by Ts3 toxin (30 nM; top panel), Ts1 toxin (1 μM; middle panel) and brevetoxin-3 (PbTx-3, 100 nM; bottom panel) in rabbit cavernosal smooth muscle contracted with phenylephrine (10 μM). Relaxant responses were obtained in the absence (white bars) and in the presence of propranolol (dotted bars), betaxolol (grey bars) or ICI 118,551 (hatched bars). Experimental values of relaxation were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. Data represent the mean±S.E.M of n experiments. **P<0.01 compared to the respective controls.

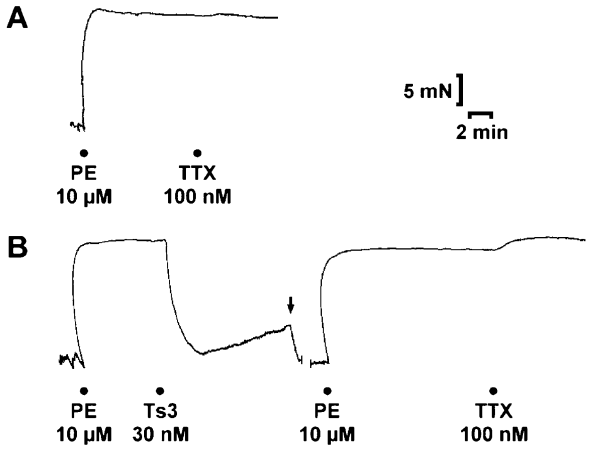

Fig. 3. Isometric force recording for Ts3 toxin (30 nM) in rabbit corpus cavernosum contracted by phenylephrine (PE, 10 μM). (Panel A) Effects of tetrodotoxin (TTX, 100 nM) on the muscle tone in strips precontracted by phenylephrine (10 μM). Addition of TTX had no effect on the tone of the preparations. (Panel B) Ts3 toxin addition to the bathing medium caused a marked and sustained relaxation of the cavernosal strips. After washing the preparations, addition of TTX (100 nM) significantly increased the tone of the tissues previously precontracted by phenylephrine. These are representative tracings of three experiments. Downward arrow denotes washout of the preparations.

3.3. Inhibitory effects of β-adrenoceptor antagonists in corpus cavernosum relaxations induced by binding site-3, -4 and -5 ligands

The binding site-3 and site-5 activators Ts3 (30 nM) and brevetoxin-3 (100 nM), respectively, produced sustained relaxations when added to the organ bath. However, the corpus cavernosum tissues did not relax to a second addition of Ts3 in the same preparation, which is indicative of tachyphylaxis. The binding site-4 activator Ts1 (1 μM) produced rapidly developing and transient relaxations. Similarly to Ts3, the relaxant response induced by Ts1 was not reproducible in the same preparation. Therefore, to investigate the effects of β-adrenoceptor antagonists on the relaxations evoked by these toxins, one single concentration was used for each corpus cavernosum strip and, therefore, experiments were run in parallel. All three toxins caused relaxations that were fully blocked by tetrodotoxin and saxitoxin (0.1 μM each).

The corpus cavernosum relaxations elicited by the Ts3 toxin (30 nM) were markedly reduced (P<0.01) by the addition of propranolol (10 μM) to the bathing medium (Fig. 2A). Next, selective β-adrenoceptor antagonists were used to evaluate if inhibition of Ts3-induced relaxations depended on a specific β-adrenoceptor subtype. Addition of either atenolol (3 μM; 59±6% in the absence and 53±5% in the presence of atenolol) or metoprolol (10 μM; 70±6% in the absence and 65±5% in the presence of metoprolol) had no effect on the relaxations elicited by Ts3. However, betaxolol (10 μM) significantly inhibited (P<0.01) the relaxations induced by this toxin (Fig. 2A). Addition of ICI 118,551 (3 μM) also markedly reduced (P<0.01) the relaxant responses evoked by Ts3 (Fig. 2A).

The cavernosal relaxations induced by Ts1 (1 μM; Fig. 2B) or brevetoxin-3 (100 nM; Fig. 2C) were blunted (P<0.01) following addition of propranolol (10 μM). Addition of either betaxolol

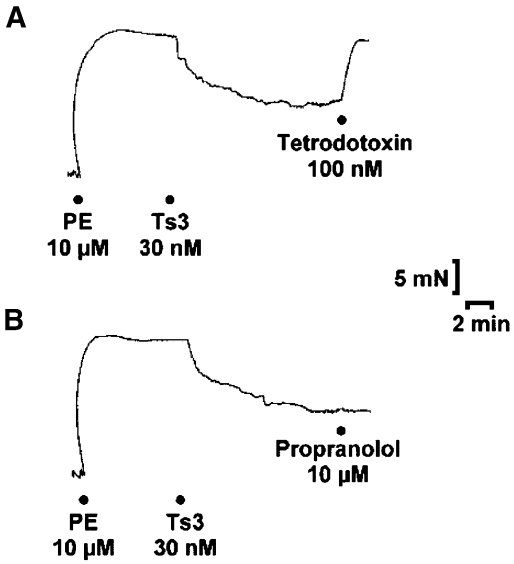

Fig. 4. Isometric force recordings for Ts3 toxin (30 nM) in the rabbit corpus cavernosum strips contracted by phenylephrine (PE, 10 μM). Effects of tetrodotoxin (0.1 μM) and propranolol (10 μM) on the established relaxation induced by Ts3 toxin. Addition of tetrodotoxin (panel A), but not propranolol (panel B), to the bathing medium promptly reversed the relaxant response. These are representative tracings of four experiments.

(10 μM) or ICI 118,551 (3 μM) also significantly reduced ($P<0.01$) the relaxant responses evoked by Ts1 or brevetoxin-3 (Fig. 2B and C).

3.4. Further investigation on the interaction of propranolol with the binding site-3 in corpus cavernosum

Tetrodotoxin (100 nM) did not cause significant alterations in the tone of precontracted cavernosal segments (Fig. 3A). The addition of Ts3 (30 nM) induced long-lasting and sustained relaxations, as previously described; however, when tissues were washed and subsequently contracted with phenylephrine, addition of tetrodotoxin elicited a slight (but significant) increase in the tone of the preparations (Fig. 3B).

In another series of experiments, tetrodotoxin (100 nM; Fig. 4A) promptly reversed the established relaxation evoked by Ts3, whereas addition of propranolol (10 μM) during the course of the Ts3-induced relaxant response did not affect its development (Fig. 4B).

As stated above, the relaxations induced by Ts3 (30 nM) are not reproducible in the same preparation, and propranolol fully prevents its relaxant actions. However, after washing the corpus cavernosum preparations previously treated with propranolol (10 μM), the addition of the same concentration of Ts3 caused a marked relaxation, in the same magnitude as that seen in the non-treated preparations (Fig. 5A). Similar results were observed using lidocaine (1 mM) instead of propranolol (Fig. 5B).

4. Discussion

Our present investigation clearly demonstrates that non-selective β- (propranolol), selective β_1- (betaxolol) and selective β_2- (ICI 118,551) adrenoceptor antagonists markedly reduce the rabbit corporeal relaxations in response to toxins acting on sites 3, 4 and 5 of voltage-gated sodium channel, namely Ts3, Ts1 and brevetoxin-3, respectively. In contrast, neither atenolol nor metoprolol (selective β_1-adrenoceptor antagonists) inhibited Ts3-induced relaxations. On the other hand, none of the β-adrenoceptor antagonists tested affected the relaxations induced by EFS or those evoked by binding site-2 neurotoxins, veratridine and aconitine.

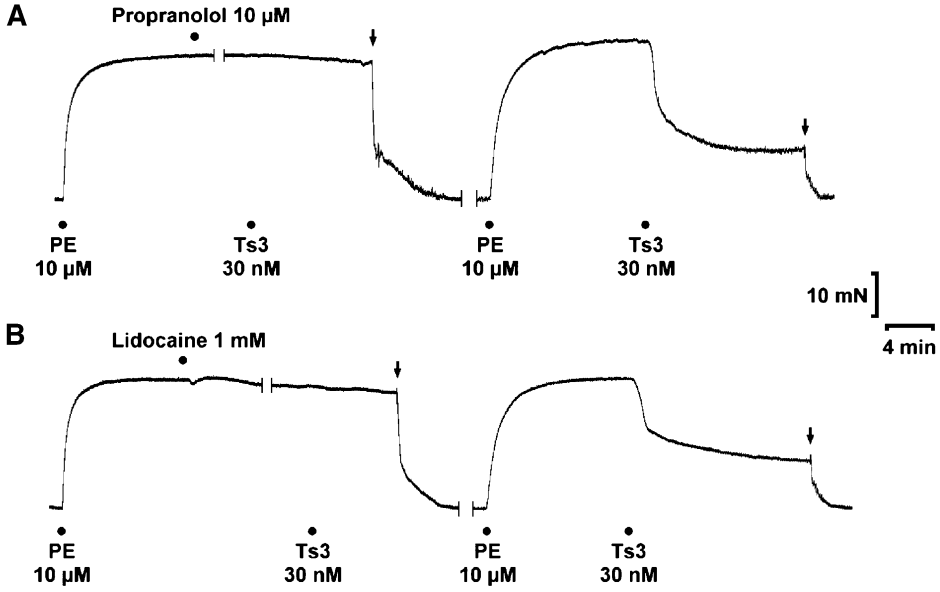

Fig. 5. Isometric force recordings for Ts3 toxin in the rabbit corpus cavernosum strips contracted by phenylephrine (PE, 10 μM). Pre-treatment of the cavernosal strips with propranolol (10 μM; panel A) or lidocaine (1 mM; panel B) virtually abolishes the relaxations evoked by Ts3 toxin. After washing the preparations, a subsequent application of the toxin markedly relaxes the phenylephrine-precontracted tissues. These are representative tracings of three experiments.

It is well established that β-adrenoceptors are widely distributed in mammalian tissues both at post- and pre-synaptic levels. In addition to their ability to antagonize β-receptors, β-adrenoceptor antagonists possess ancillary properties that may modulate their clinical effects, such as inhibition of Ca^{2+} channels, lipophilicity and membrane-stabilizing activity (Soriano et al., 1997; Kostis and Rosen, 1987). Considering that the β_1-adrenoceptor antagonists used in this study are equally effective to antagonize the β_1-receptors at the post-synaptic level (Nakane et al., 1988; Chiba and Tsukada, 1991; Longhurst and Levendusky, 1999; Mustafa et al., 1999), it is likely that inhibition of Ts3-, Ts1- and brevetoxin-3-induced relaxations by these compounds reflect β_1-independent activities, such as these ancillary properties. It is known that depolarization of nitrergic fibers lead to neural NO release as a consequence of Ca^{2+} influx, which activates the neuronal NOS (Zygmunt et al., 1995). Although the inhibition of neurotoxin-induced relaxations by the β-adrenoceptor antagonists could be ascribed to inhibition of Ca^{2+} channels, this ancillary property seems unlikely to explain the inhibitory effects of β-adrenoceptor antagonists, since relaxations evoked by both EFS and the binding site-2 toxins (veratridine and aconitine) were not affected by any β-adrenoceptor antagonist tested. With regard to lipophilicity, among the β-adrenoceptor antagonists tested, propranolol, betaxolol, metoprolol and ICI 118,551 possess high lipophilicity, whereas atenolol is hydrophilic (Bilski et al., 1983; Taylor et al., 1985; Egginger et al., 1993). Theoretically, the binding site for a β-adrenoceptor antagonist may reside on the inside surface of the cell membrane, and thus the drug will be expected to penetrate the lipid bilayer prior to reaching its binding site. However, lipophilicity itself does not explain the inhibitory actions of β-adrenoceptor antagonist in the toxin-evoked corpus cavernosum relaxations, since metoprolol, which is lipophilic, did not affect the responses. Therefore, in addition to lipophilicity, structural determinants of β-adrenoceptor antagonists might account for drug binding to Na^+ channels.

The analysis of the primary structure of voltage-gated sodium channel demonstrated that the fourth transmembrane segment from each domain (S4), which contains positively charged residues, represents the voltage sensor of the channel. Tetrodotoxin and saxitoxin binds to site-1 of the voltage-gated sodium channel causing persistent activation of channel at the resting, which block ion conductance by binding tightly to the outer vestibule of the Na^+ channel. Therefore, they are direct-acting blockers that occlude the extracellular entry to the Na^+ channel pore (Cestele and Catterall, 2000). In our study, tetrodotoxin (or saxitoxin), added prior to EFS or to addition of the toxins, fully antagonized the corpus cavernosum relaxations. Additionally, tetrodotoxin, added during the sustained relaxation evoked by Ts3, promptly reversed the relaxant response to the pre-stimulation level. This strongly indicates that the inhibitory actions of tetrodotoxin (and saxitoxin) are a consequence of physical occlusion of the channel pore in a non-competitive manner.

We next attempted to understand the interactions of propranolol with the binding sites of the voltage-gated sodium channel that lead to inhibition of neural NO release, giving particular attention to binding site-3. In our experiments, tetrodotoxin did not modify the tone of precontracted corporeal strips, confirming previous studies that the basal tone of corporeal strips is maintained through activity of endothelial NO synthase (eNOS), with no involvement of the neuronal NO synthase isoform (Andersson and Wagner, 1995). However, in corpus cavernosum strips pre-stimulated with Ts3, addition of tetrodotoxin caused an increase in the tone of the preparations, suggesting that Ts3 tightly binds to receptor site-3 of the voltage-gated sodium channel, eliciting a persistent activation of Na^+ channels and hence NO generation. This tight binding of Ts3 to receptor site-3 may also explain the lack of relaxing responses following a second addition of this toxin to the same strip.

The voltage-gated sodium channel channels exist in three functional states: resting (R), open (O) and inactivated (I) (Catterall et al., 1992). The opening of these channels provides for rapid depolarization and reversal of membrane potential. In the normal sequence of events, voltage-gated sodium channel then inactivates via closing of the inactivation gate; increasing K^+ conductance then serves to restore the resting membrane potential. Repolarization of the membrane allows the activation gate to close and the inactivation gate to reopen, restoring the channel to its resting configuration. Blockade of Na^+ channels by drugs is more likely when the channel is in I form, perhaps due to an increased affinity and accessibility of agents to channel binding sites (Catterall, 1999, 2002).

The binding of Ts3 to receptor site-3 causes depolarization of nitrergic fibers with subsequent release of NO to induce cavernosal smooth muscle relaxation (Teixeira et al., 2003, 2004). Previous studies have shown that binding of toxins to site-3 slows or blocks inactivation of the channel in excitable tissues, principally by inhibiting transitions of the channel from the open to the inactivated state (Tejedor and Catterall, 1988; Rogers et al., 1996; Benzinger et al., 1997). Thus, it is likely that Ts3 acts in the nitrergic fibers of corpus cavernosum through this mechanism, that is, it causes the inactivation gate to be held open, allowing the voltage-gated sodium channel to remain in a conducting configuration.

Similarly to tetrodotoxin, addition of propranolol prior to Ts3 abolished the toxin-induced relaxations. However, in contrast to tetrodotoxin, propranolol did not reverse the relaxant response when added during the sustained relaxation by Ts3. This excludes the possibility that the inhibitory effect of propranolol on Ts3-induced relaxations is attributed to a simple occlusion of the channel pore. This is further supported by our data showing that propranolol did not modify the inhibition of EFS-induced relaxations by tetrodotoxin. Thus, our results suggest that β-adrenoceptor

antagonists show a single affinity competitive inhibition of Ts3 activation of voltage-gated sodium channel.

Our study cannot ascertain on the exact mechanisms by which this competitive inhibition takes place, and additional studies are required. However, one may speculate that β-adrenoceptor antagonists bind at the binding site of Ts3, thus displacing Ts3, but failing itself to produce the channel activation. Alternatively, propranolol binds to an adjacent site in the voltage-gated sodium channel, which promotes an allosteric inhibition in toxin binding, thus preventing access of Ts3 to the binding site-3. Another possibility is that β-adrenoceptor antagonists bind only to a channel which is in a non-conducting configuration (e.g. gates closed), while the activator (e.g. Ts3) binds at a distinct site configuration, and thus prevents the gates from closing.

In conclusion, the inhibitory effects of β-adrenoceptor antagonists on Ts1–Ts3- and brevetoxin-3-induced corpus cavernosum relaxations reflect β_1-independent activities and may be a consequence of allosteric inhibition of toxin binding to Na^+ channels and subsequent reduction of NO release from nitrergic nerves.

Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support.

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