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β_2-Adrenoceptor-mediated inhibition of field stimulation induced contractile responses of the smooth muscle of the rat prostate gland

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Abstract

Isolated preparations of rat prostate responded to electrical field stimulation (2 strains every 60 s, 0.5 ms, 10 Hz, 80 V) with contractions. The adrenoceptor agonists adrenaline, isoprenaline and noradrenaline (0.1 nM–10 μM) elicited concentration-dependent inhibition of electrical field stimulation-induced contractions of the rat prostate. Phenylephrine had no effect on the amplitude of electrical field stimulation-induced contractions. The rank order of potency was isoprenaline ≥ adrenaline = noradrenaline > phenylephrine. Inhibition of electrical field stimulation-induced contractions by isoprenaline was attenuated by propranolol (1 μM). The selective β_1-adrenoceptor agonist (−)-1-(3,4-dimethoxy-phenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol)oxalate (RO363) and the selective β_2-adrenoceptor agonist salbutamol (1 nM–100 μM) were approximately equipotent in inhibiting electrical field stimulation-induced contractions but the selective β_3-adrenoceptor agonist sodium 4-(2-[2-hydroxy-{3-chlorophenyl}ethylamino]propyl)phenoxyacetate (BRL 37344, 1 nM–100 μM) did not inhibit electrical field stimulation-induced contractions. The selective β_2-adrenoceptor antagonist, (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI 118 551, 0.1 μM) attenuated inhibitory responses to isoprenaline and salbutamol, while the selective β_1-adrenoceptor antagonist atenolol (3 μM) did not. Contractions induced by electrical field stimulation were also inhibited by forskolin (10 nM–3 mM) but unaffected by sodium nitroprusside (10 nM–1 mM) indicating the presence of an inhibitory cAMP mechanism. These data suggest that stimulation of β_2-adrenoceptors can inhibit contractions of the rat prostate induced by electrical field stimulation.

Keywords: (Rat); Neuromuscular transmission; Inhibitory receptor; Isoprenaline; Propranolol; Salbutamol

1. Introduction

α_{1L}-Adrenoceptor-mediated contraction of the prostatic smooth muscle of a number of species is well accepted (Pennefather et al., 2000). Using functional studies with prazosin, previous studies in our laboratory showed that noradrenaline acting at α_1-adrenoceptors, was the major neurotransmitter mediating field stimulation-induced contractions in the rat (Lau et al., 1998).

Mechanisms mediating noradrenergic inhibitory effects are less well studied. However, radioligand binding studies have demonstrated the presence of populations of β-adrenoceptors in the rat prostate (Gousse et al., 1991; Fukumoto et al., 1993). Similarly, radioligand binding studies have described an abundance of β_2-adrenoceptors in porcine and human prostate (Goepel et al., 1997). This subtype of β-adrenoceptor has been shown to mediate relaxation in a variety of peripheral smooth muscle tissues (Summers et al., 1997).

Biochemical studies have also shown that β-adrenoceptor agonists are able to stimulate adenylate cyclase and therefore raise cAMP levels in the prostate of rats (Juarranz et al., 1994; Carmena et al., 1997) and guinea-pigs (Haynes and Hill, 1997). In addition, β-adrenoceptor agonists and other agents such as forskolin which stimulate cAMP accumulation directly have been shown to inhibit contractions of prostates taken from guinea-pigs (Haynes and Hill, 1997), dogs (Normandin and Lodge, 1996) and humans (Drescher et al., 1994).

Despite a large amount of research on prostatic neuromuscular transmission on the rat prostate and the demonstrated presence of β-adrenoceptors in this tissue, the effects of β-adrenoceptor stimulation on the tone of prostate

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smooth muscle from the rat have not been investigated in detail. The aim of this study was to determine whether stimulation of β-adrenoceptors caused inhibition of rat prostatic smooth muscle contraction. Classification of the β-adrenoceptors mediating these effects was also carried out using the selective β-adrenoceptor agonists RO363 (β_1), salbutamol (β_2) and BRL 37344 (β_3), and the selective β_1- and β_2-adrenoceptor antagonists atenolol and ICI 118 551, respectively.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) were housed at 22 °C and exposed to a photoperiod of 12 h light/12 h dark. Rats were allowed access to food and water ad libitum. Ethical approval was obtained from the Monash University Standing Committee of Animal Ethics in Animal Experimentation (Ethics number 2000/04).

2.2. Experimental procedure

Rats were killed by cervical dislocation. An abdominal incision was made, exposing the male urogenital tract and the left and right lobes of the prostate were removed providing two prostate preparations from each rat. The prostate lobes were placed in a Petri dish containing Krebs–Henseleit solution (mM: NaCl 118.1, KCl 4.69, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4$ 0.5, $CaCl_2$ 2.5) and the prostatic capsule was removed along with excess fat and connective tissue. The isolated prostates were set up in 5 ml isolated organ baths for the recording of changes in isometric tension. The organ baths contained Krebs–Henseleit solution, bubbled with 5% CO_2 in O_2 and maintained at 37 °C. One end of the prostate was attached to an isometric Grass FT03 force-displacement transducer, which was connected to a PowerLab data acquisition system run on a Power Macintosh 5500/225 computer. The lower end of the preparation was attached to a perspex tissue holder incorporating two parallel vertical platinum electrodes. Tissues were equilibrated for 30 min, under a resting force of 0.5 g. During the 30-min equilibration period, the bath medium was changed every 10 min due to the frothing that occurred in the organ bath as a result of spontaneous prostatic secretions (Lau et al., 1998).

Following equilibration for 30 min, nerve terminals within the tissues were field stimulated using the electrodes connected to a Grass S88 stimulator to deliver trains of 0.5 ms pulse duration, 80 V, at 10 Hz for 2 s every 60 s. This stimulation of the rat prostate produces twitch like contractions that are tetrodotoxin sensitive and attenuated by guanethidine (Lau et al., 1998), indicating that these contractions are mediated by nerves which are predominantly sympathetic in nature. Cumulative log concentration–response curves to adrenaline, noradrenaline, isoprenaline and phenylephrine were constructed on these stimulated preparations to examine the influence of inhibitory adrenoceptors on neuromuscular transmission to the rat prostate.

Cumulative log concentration–response curves to adrenaline, noradrenaline, isoprenaline, phenylephrine, the selective β_1-adrenoceptor agonist RO363, the selective β_2-adrenoceptor agonist salbutamol, the selective β_3-adrenoceptor agonist BRL 37344, the adenylate cyclase activator forskolin and the guanylate cyclase activator sodium nitroprusside (0.1 nM–1 mM) were constructed using a concentration progression ratio of half a log unit, to examine their effects and relative potencies on electrical field stimulation-induced contractions of prostatic smooth muscle. Each concentration was left in contact with the tissue for 8–10 min before the addition of the next concentration. If further drug addition was delayed beyond this contact time, the amplitude of electrical field stimulation-induced contractions recovered towards resting levels.

Cumulative log concentration–response curves to isoprenaline and salbutamol were constructed in the presence of: propranolol (1 μM, β-adrenoceptor antagonist), atenolol (3 μM, selective β_1-adrenoceptor anatagonist) or ICI 118 551 (0.1 μM; selective β_2-adrenoceptor antagonist). Concentrations of atenolol and ICI 118 551 used were approximately 100 times the previously reported pA_2 values for the β_1- and β_2-adrenoceptor subtypes, respectively, based on previous functional studies (Bylund et al., 1998; Alexander and Peters, 2000). Higher concentrations were not used so as to conserve the subtype selectivity of these two antagonists. Propranolol was used as a nonselective β-adrenoceptor antagonist to distinguish between α- and β-adrenoceptors. The concentration used was approximately 1000 times its reported pA_2 value for β-adrenoceptors. To avoid desensitisation in the rat prostate, which we had observed in pilot studies, only one concentration response curve was constructed on each tissue and time and/or vehicle (catecholamine diluent or 0.5% ethanol) control experiments were carried out on parallel preparations from the contralateral prostate. Following the 1 h initial equilibration period, the antagonist or vehicle was added to the organ bath and left in contact with the tissue for a further 30 min before a log concentration–response curve was constructed. Pilot experiments with the β-adrenoceptor antagonists revealed that equilibrium was reached after 10–15 min and remained stable for at least 1 h. Antagonists were washed out and replaced two to three times throughout the further incubation period.

Cumulative log concentration–response curves to forskolin and sodium nitroprusside were also conducted to determine whether inhibition of electrical field stimulation-induced responses could be mediated by stimulation of adenylate or guanylate cyclase, respectively. For experi-

ments using forskolin, parallel experiments were run using equivalent dilutions of vehicle (0.5% ethanol, maximum bath concentration).

In a separate set of experiments, tissues were not stimulated but instead after the 1 h equilibration period, discrete concentration–response curves to noradrenaline (1 nM–100 μM) using a dose progression ratio of half a log unit were constructed in the absence and presence of propranolol (1 μM). This protocol was conducted to examine the effects of β-adrenoceptors on the contractile action of exogenously added noradrenaline.

2.3. Measurement and analysis of data

The mean peak force of the four electrical field stimulation-induced contractions immediately preceding the addition of the first agonist concentration was determined and taken as the mean control response to electrical field stimulation. Following the addition of each concentration of agonist, five responses were recorded before the next concentration was added. The mean peak force of the two lowest contractions was then taken as the inhibitory response for that concentration of agonist. Responses were expressed as the percentage inhibition of the initial control response. Results are expressed as the mean ± standard error of the mean (S.E.M.). The value of *n* represents the number of animals used.

Graphs showing mean inhibitory log concentration response curves were constructed, using Graph Pad Prism (version 3.0). Inhibitory responses to agonists in the absence and presence of antagonists were determined by measuring the peak force developed (in *g*) to each concentration of agonist. A two-way repeated measures analysis of variance (ANOVA) was carried out to compare differences between the two treatment groups at all concentrations points on the concentration–response curve. A post hoc Bonferroni's test was conducted when multiple comparisons were made between treatment groups. These tests were carried out using Sigmastat® (version 1.0). The *P* values used to evaluate statistical significance were the probabilities of a significant interaction between dose and treatment and in all cases, $P < 0.05$ was considered significant. Estimates of the differences in agonist potency and shifts caused by antagonists were made by determining the mean concentration of agonist that produced an inhibition of 25% of the initial mean response (IC_{25}). This was determined by linear regression using Graph Pad Prism® (version 3.0). Mean and 95% confidence limits of the IC_{25} value for each agonist were then determined.

Two-tailed Student's paired *t*-tests were carried out to determine the difference in mean basal electrical field stimulation-induced contractile responses between control and treatment groups using GraphPad Prism (version 3.0). In all cases, values of $P \leq 0.05$ were considered significant.

2.4. Drugs and solutions

The following drugs were used: (−)-arterenol bitartrate (noradrenaline, Sigma), atenolol hydrochloride (ICI),sodium 4-(2-[2-hydroxy-{3-chlorophenyl}ethylamino]propyl) phenoxyacetate (BRL 37344, Tocris), (−)-epinephrine bitartrate (adrenaline, ICN), forskolin (RBI), (±)-1-[2, 3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy-3-[(1-methylethyl)amino]-2-butanol (ICI 118-551 hydrochloride, ICI), (−)-isoproterenol bitartrate (isoprenaline, Sigma), (−)-phenylephrine hydrochloride (Sigma), propranolol hydroch loride (Sigma), (−)-1-(3,4-dimethoxy-phenethylamino)-3-(3,4-dihydroxyphenoxy)-2-propanol)oxalate (RO363, Victorian College of Pharmacy), salbutamol hemisulfate (Sigma) and sodium nitroprusside dihydrate (Sigma).

Stock concentrations of noradrenaline, adrenaline, salbutamol, isoprenaline, phenylephrine and BRL 37344 were made up and diluted to the required concentration in a

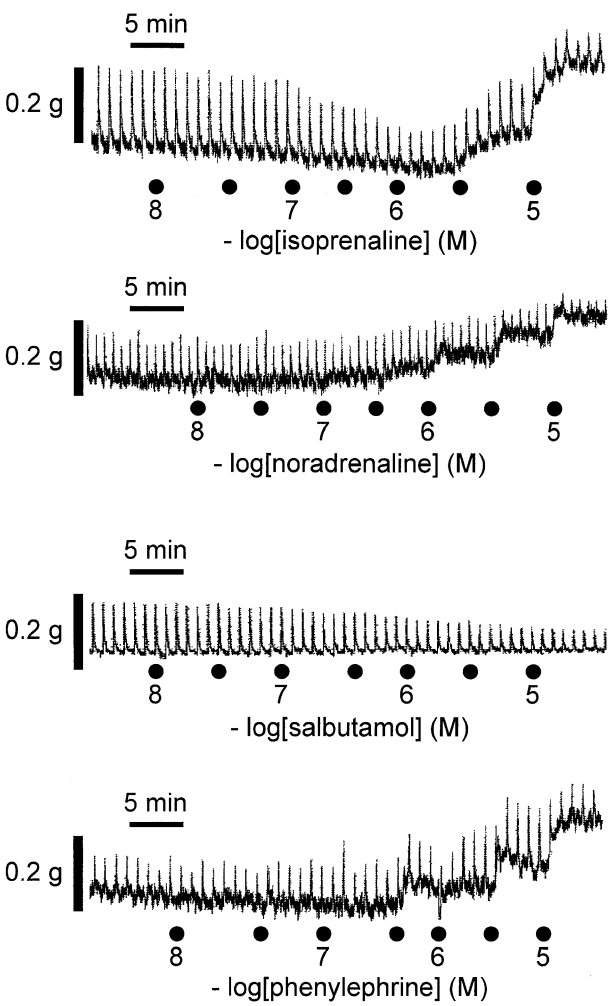

Fig. 1. Representative traces showing the effects of cumulative addition of isoprenaline, noradrenaline, salbutamol and phenylephrine on electrical field stimulation (0.5 ms pulse duration, 80 V, 10 Hz for 2 s every 60 s)-induced contractions of the isolated rat prostate.

catecholamine diluent (mM: NaCI 154.0, NaH_2PO_4 1.2, ascorbic acid 0.2). RO363 and ICI 118 551 were dissolved in 0.01 M HCl. Forskolin was dissolved in 50% ethanol. All other compounds were dissolved in distilled water. Subsequent dilutions of these drugs were made in distilled water. All drugs were made up freshly on the morning of experimentation.

3. Results

3.1. Responses to electrical field stimulation

Electrical field stimulation (10 Hz, 0.5 ms, 80 V applied for 2 s every 60 s) of nerve terminal evoked contractions of the rat prostate which remained stable in time and vehicle control experiments for the duration of the experiment (up to 6 h). Following washout after cumulative concentration–response curves to all agonists, electrical field stimulation-induced contractions returned to normal basal levels.

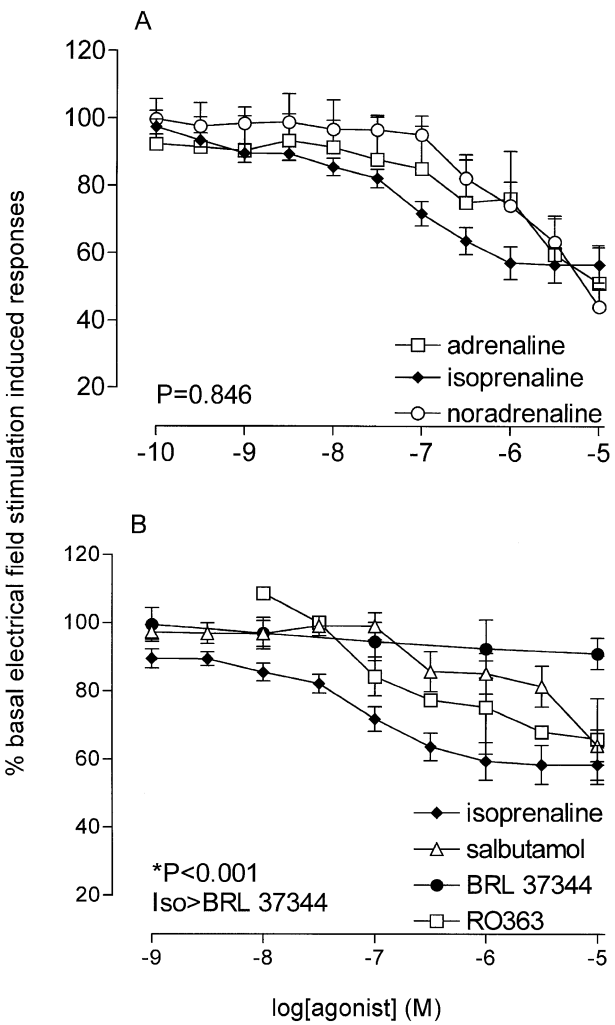

Fig. 2. Mean log concentration–response curves for the inhibitory effects of (A): adrenaline (□), isoprenaline (♦) and noradrenaline (○) and (B): isoprenaline (♦), BRL 37344 (●), salbutamol (△) and RO363 (□) on the field stimulation-induced contractions of isolated rat prostatic preparations. Results are expressed as the percentage of inhibition of basal control electrical field stimulation-induced contractile responses. Each point represents the mean ± S.E.M. of 4–18 experiments. *P* values represent the difference in the concentration–response curves according to the agonist used. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$; two-way repeated measures ANOVA, followed by post-hoc Bonferroni correction).

Table 1
Mean negative log IC_{25} values, potency ratios and mean maximum inhibition developed at β-adrenoceptors on rat prostatic smooth muscle

Agonist	$-\log IC_{25}$ (M) (95% C.I.)	Potency ratio[a]	Max. inhibition (% ± S.E.M.)
Isoprenaline	7.20 (6.85–7.56)	1.0	43.6 ± 5.0
Adrenaline	6.22 (5.07–7.37)	0.105	69.3 ± 6.9
RO363	6.14 (5.60–6.40)	0.087	47.4 ± 9.9
Noradrenaline	6.03 (5.75–6.31)	0.068	66.8 ± 3.3
Salbutamol	6.03 (5.03–7.03)	0.068	65.6 ± 3.1
BRL 37344[b]	> 5	< 0.01	N/A

$n = 4$–18 animals for each agonist.
[a] Potency ratio = antilog ((neg log IC_{25} value for analogue) − (neg log IC_{25} value for isoprenaline)).
[b] 25% Inhibition was not reached with BRL 37344 at the concentrations used in this study.

Cumulative addition of noradrenaline, adrenaline and isoprenaline produced concentration-dependent inhibition of contractions of the rat prostate induced by electrical field stimulation (10 Hz, 0.5 ms, 80 V applied for 2 s every 60 s, Fig. 1). Phenylephrine had no effect on contractions of the rat prostate induced by electrical field stimulation (Fig. 1). The adrenoceptor agonists also concentration-dependently increased baseline contractile force, however this did not affect the measurement of the amplitude of contractile responses induced by electrical field stimulation. However, it should be noted that adrenaline and noradrenaline increases in baseline contractile force were accompanied by continued inhibition of electrical field stimulation-induced contractions (Fig. 1). In contrast, the inhibition of electrical field stimulation-induced contractions caused by isoprenaline was reversed during increases in baseline contractile force (Fig. 1). Phenylephrine continued to have no effect on contractions elicited by electrical field stimulation (Fig. 1).

Isoprenaline ($n = 18$) was approximately 15-fold more potent than noradrenaline ($n = 6$) (potency ratio = 14.8 ± 1.7) in inhibiting electrical field stimulation-induced contractions of the rat prostate (Fig. 2). Although adrenaline ($n = 7$) appeared to be approximately 10-fold less potent than isoprenaline (potency ratio = 9.5 ± 1.6), the mean log concentration–response curve to adrenaline was not significantly different from isoprenaline or noradrenaline. The rank order of potency was therefore isoprenaline ≥ adrenaline = noradrenaline.

3.2. Agonist responses

The selective β_1-adrenoceptor agonist RO363 and the selective β_2-adrenoceptor agonist salbutamol also produced dose-dependent inhibition of electrical field stimulation-induced contractions (Figs. 1 and 2; $n = 4$ and 12, respectively).

Their potencies were similar (Table 1) and approximately 11- and 15-fold less than that of isoprenaline, respectively. However, the intrinsic activity of salbutamol was much greater than that of RO363 (Table 1). The β_3-adrenoceptor agonist, BRL 37344 ($n = 6$) was without

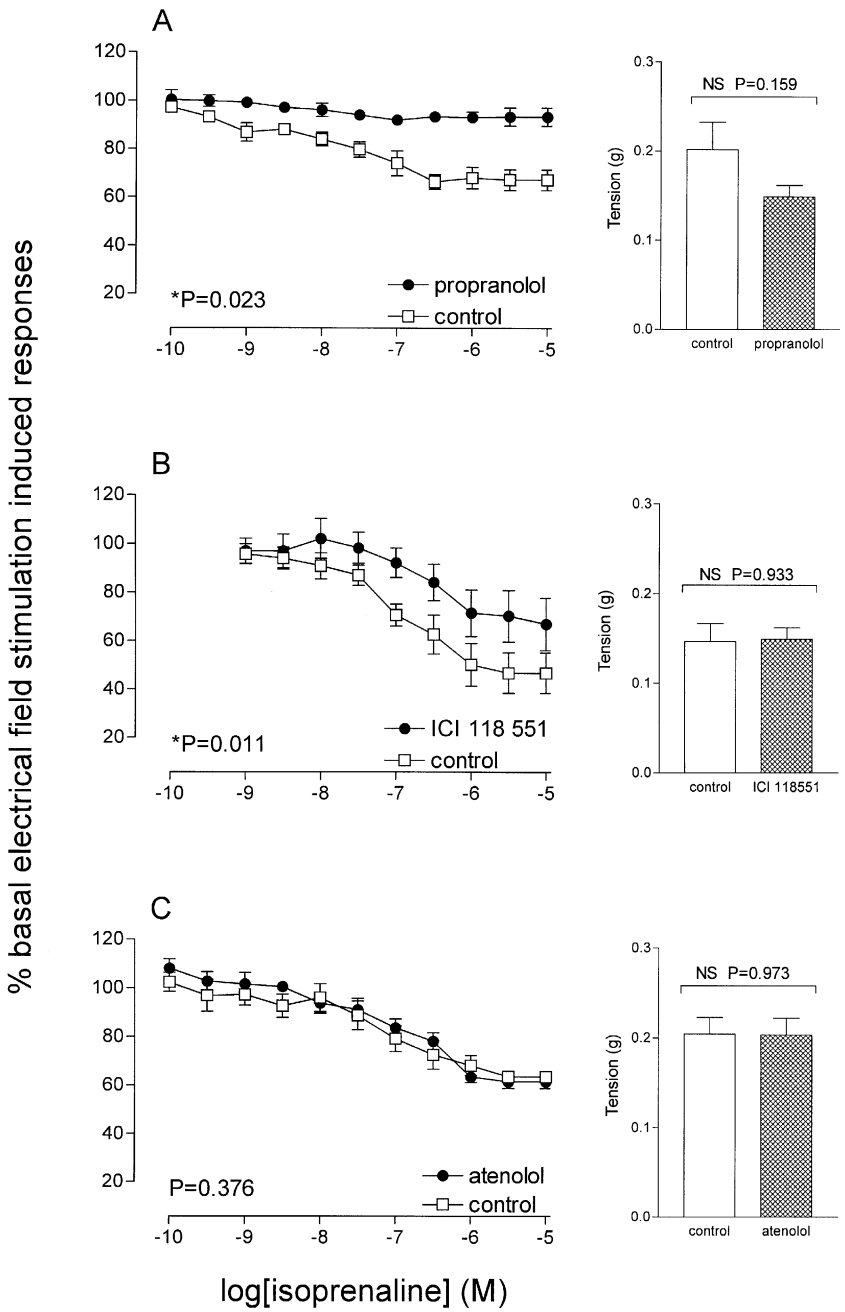

Fig. 3. Mean log concentration–response curves for the inhibitory effects of isoprenaline on field stimulation-induced contractions in rat prostatic preparations (A): in the presence (●) and absence (□) of propanolol (1 μM); (B): in the presence (●) and absence (□) of ICI 118 551 (0.1 μM); (C): in the presence (●) and absence (□) of atenolol (3 μM). Results are expressed as the percentage inhibition of basal control electrical field stimulation-induced contractile responses. Each point represents the mean ± S.E.M. of six experiments. *P* values are for the concentration × treatment interaction of a two-way repeated measures ANOVA and represent the difference in the concentration–response curves in the absence and presence of antagonist. The histogram columns represents the mean force developed by control tissues and in the presence of antagonist prior to the addition of the agonists. Vertical bars represent S.E.M. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$).

inhibitory effect on the electrical field stimulation-induced contractions of prostatic smooth muscle at the concentrations used in this study (Fig. 2; Table 1). Mean-log IC_{25} values, maximum inhibitions and potency ratios compared to isoprenaline for all agonists tested are given in Table 1.

3.3. Effects of β-adrenoceptor antagonists on agonist responses

The nonselective β-adrenoceptor antagonist, propranolol (1 μM), shifted the mean inhibitory log concentration–response curve to isoprenaline to the right (Fig. 3; $P = 0.023$; $n = 6$). Propranolol (1 μM) was without effect on basal electrical field stimulation-induced contractile responses of isolated rat prostate preparations (Fig. 3; $P = 0.159$; $n = 6$).

The selective β_1-adrenoceptor antagonist, atenolol (3 μM), did not affect inhibitory responses to either isoprenaline (Fig. 3; $P = 0.376$; $n = 6$) or salbutamol (Fig. 4; $P = 0.749$; $n = 6$). Similarly, there were no significant effects on the basal control contractile responses to electrical field stimulation by the selective β_1-adrenoceptor antagonist, atenolol (3 μM) (Figs. 3 and 4; $P \geq 0.243$; $n = 6$ for each).

ICI 118 551 (0.1 μM), a selective β_2-adrenoceptor antagonist, consistently caused approximately 10-fold shifts to the right in the mean log concentration–response curves to isoprenaline (Fig. 3, $P = 0.011$; $n = 6$) and salbutanol (Fig. 4; $P = 0.030$; $n = 6$). However, ICI 118 551 (0.1 μM) was without effect on the basal control contractile responses induced by electrical field stimulation (Figs. 3 and 4; $P \geq 0.673$; $n = 6$ for each).

3.4. Effects of forskolin and sodium nitroprusside

Forskolin (10 nM–3 mM) caused a concentration-dependent decrease in electrical field stimulation-induced contractile responses (Fig. 5), which was significantly to

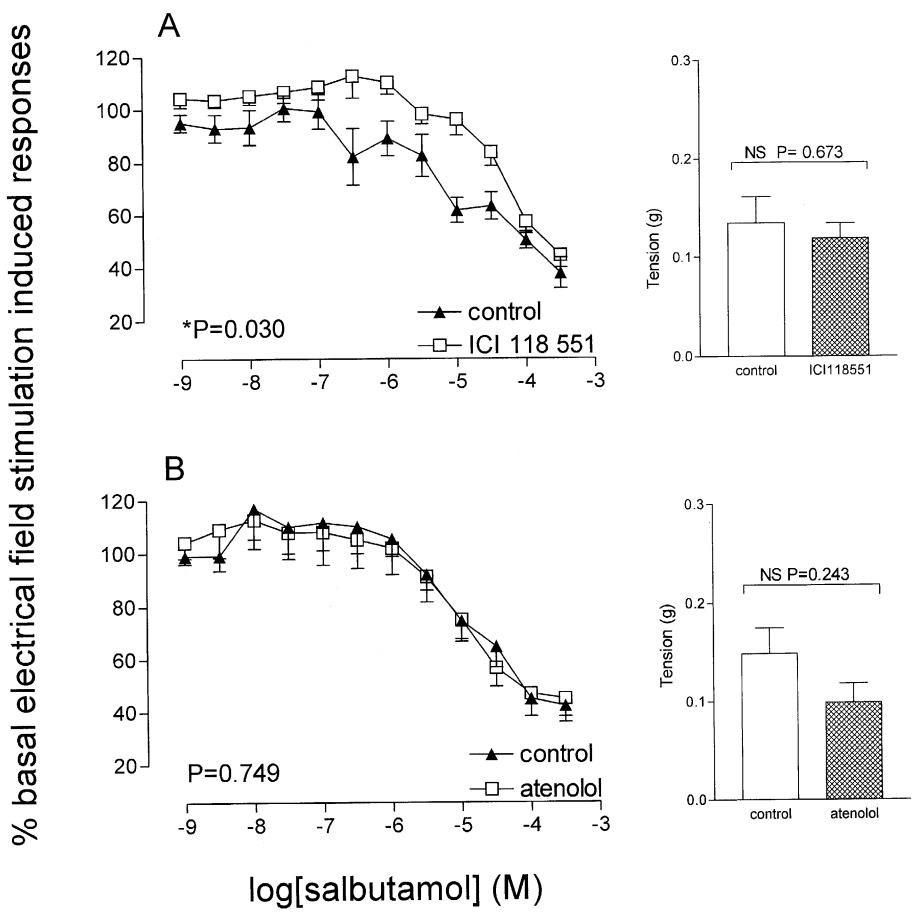

Fig. 4. Mean log concentration–response curves for the inhibitory effects of salbutamol on field stimulation-induced contractions in rat prostatic preparations (A): in the presence (□) and absence (▲) of ICI 118 551 (0.1 μM) and (B): in the presence (□) and absence (▲) of atenolol (3 μM). Results are expressed as the percentage inhibition of basal control electrical field stimulation-induced contractile responses. Each point represents the mean ± S.E.M. of six experiments. P values are for the concentration × treatment interaction of a two-way repeated measures ANOVA and represents the difference in the concentration–response curves in the absence and presence of antagonist. The histogram columns represent the mean force developed by control tissues and in the presence of antagonist prior to the addition of the agonists. Vertical bars represent S.E.M. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$).

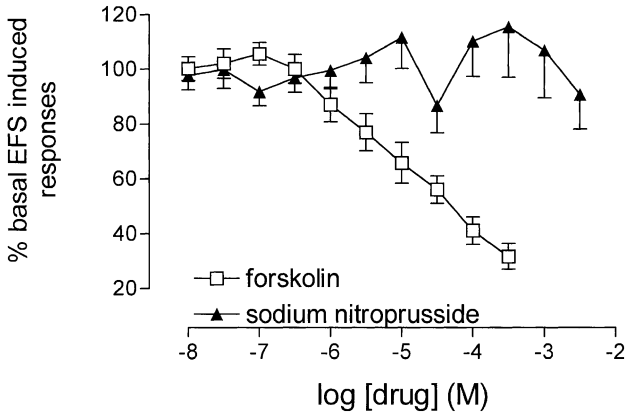

Fig. 5. Mean log concentration–response curves for the inhibitory effects of: forskolin (□) and sodium nitroprusside (▲) on the field stimulation-induced contractions of isolated rat prostatic preparations. Results are expressed as the percentage inhibition of basal control electrical field stimulation-induced contractile responses. Each point represents the mean ± S.E.M. of four experiments. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$).

the left of the vehicle control curve ($P = 0.020$; $n = 4$). The neg log IC_{25} value for forskolin was 5.50 (95% confidence limits, 5.19–5.81). Basal control contractile responses to electrical field stimulation between forskolin and vehicle control groups were not different ($P = 0.132$; $n = 4$).

Sodium nitroprusside (10 nM–1 mM) had no inhibitory effect on electrical field stimulation-induced contractile responses (Fig. 5; $n = 4$).

3.5. Effects of propranolol on contractile responses to exogenous noradrenaline

Exogenous addition of noradrenaline (1 nM–100 μM) elicited concentration-dependent contractions of isolated preparations of rat prostate. The EC_{50} for noradrenaline was 158 ± 32 nM and the maximum force generated was 0.26 ± 0.03 g. Propranolol (1 μM) did not cause a shift in the mean noradrenaline concentration–response curve ($EC_{50} = 251 \pm 49$ nM, $P = 0.072$, $n = 6$). Similarly, no difference in mean maximum responses to noradrenaline was detected for isolated prostate preparations in the presence of propranolol (1 μM, maximum response = 0.23 ± 0.04 g, $P = 0.723$, $n = 6$).

4. Discussion

The present study showed that stimulation of β-adrenoceptors causes concentration-dependent inhibition of electrical field stimulation-induced contractions of the rat prostatic smooth muscle. Thus, β-adrenoceptor stimulation has a similar effect on contractile function of prostatic smooth muscle cells in both rats, guinea-pigs (Haynes and Hill, 1997) and dogs (Normandin and Lodge, 1996). Furthermore, the effects of β-adrenoceptor stimulation on rat prostate are similar to those of calcitonin gene-related peptide (CGRP; Ventura et al., 2000) and adenosine (Preston et al., 2000) receptor stimulation, which also inhibit electrically evoked nerve-mediated contractile responses of the smooth muscle of the rat prostate.

While there is a clear inhibitory effect from stimulation of β-adrenoceptors in the rat prostate, the responsiveness of the preparation was quite variable. This may be a consequence of the low absolute magnitude of the responses to field stimulation and exogenous noradrenaline in the rat prostate. This is expected since the rat prostate has a low ratio of smooth muscle to glandular epithelium compared to the prostates of guinea-pigs, dogs and humans. Another factor which may have contributed to the variability in responses is receptor desensitisation. Isoprenaline is known to cause desensitisation after prolonged stimulation of β-adrenoceptors (Summers et al., 1997) and this phenomenon may have confounding effects. This desensitisation did not occur with noradrenaline and adrenaline and may have contributed to the observation that isoprenaline appeared to have a lower extrinsic activity than the endogenous catecholamines (see Table 1).

In addition, adrenaline, noradrenaline and to a lesser extent isoprenaline also increased the tone of the rat prostatic smooth muscle. This phenomenon was separate to their inhibitory effects on electrically evoked contractions of the rat prostate. The increase in rat prostatic smooth muscle tone by these adrenoceptor agonists is due to their concomitant stimulation of postjunctional α_1-adrenoceptors, which are the predominant excitatory contractile receptors in this preparation (Lau et al., 1998). It was not possible to avoid this occurrence by placing an α_1-adrenoceptor antagonist such as prazosin in the bathing medium as this causes almost total blockade of the electrically evoked contraction (Lau et al., 1998). The isoprenaline-induced increase in basal tone was accompanied by an increase in electrical field stimulation-induced twitch height that can be explained by the additive α_1-adrenoceptor stimulation effects of electrical field stimulation and the exogenously added agonist. However, in the cases of adrenaline and noradrenaline, agonist induced α_1-adrenoceptor-mediated tonic contraction had no effect or resulted in continued inhibition of the twitch responses to electrical field stimulation. Reducing the difference between stimulated and basal contractions may have added to the observed inhibition but this was not the case since the selective α_1-adrenoceptor agonist phenylephrine did not inhibit electrical field stimulation-induced contractions.

Forskolin, which stimulates adenylate cyclase, but not the guanylate cyclase stimulator, sodium nitroprusside, inhibited the contractile responses to electrical field stimulation of the rat prostate. Biochemical studies have shown that β-adrenoceptors agonists are able to stimulate adenylate cyclase and therefore raise cAMP levels in the prostate of rats (Juarranz et al., 1994; Carmena et al., 1997) and guinea-pigs (Haynes and Hill, 1997). This suggests that

β-adrenoceptor stimulation leads to the stimulation of adenylate cyclase and the subsequent accumulation of cAMP which inhibits smooth muscle contraction. Forskolin has also been shown to inhibit contractions of prostate tissue taken from the guinea-pig (Haynes and Hill, 1997), dog (Normandin and Lodge, 1996) and human (Drescher et al., 1994). In all of these studies, forskolin was of a comparable potency to that observed in our study.

High levels of nitric oxide synthase activity have been exhibited in prostates taken from rats (Burnett et al., 1992; Chamness et al., 1995; Di Iulio et al., 1997), and sodium nitroprusside has been shown to cause relaxation of the rat prostate at a concentration of 100 μM (Najbar-Kaszkiel et al., 1997). However, when compared with forskolin in the dog prostate, sodium nitropusside was seen to be a very much weaker (approximately 300 times less potent) relaxing agent than forskolin (Normandin and Lodge, 1996). Therefore, sodium mitroprusside's lack of relaxing activity in the rat prostate under our experimental conditions was consistent with previous reports.

Noradrenaline is the main mediator of nerve-mediated electrically evoked contractions of the rat prostate under our stimulation conditions (Lau et al., 1998). Noradrenaline acts at α_1-adrenoceptors to elicit these contractions (Lau et al., 1998) but if β-adrenoceptors are also present, then noradrenaline would also be expected to stimulate these receptors. This prompted a set of experiments to determine whether β-adrenoceptors might influence the contractile action of noradrenaline on the α_1-adrenoceptors on the smooth muscle of the rat prostate. This was not the case since contractile responses to noradrenaline were not potentiated by the β-adrenoceptor antagonist propranolol, as would be expected. Our observation that neither propranolol nor ICI 118 551 affected basal contractile responses to electrical field stimulation also implies that β-adrenoceptors are not stimulated by the endogenous noradrenaline released following nerve stimulation under the conditions used in this study.

The rank order of potency in causing inhibition of rat prostatic smooth muscle contractile responses to electrical field stimulation was: isoprenaline ≥ adrenaline = noradrenaline > phenylephrine, with isoprenaline being significantly more potent than the other agonists. This rank order of agonist potency indicates that a β rather than an α subtype of adrenoceptor (Ahlqvist, 1948) mediates adrenoceptor-induced inhibition of electrical field stimulation-induced contractions in the rat prostate. The involvement of a β-adrenoceptor was confirmed as the inhibitory effect of isoprenaline was attenuated by propranolol.

Three agonists selective for the three established subtypes of β-adrenoceptor were also employed in this study. The selective β_1-adrenoceptor agonist RO363 (Alexander and Peters, 2000), the selective β_2-adrenoceptor agonist salbutamol (Bylund et al., 1998) and the selective β_3-adrenoceptor agonist BRL 37344 (Bylund et al., 1998) were used. The finding that both RO363 and salbutamol, but not the selective β_3-adrenoceptor agonist BRL 37344, inhibited the contractile responses of the rat prostate to electrical field stimulation indicates the lack of involvement of β_3-adrenoceptors in inhibition of prostatic smooth muscle contraction in the rat.

The finding that the selective β_1- and β_2-adrenoceptor agonist RO363 and salbutamol, respectively, were approximately equipotent in causing inhibition was surprising. However, although shown to be relatively potent in this preparation by our method of analysis, the intrinsic activity of RO363 was very low. Furthermore, the experimental usefulness of RO363 in β-adrenoceptor subclassification experiments has previously been reported to be limited due to its low selectivity and efficacy (Bylund et al., 1994). In the present study, atenolol (3 μM) did not attenuate the inhibition of contractile responses of the rat prostate to electrical field stimulation by isoprenaline or salbutamol. Atenolol has been used as a pharmacological tool in binding and functional experiments to determine the nature of a β-adrenoceptor subtypes. It is selective β_1-adrenoceptor antagonist, with a $pA_2 = 7.6$ at β_1-adrenoceptors in rat atria (Bylund et al., 1998). This suggests that β_1-adrenoceptors do not mediate the inhibition of electrical field stimulation-induced contractions of the rat prostate.

The selective β_2-adrenoceptor antagonist ICI 118 551 (0.1 μM, $pA_2 = 9.3$ at β_2-adrenoceptors in guinea-pig trachea; Bylund et al., 1998), attenuated the inhibition of contractile responses to electrical field stimulation by isoprenaline and salbutamol. This implies that the inhibition of electrical field stimulation-induced responses by the β-adrenoceptor agonists used in the study were mediated by stimulation of β_2-adrenoceptor. This is in contrast to an earlier study investigating β-adrenoceptor-mediated inhibition of field stimulation-induced contractions in the prostate of the guinea-pig (Haynes and Hill, 1997). These authors reported a β_1-like adrenoceptor-induced inhibition which was based on the observation that inhibition of electrical field stimulation-induced contractions by isoprenaline was antagonised by atenolol but not by ICI 118 551. This is in contrast to what we observed despite using similar concentrations of the antagonists. Higher concentrations of ICI 118 551 may have produced greater and more impressive rightward shifts in the concentration–response curves to isoprenaline and salbutamol but this would have been at the expense of subtype specificity and consistency with earlier reports of β-adrenoceptors in the prostate gland.

Previous competition radioligand binding studies on the homogenates of rat prostate indicated a population of β-adrenoceptors (Gousse et al., 1991; Fukumoto et al., 1993). These results are supported by the present study, where clearly, β-adrenoceptors play some role in modulating prostate contractions in the rat. Furthermore, binding studies in pig and human have described a predominance of β_2-adrenoceptors and only a smaller subpopulation of β_1-adrenoceptors (Goepel et al., 1997). In this study, the subtype of β-adrenoceptor mediating inhibition of the rat

prostate was also shown to be of the β_2-adrenoceptor subtype.

Many diverse pharmacological agents including drugs which block β-adrenoceptors are used in the treatment of hypertension. Based on the current results, the possibility exists that nonselective β-adrenoceptor antagonist therapy could have a negative impact on urinary symptoms in patients with benign prostatic hyperplasia.

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