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β-Adrenoceptor-mediated inhibition of α_1-adrenoceptor-mediated and field stimulation-induced contractile responses in the prostate of the guinea pig

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1 The prostate of the guinea pig responds to electrical field-stimulation (2 s trains, 0.1 ms pulses at 3–60 Hz, supramaximal voltage) with contractile responses. At 18 Hz these responses were inhibited ($82 \pm 2\%$) by the L-type Ca^{2+} channel blocker, nifedipine (10 μM) and (by 100%) by the neurotoxin, tetrodotoxin (500 nM). The α_{1A}-selective adrenoceptor antagonist, 5-methylurapidil, inhibited responses to field stimulation in the absence and presence of nifedipine (10 μM) with $-\log$ molar (p) IC_{50} ($\pm$s.e.mean) values of 7.95 ± 0.14 and 7.01 ± 0.07, respectively.

2 The non-selective β-adrenoceptor agonist, isoprenaline, reduced ($56 \pm 8\%$) field stimulation induced contractile responses (pEC_{50} 6.91 ± 0.11). The non-selective β-adrenoceptor antagonist propranolol (50 nM) and the β_1-adrenoceptor selective antagonist, atenolol (3 μM), but not the β_2-adrenoceptor antagonist ICI 118,551 ((±)-1 -[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxyl]-3-[1-methylethyl)amino]-2-butanol HC1; 100 nM) antagonized this effect (apparent pK_B values 8.44 ± 0.22 and 6.92 ± 0.21, respectively) indicating an effect mediated through β_1-like adrenoceptors. In the presence of nifedipine (10 μM) isoprenaline (up to 10 μM) did not inhibit the remaining response to field-stimulation.

3 Phenylephrine elicited contractile responses (pEC_{50} 4.47 ± 0.30) from preparations of guinea pig prostate which were reduced ($63 \pm 25\%$) by nifedipine (10 μM). This response was antagonized by 5-methylurapidil (100 nM, apparent pK_B 8.24 ± 0.33), but was not affected by preincubation chloroethylclonidine (50 μM, 30 min). Responses to phenylephrine (30 μM) were inhibited (by up to $52 \pm 5\%$) by isoprenaline (pIC_{50} 6.40 ± 0.35, the β_2-adrenoceptor selective agonist, salbutamol was weakly effective). Propranolol (300 nM), ICI 118,551 (100 nM) and atenolol (3 μM) shifted isoprenaline concentration–response curves to the right (apparent $pK_B \pm$s.e. values 7.68 ± 1.10; 8.00 ± 0.72 and 6.62 ± 0.95, respectively). In the presence of nifedipine (10 μM) responses to phenylephrine (30 μM,) were inhibited (by up to $51 \pm 4\%$) by isoprenaline (pIC_{50} 6.88 ± 0.17): propranolol (300 nM) and ICI 118,551 (100 nM), but not atenolol (3 μM) antagonized this effect (apparent pK_B values 8.85 ± 1.53 and 8.35 ± 1.18, respectively). Thus β_1-like and β_2-like adrenoceptors may be involved in the isoprenaline-stimulated inhibition of phenylephrine concentration–response curves.

4 Phenylephrine stimulated [^{3}H]-inositol phosphate accumulation (pEC_{50} 4.47 ± 0.83), an effect insensitive to chloroethylclonidine pre-treatment (50 μM, 30 min) and to nifedipine (10 μM), but inhibited by 5-methylurapidil (apparent pK_D 7.90 ± 0.22). Isoprenaline (up to 1 μM) did not affect the phenylephrine-stimulated maximal increase in [^{3}H]-inositol phosphates but did increase [^{3}H]-cyclic adenosine monophosphate ([^{3}H]-cAMP) accumulation (pEC_{50} 6.77 ± 0.66); propranolol (30 nM) and ICI 118,551 (110 nM), but not atenolol (up to 3 μM), antagonized this effect. These responses may therefore be mediated through β_2-like adrenoceptors.

5 These results show that the α_1-adrenoceptor mediated and field stimulation-induced contractions of the guinea pig prostate are partly dependent upon intracellular and extracellular sources of Ca^{2+}. We conclude that both β_1- and β_2-like adrenoceptors inhibit responses to phenylephrine in the prostate of the guinea pig. The β_1-like adrenoceptor-mediated inhibition of these responses is evident upon the field stimulation-induced and nifedipine-sensitive component of the response to phenylephrine and may not involve the activation of adenylyl cyclase. The β_2-like adrenoceptor may inhibit both nifedipine sensitive and insensitive components of the response to phenylephrine, possibly through the activation of adenylyl cyclase, but not through the inhibition of inositol phosphate accumulation.

Keywords: Guinea pig prostate; α-adrenoceptor; β-adrenoceptor; inositol phosphate; cAMP

Introduction

α_1-Adrenoceptors have been identified in bovine, canine, rat and rabbit prostatic tissue (Maruyama *et al*., 1992; Couldwell *et al*., 1993; Testa *et al*., 1993; Faure *et al*., 1994; Rokosh *et al*., 1994; Hiraoka *et al*., 1995). In humans, functional, molecular and radioligand binding studies have variously described the α_1-adrenoceptors of the prostate as mixed populations of α_{1A}-, α_{1B}-/α_{1C}- (Guh *et al*., 1995), α_{1C}- and α_{1L} - (Muramatsu *et al*., 1994), α_{1C}- (Faure *et al*., 1994) and α_{1C}- (Lepor *et al*., 1993; Forray *et al*., 1994). More recent studies indicate that the human prostate may contain α_1-adrenoceptors of the α_{1L}-subtype (Ford *et al*., 1996). In contrast to other species, few studies have demonstrated the presence of α_1-adrenoceptors in the prostate of the guinea pig.

In a recent report we have shown that α_2-adrenoceptors, negatively coupled through $G_{i/o}$, to adenylyl cyclase potentiate α_1-adrenoceptor-mediated contractile responses in the epididymis of the guinea pig (Haynes & Hill, 1996). This finding is significant as the prostatic tissue of the human and rat have been shown to contain α_2- (Chapple *et al*., 1989) and β-adrenoceptors (Shima *et al*., 1985; Chen *et al*., 1995), respectively. In this study we investigate the interaction between α- and β-adrenoceptor activation in the contractile response of the

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prostate of the guinea pig using classical pharmacology, [^{3}H]-inositol phosphate and [^{3}H]-cAMP accumulation studies.

Methods

Animals

Male Duncan-Hartley guinea pigs (600 g to 1 kg; 12–20 weeks) were housed in open runs (21°C) with a 12 h light–dark cycle. Food consisted of BeKay pellets with green vegetables and water *ad libitum*. On the day of use animals were killed and the ventral and dorsal lobes of the prostate were removed.

Contractility studies

Preparations of prostate were tied (with a silk thread) to a tissue holder held between parallel platinum electrodes and placed into organ baths containing modified Krebs solution (of composition, mM; NaCl 118; KCl 4.7; $MgSO_4$ 0.45; K_2HPO_4 2.5; $NaHCO_3$ 25; $CaCl_2$ 1.9; glucose 11) gassed with O_2:CO_2 (95:5), at 36°C. The upper end of each preparation was connected to a Grass FT03 force-displacement transducer via another silk thread. Preparations were suspended under 0.35 *g* resting force. Recordings of contractile force were made using a Grass (model 79D) chart recorder. Tissues were allowed at least 40 min equilibration time prior to the addition of agonists or field stimulation. Only one concentration–response curve was constructed on any tissue.

Responses to field stimulation

Preparations were electrically stimulated (using an SRI square wave variable pulse stimulator coupled to a BBC computer) with trains of field stimulation (2 s at 0.1 ms duration; 1, 3, 9, 18, 30 or 60 Hz and supramaximal voltage) and responded with contractions (see Figure 1 for typical responses); at 18 Hz these responses were submaximal (37±5% of the responses at 60 Hz, $n=6$). Preparations were field stimulated (2 s trains of pulses, 0.1 ms duration, 18 Hz, supramaximal voltage) for 30 min prior to the cumulative addition of either the α_{1A}-adrenoceptor antagonist, 5-methylurapidil (100 pM to 10 μM), or the non-selective β-adrenoceptor agonist isoprenaline (1 nM to 10 μM). For some experiments propranolol (50 nM), ICI 118, 551 (100 nM), atenolol (3 μM) or nifedipine (10 μM) were added to preparations at least 15 min prior to the addition of isoprenaline. In some experiments nifedipine (10 μM) was added to preparations prior to the cumulative addition of 5-methylurapidil (100 pM to 10 μM). Responses to field stimulation were allowed to plateau or allowed 10 min, following the addition of isoprenaline or 5-methylurapidil, before subsequent concentrations of agonist or antagonist were added.

The (mean of the two) minimum responses to field stimulation obtained at each concentration of agonist or antagonist was calculated as a percentage of the mean (of the two) responses obtained before agonist or antagonist addition (S1). A similar analysis was undertaken for time-matched control tissues responses. Results are expressed as the difference between responses measured in the presence of agonist/antagonist and those of time control preparations. In cases where antagonists or nifedipine were used in addition to isoprenaline, the responses to field stimulation in the presence antagonist or nifedipine are taken as the control response (S1).

In some experiments preparations of field stimulated prostate were allowed to equilibrate (40 min) prior to the addition of prazosin (1 μM), atropine (1 μM) or suramin (100 μM), tetrodotoxin (500 nM) or nifedipine (10 μM) which remained in contact with the preparations for the remainder of the experiment (at least 90 min).

Responses to phenylephrine

Following the equilibration period KCl (60 mM) was added to preparations to ensure tissue viability and to provide an index of tissue contractility, the maximum response was recorded and the tissues washed and left for 15 min. Phenylephrine was added to organ baths (120 s contact period every 15–17 min; see Figure 1 for typical responses), tissues were then washed 2–5 times (bath volume) with fresh Krebs solution. The addition of the neuronal uptake inhibitor cocaine (10 μM) and the extraneuronal uptake inhibitor β-oestradiol (10 μM) significantly potentiated responses to phenylephrine (30 μM) (Table 1); however, cocaine and β-oestradiol also significantly increased spontaneous activity of these preparations (by up to 70±25 mg force, $n=5$) and were not therefore routinely included in subsequent experiments to minimize the effects of endogenous noradrenaline. The β-adrenoceptor antagonist, propranolol (300 nM) and the P_2 purinoceptor antagonist, suramin (100 μM), did not affect the responses to phenylephrine (Table 1).

For some experiments preparations were incubated with nifedipine (10 μM) or were preincubated with chloroethylclonidine (50 μM, 30 min) prior to the construction of phenylephrine concentration–response curves. In an alternative series of experiments isoprenaline (1 nM to 100 μM) was added to the preparations (90 s) prior to the addition of phenylephrine (30 μM), some preparations were also incubated with either propranolol (300 nM), atenolol (3 μM), ICI 18551 (100 nM) or nifedipine (10 μM) throughout the experiment.

[^{3}H]-inositol phosphate accumulation

Preparations were obtained as described above and incubated (37°C) in Krebs buffer containing (0.45 μCi) [^{3}H]-*myo*-inositol (NEN, Dupont) for 4.5 h (in an O_2 (95): CO_2 (5) atmosphere),

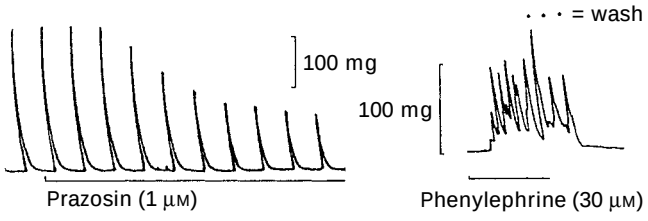

Figure 1 Typical responses to field stimulation (2 s trains, 0.1 ms duration, 18 Hz, supramaximal voltage every 30 s) and phenylephrine (30 μM) evoked contractile responses in the prostate of the guinea pig (left and right panels, respectively). The horizontal bar indicates the addition of prazosin (1 μM) or phenylephrine (30 μM, 90 s contact time). (●) Indicates wash.

Table 1 Effects of changing incubation conditions upon responses to phenylephrine

	Control response (%) (mean±s.e.mean)	*n*
Time control	95±5	5
Propranolol*	99±5	3
Suramin	106±4	4
β-Oestradiol/cocaine	149±8	5
Xylazine	101±7	6

Antagonists were allowed at least 30 min to incubate prior to second addition of phenylephrine. Xylazine was added 2 min prior to the second addition of phenylephrine. *These preparations received phenylephrine (300 μM). n=the number of replicate experiments. Control responses to phenylphrine (30 or 300* μM) were established prior to, and then following the addition of either propranolol (300 nM), β-oestradiol and cocaine (both 10 μM), suramin (100 μM) or xylazine (1 μM).

30 min prior to the end of this incubation chloroethylclonidine was added to some preparations (giving a final concentration of 50 μM). All tissues were then rinsed in fresh Krebs solution, blotted dry and allowed to incubate for 25 min in fresh Krebs buffer (35–36°C) containing LiCl (20 mM) and, where indicated, 5-methylurapidil or nifedipine. Phenylephrine was added and the preparations allowed to incubate for a further 15 min prior to transfer to 1 ml ice-cold methanol: (0.12 M) HCl (1:1). Tissues were then stored −20°C for up to 4 days before being homogenized (Kinematica PT 10–35), centrifuged 20 000 *g* (10 min in a Sigma Howe 3K20 centrifuge). The supernatant was neutralized with 25 mM) Tris: (0.5 M) NaOH: H_2O (55:7:170). Total [^{3}H]-inositol phosphates were separated from free [^{3}H]-*myo*-inositol by anion exchange chromatography (Hall & Hill, 1988). Tritium levels in supernatant were determined by liquid scintillation counting.

[^{3}H]-cAMP accumulation

This protocol is essentially a modification of that outlined by Ruck *et al.* (1991). Briefly, preparations of prostate were obtained as described above and incubated (37°C) in Krebs buffer containing (0.3 μCi) [^{3}H]-adenine (NEN, Dupont) for 2 h. Preparations were washed once in 2 ml fresh Krebs and incubated for 15 min in fresh Krebs (35–36°C) containing the phosphodiesterase inhibitor, rolipram (100 μM). Agonists or forskolin were added and preparations left for 10 min. The reaction was terminated by the addition of concentrated HCl (5% of incubation volume). Tissues were frozen (−20°C) overnight and [^{3}H]-cAMP extracted from the incubation media using acidic alumina columns (Johnson *et al.*, 1994). Tritium levels in samples was determined by liquid scintillation counting.

Statistics

Estimates of −log molar (p) EC_{50} or pIC_{50} were generated using a four-parameter logistic curve fitting and graphics programme PRISM v1.0 (GraphPad Software Inc., San Diego). Comparison between concentration–response curves were determined by the use of an iterative curve fitting programme, FLEXIFIT (see Guardabasso *et al.*, 1988), significant changes were determined with an *F* test. One-way ANOVA, Student's *t* test and Dunnett's test were used to determine changes between data sets. In all cases $P<0.05$ was taken as the level of significance. Apparent K_B values were determined using the Gaddum equation:

$$pK_B = \log\,[\text{concentration ratio} - 1] - \log\,[\text{antagonist concentration}]$$

An apparent pK_D value was calculated for the inhibition of phenylephrine stimulated [^{3}H]-inositol phosphate accumulation by 5-methylurapidil using a previously described modification (Dickenson & Hill, 1993) of the null equation, i.e. $K_D = (C'/C-1)*(IC_{50})^{-1}$ where C'/C is the ratio of equieffective agonist concentrations in the absence and presence of antagonist and the IC_{50} is that of the antagonist.

Drugs

(-)-Atenolol; chloroethylclonidine HCl; cocaine HCl; forskolin and idazoxan HCl (Research Biochemicals Inc., Natick, U.S.A.): atropine SO_4, (±)-isoprenaline HCl; nifedipine; β-oestradiol; phenylephrine HCl; (±)-propranolol HCl; salbutamol; tetrodotoxin and xylazine HCl (Sigma Chemical Co., U.K.): ICI 118,551 ((±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxyl]-3-[1-methylethyl)amino]-2-butanol HCl), a gift from ICI, U.K.): 5-methylurapidil (a gift from B.K.G. Germany): rolipram (a gift from Schering AG Berlin, Germany): suramin sodium (ICN Biomedicals Inc., U.S.A.).

Tetrodotoxin was dissolved in acidified ethanol; nifedipine and β-oestradiol were dissolved in DMSO; forskolin and salbutamol were dissolved in ethanol; isoprenaline was made up in 0.01 N HCl containing ascorbic acid (100 μM). All other drugs were dissolved in distilled water and made up to volume with Krebs solution.

Results

Responses to field stimulation

Preparations of prostate responded to trains of field stimulation (2, 10 or 30 s at 0.1 ms duration; 1, 3, 9, 18, 30 or 60 Hz and supramaximal voltage) with contractile responses (see Figure 1). The electrically evoked responses (to 2 s trains at 18 Hz) could be completely blocked by tetrodotoxin (500 nM; $n=4$, data not shown) and inhibited (by 82±2%) by nifedipine (10 μM, $n=4$, for typical trace see Figure 1).

The α_{1A}-selective adrenoceptor antagonist 5-methylurapidil inhibited field-stimulation evoked contractile responses in the absence and presence of nifedipine (pIC_{50} 7.92±0.14, $n=6$; and 7.01±0.07, $n=5$, respectively; Figure 2).

The P_2-purinoceptor antagonist, suramin (100 μM), the muscarinic antagonist, atropine (1 μM), and the non-selective α_1-adrenoceptor antagonist, prazosin (1 μM) also reduced responses to field stimulation (Table 2), the effects of prazosin and 5-methylurapidil (both at 1 μM) were not significantly different (Student's *t* test).

Effects of isoprenaline

The non-selective β-adrenoceptor agonist, isoprenaline inhibited responses to field stimulation (pEC_{50} 6.91±0.11; $n=6$).

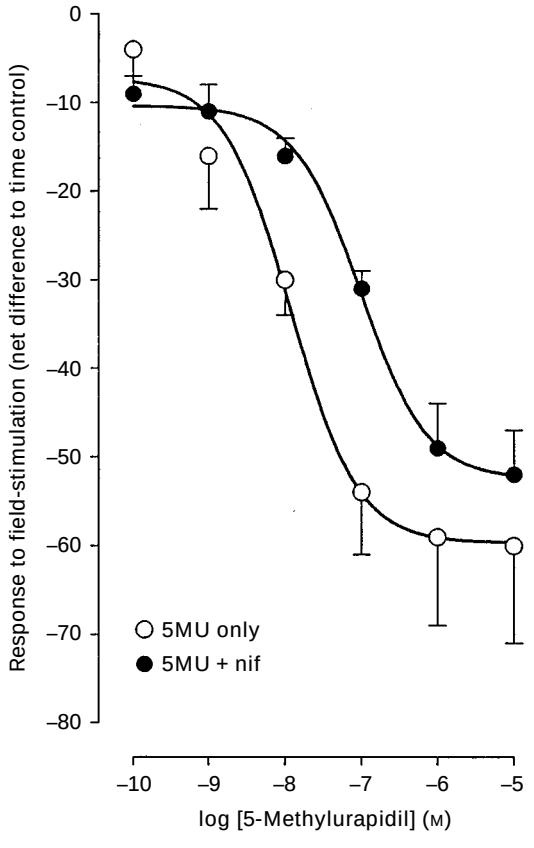

Figure 2 Effects of 5-methylurapidil on field stimulation-evoked contractile responses in preparations of prostate in the absence and presence of nifedipine (10 μM; nif). Results were calculated as a percentage of the initial (antagonist- or vehicle-free) response to field stimulation (S1). The symbols show the net difference between responses in the presence of antagonist and vehicle only (time control) responses. Bars (some omitted for clarity) represent s.e.mean of 5–6 replicate experiments.

This effect could be blocked by propranolol (50 nM) and by atenolol (3 μM, mean apparent pK_B values of 8.44±0.22 and 6.92±0.21; $n=5$), respectively, but not by ICI 118,551 (100 nM, $n=5$, Figure 3a), also see Table 3. In the presence of nifedipine (10 μM), isoprenaline did not significantly inhibit responses to field stimulation ($n=5$, Figure 3b).

The diterpine, forskolin inhibited responses to field stimulation (10 μM, Figure 3a). In the presence of nifedipine (10 μM) forskolin (100 μM) inhibited responses to field stimulation by 32±8% of control (S1, $n=3$).

Responses to phenylephrine and the effect of isoprenaline

Preparations of prostate responded to the addition of phenylephrine with contractile responses (pEC_{50} 4.47±0.30, see Figure 1 for typical responses and Figure 4). Pretreatment of tissues with the $\alpha_{1B/D}$-adrenoceptor alkylating agent chloroethylclonidine (50 μM, 30 min) did not affect responses to phenylephrine (pEC_{50} 4.15±0.33, Figure 4a). Nifedipine (10 μM) significantly (F test, $P<0.05$, d.f. = 1,56) reduced the maximal response to, but not the pEC_{50} (4.57±0.20) of phenylephrine concentration–response curves (Figure 4a). 5-Methylurapidil shifted phenylephrine concentration–response curves to the right with a mean apparent pK_B of 8.24±0.33 ($n=5$; Figure 4b). The α_2-adrenoceptor agonist xylazine (1 μM) did not potentiate responses to phenylephrine (30 μM, Table 1).

Isoprenaline inhibited (by 52±5%) responses to phenylephrine (30 μM) in preparations of prostate (pEC_{50} 6.40±0.35, $n=6$), this effect was antagonized by propranolol (300 nM), ICI 118,551 (100 nM) and by atenolol (3 μM) with apparent pK_B (±s.e.) values of 7.68±1.10, 8.00±0.72 and 6.62±0.95, respectively (Figure 5a). In the presence of nifedipine (10 μM) responses to phenylephrine (30 μM), were inhibited (by up to 51±4%) by isoprenaline (pIC_{50} 6.88±0.17): ICI 118,551 (100 nM) and propranolol (300 nM), but not atenolol (3 μM) antagonized this effect (apparent pK_B±s.e. values 8.35±1.18 and 8.85±1.53; Figure 5b). The pEC_{50} values of isoprenaline under these varying conditions is also shown in Table 3. The β_2-adrenoceptor selective agonist, salbutamol, inhibited responses to phenylephrine (significant at 100 μM $P<0.05$, Student's t test) in the absence ($n=6$) and presence ($n=5$) of nifedipine (10 μM) (Figure 5c).

Table 2 Effects of antagonists upon responses to field stimulation (2 s trains, 0.1 ms duration, 18 Hz, supramaximal voltage every 30 s) in preparations of the prostate of the guinea pig

	Mean control response(%) (mean±s.e.mean)	*n*
Time control	97±4	5
Suramin (100 μM)	43±6*	6
Prazosin (1 μM)	46±7*	6
Atropine (1 μM)	35±3*	4

Responses to field stimulation in the presence of antagonists were allowed to plateau prior to measurement. Responses to field stimulation in the presence of antagonists are expressed as a percentage of the mean response (of the two) contractions prior to the addition of antagonist (S1). *Significantly different to S1 ($P<0.05$, Student's t test). n=the number of replicate experiments.

[^{3}H]-inositol phosphate accumulation

Phenylephrine elicited concentration-dependent increases in the accumulation of [^{3}H]-inositol phosphates, pEC_{50} 4.47±0.83 (Figure 6a). The maximal increase in [^{3}H]-inositol phosphate production was unaffected by the preincubation of tissues with cloroethylclonidine (50 μM, 30 min, $n=6$, Figure 6a) nor was it affected by nifedipine (10 μM, $n=5$ Figure 6a). The α_{1A}-adrenoceptor selective antagonist, 5-methylurapidil, inhibited this response (with an apparent pK_D of 7.90±0.22, Figure 6b).

Preparations of prostate responded to the addition of phenylephrine (300 μM) with an increase in basal [^{3}H]-inositol phosphates accumulation (268±45 to 929±294 d.p.m. mg^{-1}, both $n=6$), in preparations receiving isoprenaline (1 μM, 90 s

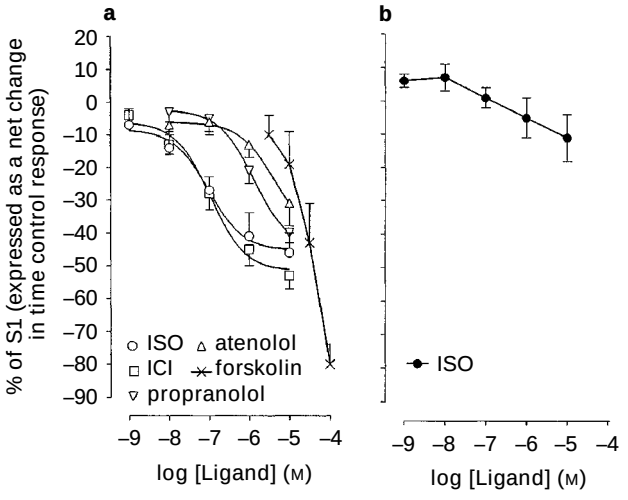

Figure 3 Effects of isoprenaline on field stimulation (2 s trains, 0.1 ms duration, 18 Hz, supramaximal voltage every 30 s) -evoked contractile responses in preparations of prostate. (a) Effects of isoprenaline on responses to field stimulation in the absence (ISO) and presence of propranolol (50 nM), atenolol (5 μM); ICI 118,551 (100 nM, ICI), also shown is the effect of forskolin. (b) Effects of isoprenaline (ISO) on responses to field stimulation in the presence of nifedipine (10 μM). Results were calculated as a percentage of the mean of the two initial (antagonist- or vehicle-free) responses to field-stimulation (S1). The data are shown as the net difference between responses in the presence of antagonist and vehicle only (time control) responses. Bars (some omitted for clarity) represent s.e.mean of 5–6 replicate experiments.

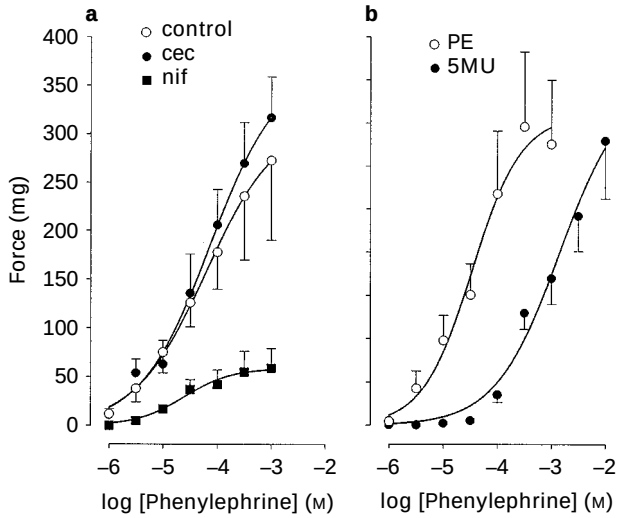

Figure 4 Effect of chloroethylclonidine, nifedipine and 5-methylurapidil phenylephrine concentration-response curves in the prostate of the guinea pig. (a) Concentration–response curves to phenylephrine alone (control), during the incubation with nifedipine (10 μM; nif) or following the preincubation with chloroethylclonidine (50 μM, 30 min; cec). (b) Shows phenylephrine concentration–response curves in the absence (PE) or presence of 5-methylurapidil (100 nM; 5 MU). All symbols and bars (some omitted for clarity) represent mean and s.e.mean of 5–6 replicate experiments.

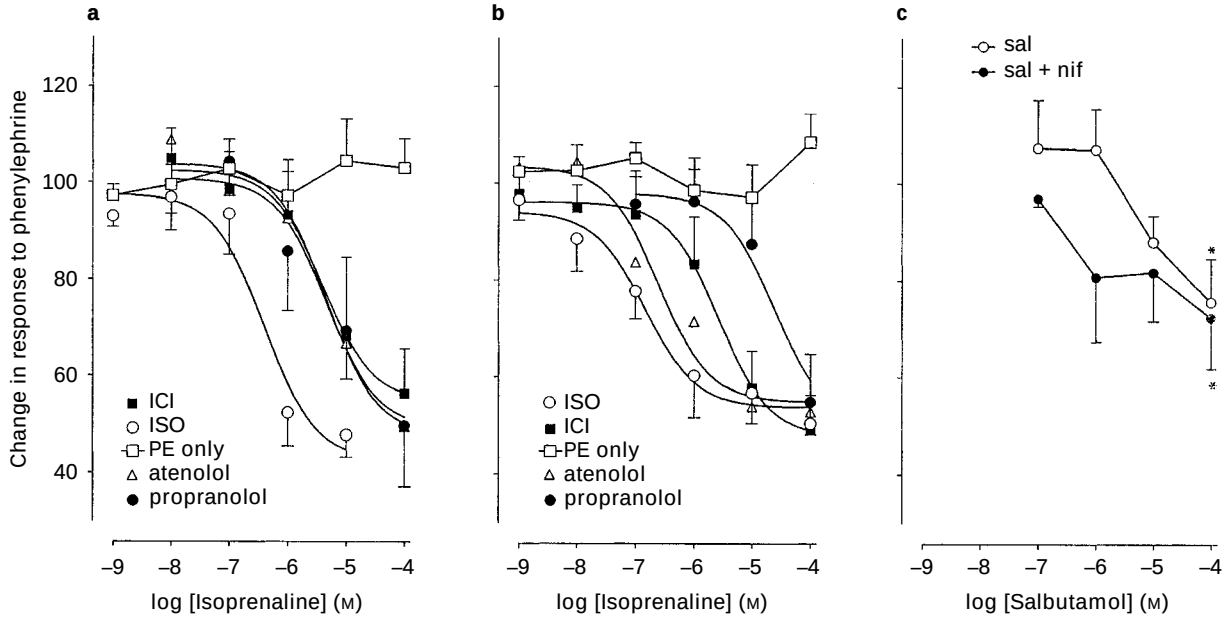

Figure 5 Effect of isoprenaline upon responses to phenylephrine (30 μM) in the prostate of the guinea pig. The symbols (a) show the inhibition of responses to phenylephrine (30 μM) following the addition of isoprenaline (ISO), alone or in the presence of either propranolol (300 nM), ICI 118,551 (100 nM; ICI) or atenolol (3 μM). (b) Same agonist, antagonist combinations all in the presence of nifedipine (10 μM). (c) Effects of the β_2-adrenoceptor agonist, salbutamol, on responses to phenylephrine (30 μM) in the absence (sal) and presence of nifedipine (10 μM; sal + nif). (a) and (b) Time control responses (to phenylephrine, 30 μM) are also shown (PE only). Results are expressed as a percentage of their first response to phenylephrine (30 μM). It should be noted that compared to vehicle controls nifedipine reduces the maximal response to phenylephrine by 63±25% ($n=6$). All symbols and bars (some omitted for clarity) represent mean and s.e.mean of 5–7 replicate experiments. *Significant ($P<0.05$, Student's t test) difference from S1.

Table 3 Effects of isoprenaline upon responses to field (2 s trains, 0.1 ms duration, 18 Hz, supramaximal voltage every 30 s)-stimulation (stim) and phenylephrine (pe, 30 μM)-stimulation in preparations of the prostate of the guinea pig

	pEC_{50}			
Stimuli	*iso only*	*iso + prop*	*iso + ICI*	*iso + aten*
stim	6.91±0.11	5.89±0.03	6.97±0.17	5.37±0.19
pe	6.40±0.35	5.33±0.30	5.37±0.13	5.35±0.18
pe + nifedipine	6.88±0.17	4.61±0.17	5.57±0.06	6.61±0.26

In all cases the values shown were obtained from between 5–7 replicate experiments. Each value represents the mean apparent pEC_{50} (±s.e.mean) values of isoprenaline in the absence and presence of nifedipine (10 μM), propanolol (prop, 300 nM), (ICI, 100 nM) and atenolol (aten, 3 μM).

earlier) phenylephrine (300 μM) still increased [^{3}H]-inositol phosphate accumulation (to 737±136 d.p.m. mg^{-1}, $n=6$).

[^{3}H]-cAMP accumulation

Isoprenaline elicited concentration-dependent increases in [^{3}H]-cAMP in preparations of prostate (pEC_{50} 6.77±0.66, $n=6$, Figure 7a). The non-selective β-adrenoceptor antagonist propranolol (30 nM, $n=5$) and the β_2-adrenoceptor antagonist ICI 118,551 (100 nM, $n=5$) significantly ($P<0.05$, Dunnett's test) inhibited the isoprenaline (1 μM)-stimulated accumulation of [^{3}H]-cAMP (Figure 7b and c, respectively), the β_1-adrenoceptor antagonist atenolol (up to 3 μM, $n=5$) was without significant (Dunnett's test) effect (Figure 7d).

Discussion

These studies have demonstrated that the prostate of the guinea pig responds to field stimulation with contractions sensitive to the neurotoxin, tetrodotoxin, indicating that they are mediated through the release of neurotransmitter rather than a direct depolarizing effect upon the prostatic smooth muscle. The α_1-adrenoceptor antagonist, prazosin, and the α_{1A}-selective adrenoceptor antagonist, 5-methylurapidil, both inhibited responses to field stimulation indicating that a component of this response was mediated through activation of α_1-adrenoceptors. The inhibition of the responses to field stimulation by the L-type voltage operated Ca^{2+} channel (VOCC) blocker, nifedipine, indicates that a significant component of these contractions is dependent upon the influx of extracellular Ca^{2+}.

In addition to field stimulation-induced contractile responses, preparations of prostate also respond to the addition of the α_1-adrenoceptor agonist phenylephrine with contractions which were antagonized by 5-methylurapidil, unaffected by incubation of tissues with the $\alpha_{1B/D}$-adrenoceptor selective (Hieble *et al*., 1995) alkylating agent chloroethylclonidine but were reduced by nifedipine, a finding consistent with that reported in human prostate, where nifedipine reduced phenylephrine-stimulated contractile responses by 70% (Drescher *et al*., 1994a, b; Eckert *et al*., 1994). Phenylephrine also increased the accumulation of [^{3}H]-inositol phosphates in the prostate of the guinea pig, an effect antagonized by 5-methylurapidil, but insensitive to both nifedipine and to the preincubation of tissues with chloroethylclonidine. This later finding is in contrast to earlier findings from this laboratory where the phenylephrine-stimulated [^{3}H]-inositol phosphate accumulation in the epididymis was sensitive to the preincubation of tissues with chloroethylclonidine (Haynes & Hill, 1996), indicating that epididymal and prostatic α_1-adrenoceptors may represent distinct populations. Although the data from the present study are consistent with the hypothesis that the prostate of the guinea pig contains α_1-adrenoceptors (Cohen & Drey, 1988) our evidence is not sufficient to determine whether the prostatic α_1-adrenoceptor(s) mediating Ca^{2+} influx, through L-type Ca^{2+} channels and inositol phosphate accumulation represent the same or different subtypes. Also in contrast with

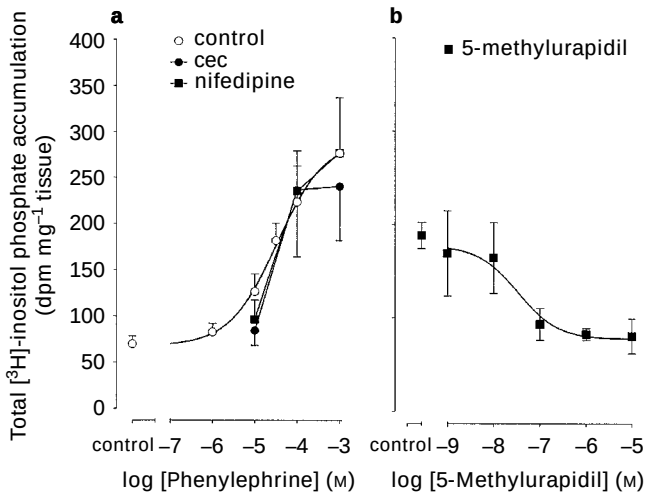

Figure 6 Phenylephrine and 5-methylurapidil effects on [^{3}H]-inositol phosphate accumulation in preparations of prostate. (a) Effects of phenylephrine alone (control), in the presence of nifedipine (10 μM) or following preincubation with chloroethylclonidine (50 μM; cec). (b) Effect of 5-methylurapidil upon the phenylephrine (30 μM) mediated accumulation of [^{3}H]-inositol phosphates. All symbols and bars (some omitted for clarity) represent mean and s.e.mean of 5–7 replicate experiments.

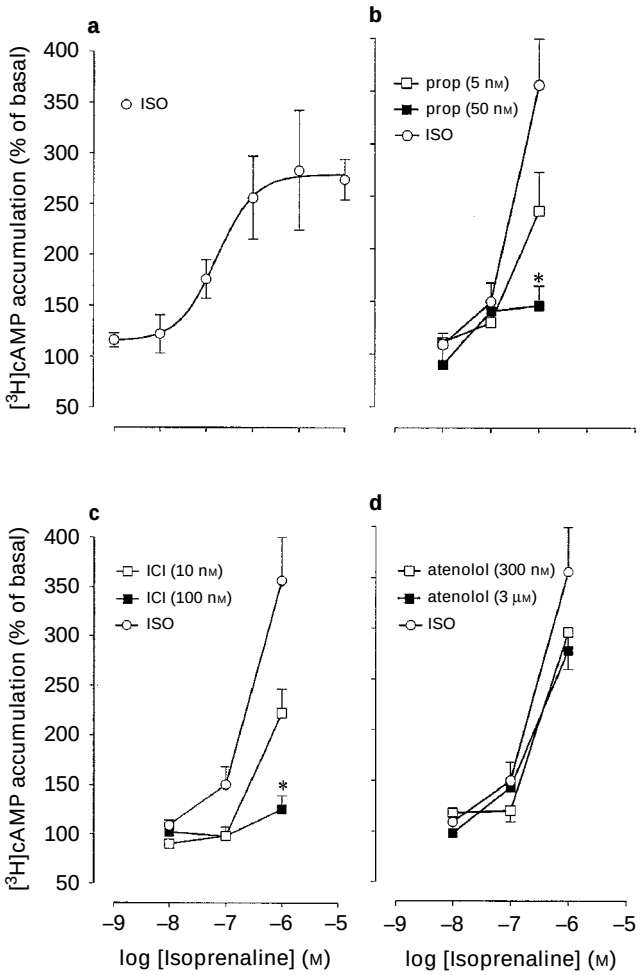

Figure 7 Isoprenaline effects upon [^{3}H]-cAMP accumulation in preparations of prostate. All panels show the isoprenaline (ISO)-mediated [^{3}H]-cAMP accumulation. The effects of the non-selective β-adrenoceptor antagonist propranolol (3 and 30 nM; prop), the β_2-adrenoceptor antagonist ICI 118,551 (10 and 100 nM; ICI) and the β_1-adrenoceptor antagonist atenolol (300 nM and 3 μM) are shown in b, c and d, respectively. In all panels the high concentrations of antagonist are represented by filled symbols. All symbols and bars (some omitted for clarity) represent mean and s.e.mean of 5 experiments. *Significant ($P<0.05$, Dunnett's test) difference from agonist only.

our earlier study (Haynes & Hill, 1996), we have not been able to demonstrate a significant potentiation of α_1-adrenoceptor-mediated responses by the α_2-adrenoceptor agonist, xylazine, this finding may indicate either that the smooth muscle of the prostate of the guinea pig contains no α_2-adrenoceptors or that activated α_2-adrenoceptors do not potentiate responses to phenylephrine in this tissue.

We have shown that isoprenaline inhibits both phenylephrine and field stimulation evoked contractile responses of prostatic smooth muscle and also stimulates the accumulation of [^{3}H]-cAMP in preparations of prostate. These findings are consistent with those reported by Drescher *et al.*, (1994a, b) who demonstrated that phosphodiesterase inhibitors, the cell permeable cAMP analogue dibutyryl-cAMP and forskolin all reduced the contractile response to phenylephrine in human prostatic tissue. In the present study we report that the isoprenaline-mediated inhibition of responses to field stimulation is sensitive to both the non-selective β-adrenoceptor antagonist, propranolol, and the β_1-adrenoceptor selective antagonist, atenolol, their apparent pK_B values and the ineffectiveness of the β_2-adrenoceptor selective antagonist ICI 118,551, are consistent with the action of isoprenaline at β_1-like rather than β_2-like or β_3-like adrenoceptors (Bylund *et al.*, 1994). In contrast, propranolol, ICI 118,551 and atenolol all antagonize the isoprenaline-mediated inhibition of phenylephrine-stimulated contractions, except in the presence of nifedipine where atenolol becomes ineffective and propranolol more effective. Our explanation for these findings is that the prostate of the guinea pig contains populations of both β_1- and β_2-like adrenoceptors; the β_1-like adrenoceptors are effective at inhibiting nifedipine-sensitive responses and that β_2-like adrenoceptors are more effective against nifedipine-insensitive responses. Thus in the absence of nifedipine the β-adrenoceptor agonists act through both β_1 and β_2-adrenoceptors. From our studies of prostatic tissue second messenger function it is clear that the prostatic β_2-like adrenoceptors are coupled to adenylyl cyclase but that their activation does not lead to a gross inhibition of phospholipase C since isoprenaline had no effect upon the phenylephrine-stimulated [^{3}H]-inositol phosphate accumulation. It is unlikely that uptake processes account for the different effects of the β-adrenoceptor antagonists upon [^{3}H]-cAMP accumulation and the inhibition of phenylephrine-mediated contractility as the protocols for determining [^{3}H]-cAMP accumulation and the inhibition of contractile responses are quite similar and isoprenaline is not a good substrate for extraneuronal uptake (Mammen, 1987).

Prostate β_1-like adrenoceptors, in contrast to β_2-like adrenoceptors, do not appear to couple to adenylyl cyclase and may rely upon an alternative mechanism of action to exert their effect. β-adrenoceptors are generally thought to act through Gs proteins (Bylund *et al.*, 1994) and since Kume *et al.* (1992, 1994, 1995) demonstrated that the alpha subunit of Gs can open potassium channels in airway smooth muscle, leading to relaxation, it is possible that these prostatic β_1-like adrenoceptors also act directly upon ion channels to exert their effect upon contractility. The significance of this hypothesis with regard to our evidence indicating that β_1-like adrenoceptors only inhibit contractile responses mediated through the activation of VOCCs is currently under investigation. The lack of effect of isoprenaline on field stimulation-mediated responses in the presence of nifedipine is not consistent with the effect of isoprenaline on phenylephrine-mediated contractile responses in the presence of nifedipine. It is possible that confounding factors such as transmitter release versus agonist addition (noradrenaline versus phenylephrine), the time course

of transmitter versus phenylephrine addition (2 s trains versus 60–90 s contact time) and the magnitude of the nifedipine-sensitive component of the responses to field-stimulation and to phenylephrine (82 versus 63%) reduce the impact of isoprenaline upon nifedipine-insensitive responses to field stimulation. In addition it is also possible that other neurotransmitters may have some influence upon the activity of isoprenaline in field stimulated tissues.

In addition to adrenoceptor-mediated responses we have observed that the muscarinic receptor antagonist, atropine, and the P_2-purinoceptor antagonist, suramin, both reduce responses to field stimulation in the prostate of the guinea pig. The latter finding is interesting because in the smooth muscle of the guinea pig vas deferens, the activation of α_1-adrenoceptors stimulates the release of ATP (von Kugelgen & Starke, 1994). In this study, however, suramin does not affect responses to phenylephrine leaving us to conclude that α_1-adrenoceptor-mediated contractions do not involve ATP release from this tissue.

These studies have demonstrated that the prostatic tissue of the guinea pig responds to electrical field stimulation with contractile responses which are sensitive to the effects of β-adrenoceptor agonists, nifedipine, forskolin, prazosin, 5-methylurapidil, atropine and suramin and tetrodotoxin. The α_1-adrenoceptor agonist, phenylephrine, elicits nifedipine-sensitive and nifedipine-insensitive contractile responses from this tissue, both nifedipine-sensitive and nifedipine-insensitive components of this response are sensitive to 5-methylurapidil and are therefore likely to be mediated through α_1-adrenoceptor activation. The stimulation of prostatic β-adrenoceptors inhibits both the responses to field stimulation and the responses to phenylephrine, probably through the activation of β_1- and β_2-like adrenoceptors. The β_1-like adrenoceptor-mediated inhibition of contractile responses does not appear to involve adenylyl cyclase and is evident only on VOCC-dependent contractile responses. In contrast the β_2-like adrenoceptor inhibition of contractility may involve adenylyl cyclase, but is not mediated through a direct effect on PLC.

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