British Journal of Pharmacology (1996) 119, 1203-1210

α-Adrenoceptor mediated responses of the cauda epididymis of the guinea-pig

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1 The subtypes of α-adrenoceptor mediating the contractile responses of the cauda epididymis of the guinea-pig were investigated. The α_1-adrenoceptor agonist phenylephrine, but not the α_2-adrenoceptor agonist, xylazine (up to 10 μM), elicited concentration-dependent contractions from preparations of cauda epididymis (EC_{50} 3.4 μM). The L-type Ca^{2+} channel antagonist, nifedipine (10 μM), reduced the maximal response to phenylephrine (by 77%). Preincubation of tissues with the α_{1B}-adrenoceptor-alkylating agent, chloroethylclonidine (50 μM, 30 min), shifted phenylephrine concentration-response curves to the right (4 fold) only when the α_2-adrenoceptor antagonist idazoxan (100 nM) was included during the pre-incubation with chloroethylclonidine.

2 Xylazine (1 μM) significantly shifted phenylephrine concentration-response curves to the left (3 fold); this effect was attenuated by idazoxan (100 nM). Both the incubation of preparations with nifedipine (10 μM) and the pre-incubation of preparations with chloroethylclonidine (50 μM, 30 min) attenuated the potentiating effects of xylazine (1 μM). Protection of α_2-adrenoceptors with idazoxan (100 nM) during the chloroethylclonidine (50 μM, 30 min) incubation restored the xylazine-mediated enhancement of phenylephrine concentration-response curves. Pertussis toxin (200 ng ml^{-1}, 24 h) attenuated the xylazine (1 μM)-mediated potentiation of phenylephrine concentration-response curves.

3 Following the pre-incubation of preparations with chloroethylclonidine (50 μM, 30 min) 5-methylurapidil (10 nM to 3 μM) shifted phenylephrine concentration-response curves, in parallel, to the right with mean pK_B values in the range of 8.27 (at 10 nM 5-methylurapidil) to 7.76 (at 3 μM 5-methylurapidil), the addition of idazoxan (100 nM) to the incubation medium did not significantly affect the 5-methylurapidil (10 to 300 nM) pK_B values (8.41 to 7.64, respectively). In the presence of both idazoxan (100 nM) and nifedipine (10 μM), and following the pre-incubation with chloroethylclonidine (50 μM, 30 min), 5-methylurapidil (30 to 300 nM) still shifted phenylephrine concentration-response curves to the right (pK_B values 7.77 to 7.36, respectively).

4 Phenylephrine (1 μM to 1 mM) increased the accumulation of [^{3}H]-inositol phosphates (10 fold) in preparations of cauda epididymis (EC_{50} 12 μM). This effect was sensitive to chloroethylclonidine pretreatment (50 μM, 30 min), antagonized with low affinity by 5-methylurapidil (− log pK_i 7.8), but not potentiated by xylazine (1 μM). Xylazine (10 nM − 100 μM) reversed the forskolin (10 or 30 μM) stimulated accumulation of [^{3}H]-adenosine 3′:5′-cylic monophosphate (cyclic AMP) in preparations of cauda epididymis (by approximately 45%). Incubation of tissues with both pertussis toxin (200 ng ml^{-1}, 24 h) and pertussis toxin vehicle increased the basal activity of adenylate cyclase (3 fold) but did not increase the capacity of forskolin (30 μM) to stimulate the accumulation of [^{3}H]-cyclic AMP in these tissues. Xylazine did not significantly inhibit the forskolin-stimulated accumulation of [^{3}H]-cyclic AMP in either vehicle or pertussis toxin treated tissues.

5 These studies indicate that the epididymis of the guinea-pig contains α_1- and α_2-adrenoceptors. On the basis of the actions of chloroethylclonidine and 5-methylurapidil the α_1-adrenoceptors of this tissue may be of the α_{1A}- and α_{1B}-subtypes and are linked to both the influx of extracellular Ca^{2+} and to phospholipase C. The α_2-adrenoceptors of this tissue are negatively coupled to adenylate cyclase, sensitive to pertussis toxin, but do not amplify phenylephrine-stimulated [^{3}H]-inositol phosphate accumulation. Stimulation of the α_2-adrenoceptors of this tissue may selectively potentiate the influx of extracellular Ca^{2+}.

Keywords: Guinea-pig epididymis; α_{1A}-adrenoceptor; α_{1B}-adrenoceptor; 5-methylurapidil; nifedipine; chloroethylclonidine

Introduction

For many years the vas deferens of both the guinea-pig and rat have been widely used to investigate peripheral adrenergic mechanisms (Saxena, 1970; Swedin, 1971; Anstey & Birmingham, 1978; 1980; Fukushi *et al.*, 1988; Eltze & Boer, 1992; Bultman *et al.*, 1994). Many studies have demonstrated that, in the rat, this tissue contains a functionally predominant population of α_{1A}-adrenoceptors (Mallard *et al.*, 1992; Ohmura *et al.*, 1992; Teng *et al.*, 1994; Burt *et al.*, 1995). Although the vas deferens has been widely utilized by many research groups, relatively little is known of the α-adrenoceptor subtypes in the cauda epididymis, a tissue which is automatically contiguous with the vas deferens and which, like the vas deferens, receives innervation from the hypogastric nerve (Mitchell, 1935). Although recent studies have demonstrated that, in the rat, the cauda epididymis contains postjunctional α_1-, but not α_2-adrenoceptors (Ventura & Pennefather, 1991; 1994), there is no evidence indicating which α-adrenoceptor subtypes mediate contractile responses of the cauda epididymis of the guinea-pig.

Since the α-adrenoceptors of the rat vas deferens have been shown to stimulate inositol phosphate production (Minneman *et al.*, 1988) and to be sensitive to the effects of L-type voltage operated Ca^{2+} channel blockers (Teng *et al.*, 1994) we have combined classical pharmacology, [^{3}H]-inositol phosphate and [^{3}H]-adenosine 3′:5′-cyclic monophosphate ([^{3}H]-cyclic AMP) accumulation studies to characterize the α-adrenoceptor subtypes and their mechanism of action in the cauda epididymis of the guinea-pig.

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Methods

Animals

Male Duncan-Hartley guinea-pigs (600–1000 g) 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 by cervical dislocation, both vasa deferentia and testis were removed. The vasa deferentia were cut from the testis and the cauda epididymis unravelled (with the aid of a N.U.M.S. HM 1 binocular microscope). For contractility and second messenger studies the epididymis was cut into sections approximately 15 or 5 mm long, respectively.

Contractility studies

Tissues were tied (with a silk thread), to a tissue holder and then placed into organ baths containing modified Krebs solution (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 0.45, K_2HPO_4 25, $NaHCO_3$ 25, $CaCl_2$ 1.9, glucose 11) gassed with O_2:CO_2 (95:5), at 35–36°C. The upper end of each preparation was connected to a Grass FTO3 force-displacement transducer via another silk thread. Preparations were suspended under 0.35 g resting force. Recordings of contractile force were made with a Grass (model 79D) chart recorder.

To minimize possible heterogeneity of the responses along the length of epididymis, sections of tissue were randomly assigned within each experimental paradigm. Tissues were allowed at least 40 min equilibration time prior to the addition of agonists. Only one concentration-response curve was constructed on any tissue. KCl (60 mM) was added to tissue baths to ensure tissue viability and to provide an index of tissue contractility.

Addition of phenylephrine

Following the equilibration period phenylephrine was added to organ baths (90–120 s contact period every 15–17 min), tissues were then washed 3–5 times (bath volume) with fresh Krebs solution.

Addition of chloroethylclonidine, nifedipine and 5-methylurapidil

Preparations were incubated with nifedipine (10 μM) or were preincubated with chloroethylclonidine (50 μM, 30 min) before the construction of phenylephrine concentration-response curves. In one set of experiments preparations of epididymis were pre-incubated with chloroethylclonidine (50 μM, 30 min) before the addition of either nifedipine (10 μM), 5-methylurapidil (10 nM–1 μM), 5-methylurapidil (10 nM–1 μM) and idazoxan (100 nM) or 5-methylurapidil (10 nM–1 μM) plus idazoxan (100 nM) and nifedipine (10 μM).

To reduce the potential effect of chloroethylclonidine at α_2-adrenoceptors some tissues were co-incubated with chloroethylclonidine (50 μM, 30 min) and idazoxan (100 nM; added 15 min before the addition of chloroethylclonidine). Following this incubation period the preparations were washed (4×) with fresh Krebs solution and left for 10 min before the addition of agonists.

[^{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), 30 min before the end of this incubation chloroethylclonidine was added to some preparations (giving a final concentration of 50 μM).

Following the [^{3}H]-*myo*-inositol incubation the tissues were allowed to incubate for 25 min in Krebs buffer (35–36°C) containing LiCl (20 mM) and, where indicated, 5-methylurapidil or nifedipine. Phenylephrine was added to preparations and the tissues were left for 15 min before being transferred 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 the supernatant were determined by liquid scintillation counting.

[^{3}H]-cyclic AMP accumulation

This protocol is essentially a modification of that outlined by Ruck *et al.* (1991). Briefly, preparations of epididymis 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 were added, (1–2 min) before the addition of forskolin (3–300 μM), and preparations were left for a further 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]-cyclic AMP extracted from the incubation media by use of acidic alumina columns (Johnson *et al.*, 1994). Tritium levels in samples was determined by liquid scintillation counting.

Pertussis toxin treatment

Preparations were incubated (for 24 h at 37°C, under an O_2 (95%): CO_2 (5%) atmosphere) in Dulbecco's modified Eagles medium (Gibco, U.K.) with pertussis toxin (200 ng ml^{-1}) or vehicle before use in contractility or [^{3}H]-cyclic AMP accumulation studies (as described above).

Statistics

Estimates of −log molar EC_{50} (pEC_{50}), −log molar K_i (pK_i), slope, maximum response were generated by use of 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 programm, FLEXIFIT (see Guardabasso *et al.*, 1988), significant changes were determined with an F-test. pA_2 values were calculated according to the method of Arunlakshana and Schild (1959), by use of the statistical package of Tallarida and Murray (1981). One-way ANOVA and the Student's *t* test were used to determine changes between data sets. In all cases $P<0.05$ was taken as the level of significance. Appparent pK_B values were determined by the Gaddum equation:

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

Drugs

Chloroethylclonidine HCl, forskolin and idazoxan HCl (Research Biochemicals Inc., Natick, U.S.A.); nifedipine, (−)-phenylephrine HCl and xylazine HCl (Sigma Chemical Co., U.K.); 5-methylurapidil (a gift from B.K.G. Germany); pertussis toxin (Porton Products/Speywood, U.K.).

Forskolin was stored as a stock solution in ethanol (−20°C). Nifedipine was dissolved in dimethylsulphoxide (DMSO) (the final concentration of DMSO never exceeded 0.1% of tissue bath volume). Other drugs were dissolved in distilled H_2O. All drugs were made up to volume in buffer on the day of use.

Results

Responses to phenylephrine

The α_1-adrenoceptor agonist phenylephrine elicited concentration-dependent contractile responses from preparations of cauda epididymis (pEC_{50} 5.59 (95% confidence limits, 5.28, 5.91), maximum response 231 (95% confidence limits 193, 268) mg force, $n=12$; see Figure 1 for typical responses). The incubation of preparations with nifedipine (10 μM) significantly (F-test, $P<0.05$, d.f. = 1, 69) reduced the maximal response to, but not the EC_{50} of phenylephrine concentration-response curves (Figure 2a).

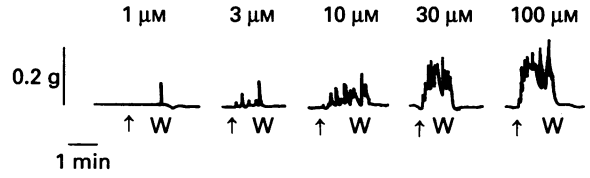

Figure 1 Typical responses of preparations of cauda epididymis to the addition of different concentrations of phenylephrine added at the arrrow. Preparations were washed at W.

The pre-incubation of preparations of cauda epididymis with chloroethylclonidine (50 μM, 30 min) did not significantly affect responses to phenylephrine (Figure 2b). The addition of nifedipine (10 μM) to tissues pre-incubated with chloroethylclonidine (50 μM, 30 min), further reduced (F-test, $P<0.05$, d.f. = 1, 58) the maximal response to phenylephrine (Figure 2c).

The concomitant pre-incubation of tissues with chloroethylclonidine (50 μM, 30 min) and idazoxan (100 nM) caused a significant 4 fold rightward shift (F-test, $P<0.05$, d.f. = 1, 68) of phenylephrine concentration-response curves compared to tissues pre-incubated with idazoxan (100 nM) only (Figure 2d).

Responses to xylazine

Xylazine (at concentrations up to 10 μM) did not elicit contractions from preparations of cauda epididymis ($n=4$, data not shown) but did (at 1 μM) significantly (F-test, $P<0.05$,

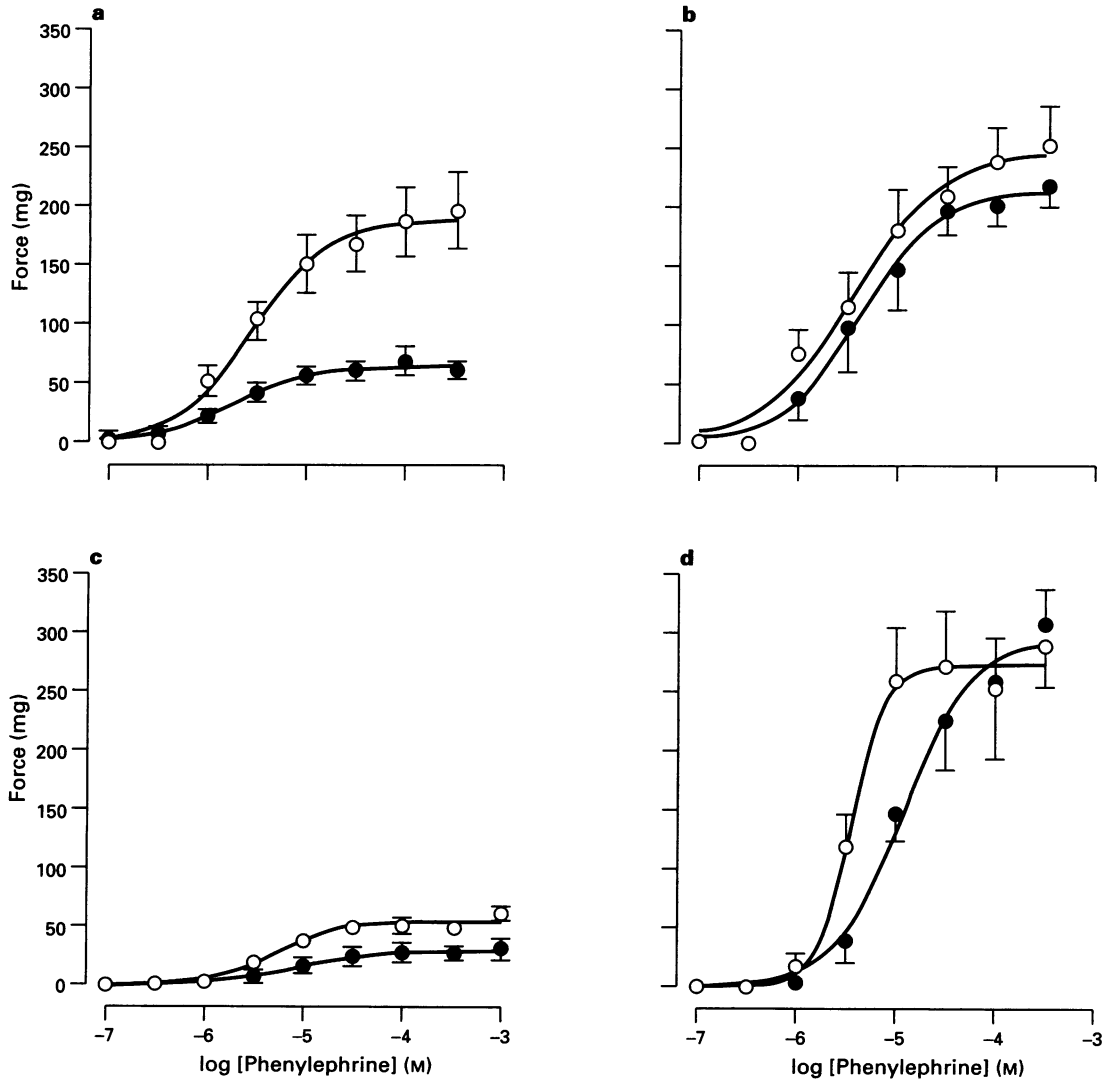

Figure 2 Concentration-response curves to phenylephrine in preparations of cauda epididymis. (a) The responses to phenylephrine in the absence (○) and presence (●) of nifedipine (10 μM); (b) responses to phenylephrine in control (○) and in tissues pre-incubated with chloroethylclonidine (50 μM, 30 min; ●); (c) responses to phenylephrine in the presence of nifedipine (10 μM; ○) and in the presence of nifedipine (10 μM) following pre-incubation with chloroethylclonidine (50 μM, 30 min; ●); (d) responses to phenylephrine following the pre-incubation of tissues with either chloroethylclonidine (50 μM, 30 min; ○) or both chloroethylclonidine (50 μM, 30 min) and idazoxan (100 nM) (●). All symbols represent the mean and vertical lines s.e. mean of 5–12 replicate experiments. In some cases error bars have been omitted for clarity.

d.f. = 1, 63) shift phenylephrine concentration-response curves 3 fold to the left (Figure 3a). This effect could be attenuated by the addition of idazoxan (100 nM; $n = 5$, data not shown). The incubation of preparations with nifedipine (10 μM) or the pre-incubation of preparations with chloroethylclonidine (50 μM, 30 min) attenuated the potentiating effects of xylazine (1 μM; Figure 3b and c, respectively). In preparations pre-incubated with both chloroethylclonidine (50 μM, 30 min) and idazoxan (100 nM) xylazine (1 μM) significantly (F-test, $P < 0.05$, d.f. = 1, 57) potentiated phenylephrine concentration-response curves 2 fold (Figure 3d).

Incubation of tissues with pertussis toxin (200 ng ml^{-1} for 24 h), but not with pertussis toxin vehicle, attenuated the xylazine (1 μM)-mediated potentiation (7.1 fold) of phenylephrine concentration-response curves (Figure 4b and a, respectively).

Addition of 5-methylurapidil

In preparations of cauda epididymis (pre-incubated with chloroethylclonidine; 50 μM, 30 min) the lowest concentration of 5-methylurapidil (10 nM) shifted phenylephrine concentration-response curves significantly (F-test, $P < 0.05$, d.f. = 1, 69) to the right (2.9 fold). Increasing concentrations of 5-methylurapidil (up to 3 μM) elicited greater shifts, Schild analysis of this data (not shown) indicated a slope of less than unity. To reduce any potential interaction of phenylephrine with α_2-adrenoceptors in the epididymis, preparations were pre-incubated with chloroethylclonidine (50 μM, 30 min) before the addition of idazoxan (100 nM) and 5-methylurapidil (10–300 nM). To determine the effects of 5-methylurapidil on the nifedipine-insensitive component of the contractile response to phenylephrine in this tissue, preparations were pre-incubated with chloroethylclonidine (50 μM, 30 min) before the addition of idazoxan (100 nM), nifedipine (10 μM) and 5-methylurapidil (10–300 nM). Table 1 shows the apparent pK_B values for 5-methylurapidil following the pre-incubation of preparations with chloroethylclonidine (50 μM, 30 min) and also in the presence of idazoxan and/or nifedipine.

[^{3}H]-inositol phosphate accumulation

Phenylephrine elicited concentration-dependent increases in [^{3}H]-inositol phosphate accumulation in preparations of the

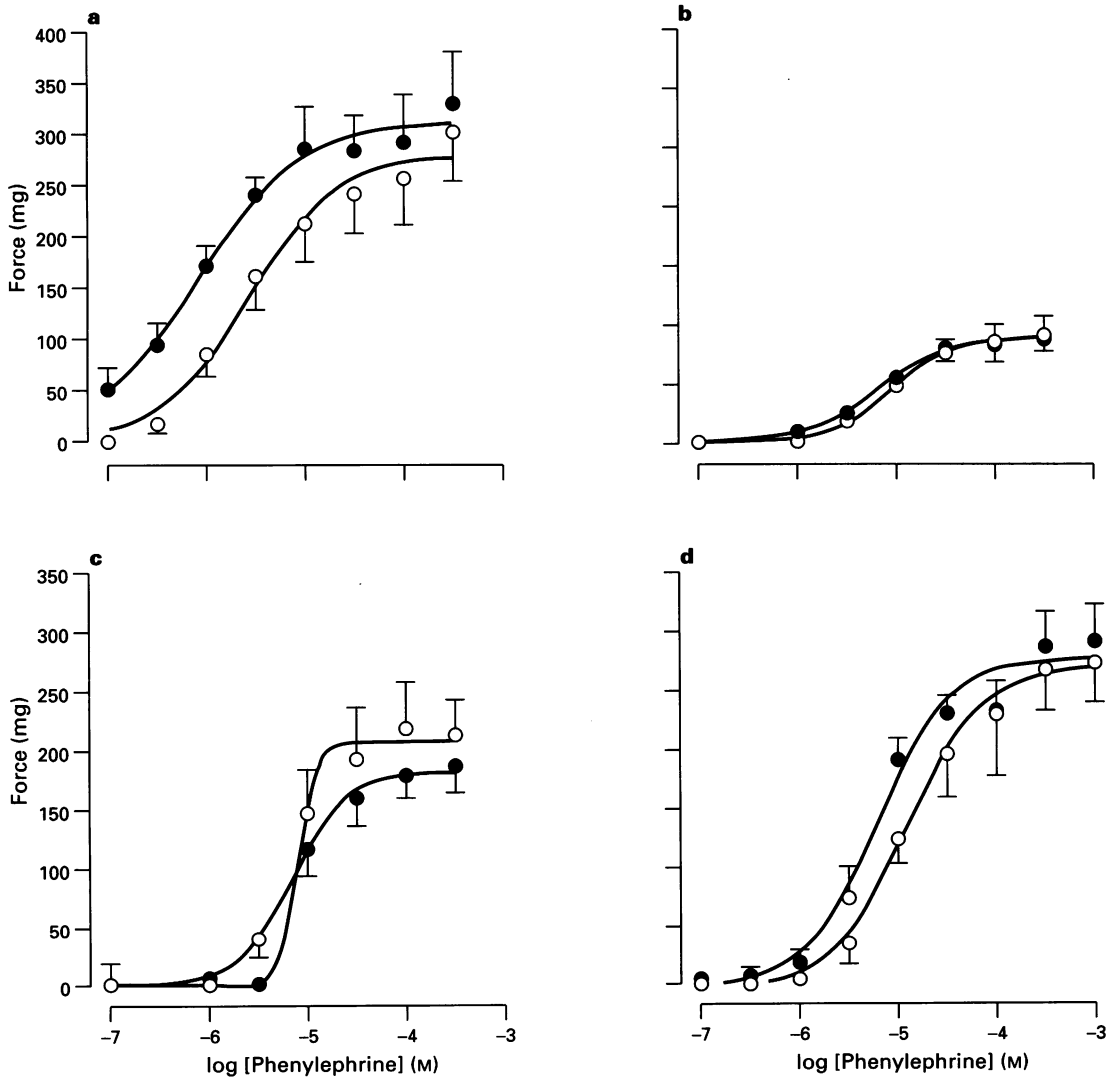

Figure 3 Concentration-response curves to phenylephrine in preparations of cauda epididymis: the effects of xylazine. (a) The responses to phenylephrine in the absence (○) and presence (●) of xylazine (1 μM); (b) phenylephrine concentration-response curves in the presence of either nifedipine (10 μM; ○) or both nifedipine (10 μM) and xylazine (1 μM) (●); (c) responses to phenylephrine in tissues pre-incubated with chloroethylclonidine (50 μM, 30 min; ○) and in tissues pre-incubated with chloroethylclonidine (50 μM, 30 min) and incubated with xylazine (1 μM) (●); (d) responses to phenylephrine following the pre-incubation of tissues with both chloroethylclonidine (50 μM, 30 min) and idazoxan (10 μM) (○) and the effects of xylazine (1 μM) in tissues pre-incubated with both chloroethylclonidine (50 μM, 30 min) and idazoxan (10 μM) (●). All symbols represent the mean and vertical lines s.e. mean of 5–6 replicate experiments. In some cases error bars have been omitted for clarity.

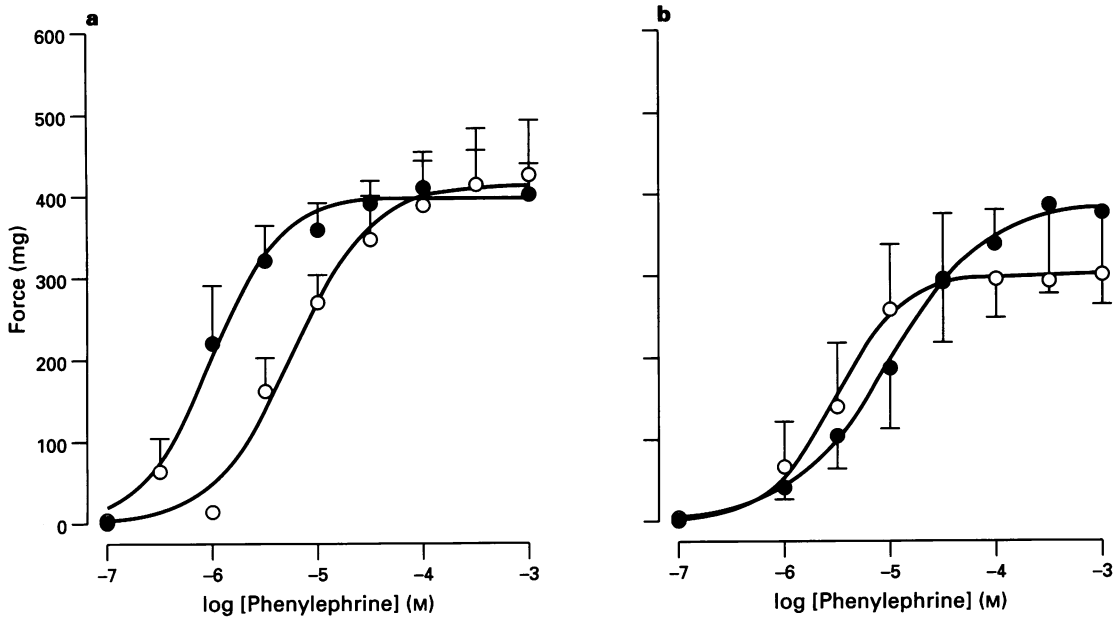

Figure 4 Effects of pertussis toxin (200 ng ml^{-1}, 24 h) or vehicle on the potentiation of phenylephrine concentration-response curves by xylazine (1 μM). (a) The effects of vehicle incubation on responses to phenylephrine in the absence (○) and presence (●) of xylazine. (b) The effects of pertussis toxin on the responses to phenylephrine in the absence (○) and presence (●) of xylazine (1 μM). All symbols represent the mean and vertical lines s.e. mean of 5 replicate experiments. In some cases error bars have been omitted for clarity.

cauda epididymis (pEC_{50} value (95% confidence limits) 4.55 (4.21, 5.05), $n=8$; Figure 5a). The pre-incubation of preparations with chloroethylclonidine (50 μM, 30 min) significantly reduced the phenylephrine (100 μM)-stimulated accumulation of [^{3}H]-inositol phosphates ($P<0.05$, Student's t test, $n=7$; Figure 5a). Nifedipine (10 μM) did not affect phenylephrine (10 μM – 1 mM)-stimulated [^{3}H]-inositol phosphate production in preparations of cauda epididymis (one-way ANOVA, d.f. = 1, 22 and 1, 14 respectively, data not shown). 5-Methylurapidil inhibited the phenylephrine (30 μM)-stimulated increase in [^{3}H]-inositol phosphate accumulation in preparations of cauda epididymis (pK_i value (95% confidence limits) 7.7 (8.8, 6.67), $n=7$; Figure 5b). The addition of xylazine (1 μM, 1–2 min) before the addition of phenylephrine (1 μM – 1 mM) did not significantly increase the accumulation of [^{3}H]-inositol phosphates ($n=6$, data not shown).

[^{3}H]-cyclic AMP accumulation

Forskolin (3–300 μM) stimulated the accumulation of [^{3}H]-cyclic AMP in preparations of cauda epididymis (Figure 6a). Xylazine inhibited the forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP (pIC_{50} 6.73 (8.30, 5.19); Figure 6b). When compared to fresh tissue, the overnight incubation of tissues in vehicle or in pertussis toxin increased the basal accumulation of [^{3}H]-cyclic AMP, but did not increase the forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP (Table 2). Xylazine (10 μM) did not significantly inhibit the forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP in either vehicle or pertussis toxin treated tissues ($n=6$, data not shown).

Discussion

These studies have shown that phenylephrine elicits concentration-dependent responses from the cauda epididymis of the guinea-pig. These responses, greatly reduced in the presence of the L-type (voltage sensitive) Ca^{2+} channel antagonist, nifedipine, but not affected by the α_{1B}-adrenoceptor alkylating agent chloroethylclonidine, are consistent with those

Table 1 The effects of 5-methylurapidil (5-MU) on phenylephrine concentration-response curves in preparations of epididymis pre-incubated with chloroethylclonidine (50 μM, 30 min)

5-MU (nM)	*Apparent pK_B*		
		Idazoxan	*Idazoxan + nifedipine*
10	8.27±0.16	8.41±0.19	NS
30	8.05±0.20	7.99±0.11	7.77±0.10
100	7.86±0.27	7.94±0.19	7.61±0.10
300	7.86±0.13	7.64±0.15	7.36±0.06
1000	7.76±0.15	ND	ND

Preparations of epididymis were either pre-incubated with chloroethylclonidine, or were pre-incubated with chloroethylclonidine before the addition of either idazoxan (100 nM) or the combination of idazoxan (100 nM) and nifedipine (10 μM). Numbers show the mean (±s.e. values) apparent pK_B values of between 4–6 replicate experiments. NS – no significant shift at this concentration of 5-methylurapidil; ND – not determined.

previously shown in the rat vas deferens (Mallard *et al.*, 1992; Ohmura *et al.*, 1992; Teng *et al.*, 1994; Burt *et al.*, 1995). We have also shown that the antagonism of phenylephrine by low concentrations of 5-methylurapidil (10 nM) is attenuated by nifedipine and that the nifedipine-insensitive component of the response to phenylephrine is sensitive to the effects of chloroethylclonidine. Although these findings may indicate α_1-adrenoceptor heterogeneity in the epididymis of the guinea-pig it should be noted that recent studies have questioned the suitability of chloroethylclonidine as an α_1-adrenoceptor subtype selective tool (Han *et al.*, 1994). In terms of the current nomenclature (Bylund *et al.*, 1994; Hieble *et al.*, 1995), these receptors could be classified as α_{1A}-like and either (or both) α_{1B}- or α_{1D}-like adrenoceptors. The weak effect of chloroethylclonidine on phenylephrine concentration-response curves may be attributable to the limited penetration of the highly lipophilic chloroethylclonidine into tissues rather than to a small population of α_{1B}- or α_{1D}-like adrenoceptors.

However, chloroethylclonidine was very effective at blocking [^{3}H]-inositol phosphate accumulation. A similar observation has also been described in the rat vas deferens where chloroethylclonidine reduced noradrenaline-mediated [^{3}H]-inositol phosphate accumulation in ring segments of rat vas deferens (Minneman *et al.*, 1988) even though it had no effect on α_1-adrenoceptor agonist-mediated contractile responses (Minneman *et al.*, 1988; Mallard *et al.*, 1992; Ohmura *et al.*, 1992; Teng *et al.*, 1994; Burt *et al.*, 1995). One possible explanation for these findings may be that chloroethylclonidine, in addition to its alkylating action at α_{1B}-adrenoceptors, binds as an agonist to α_2-adrenoceptors (as has been previously found; Bultmann & Starke, 1993) and potentiates α_{1A}-adrenoceptor-mediated contractile responses. The net effect of the chloroethylclonidine pretreatment may therefore be negligible. This hypothesis is consistent with our findings, where chloroethylclonidine inhibited the α_1-adrenoceptor-mediated accumulation of [^{3}H]-inositol phosphates but only showed significant inhibition of contractile responses when α_2-adrenoceptors were protected from chloroethylclonidine by idazoxan.

Evidence that the cauda epididymis contains postjunctional α_2-adrenoceptors is evident from our studies showing that the α_1-adrenoceptor-mediated responses are potentiated by the α_2-adrenoceptor selective agonist xylazine; an effect blocked by the α_2-adrenoceptor antagonist idazoxan and sensitive to pre-

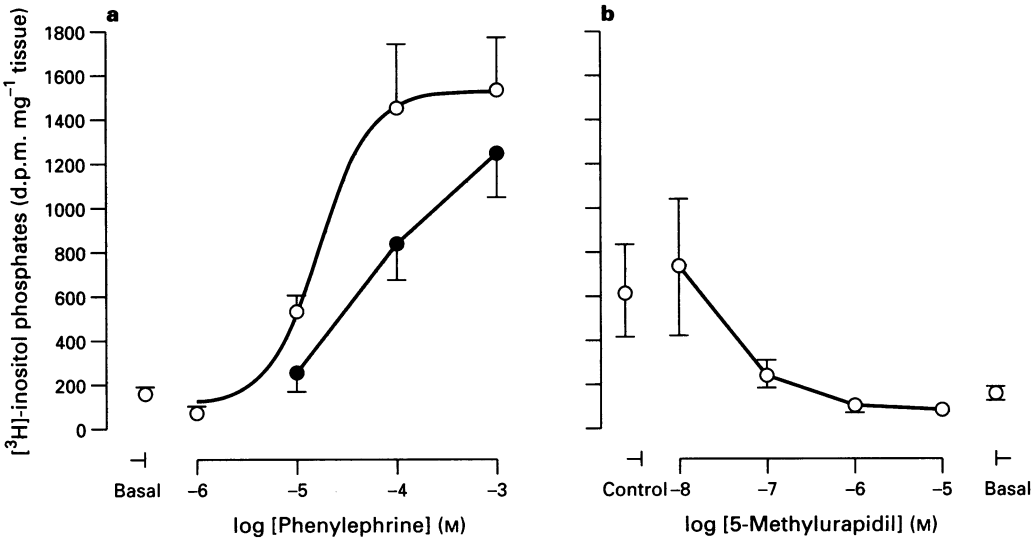

Figure 5 The effects of chloroethylclonidine (50 μM, 30 min) and 5-methylurapidil on the phenylephrine-mediated accumulation of [^{3}H]-inositol phosphates in preparations of the cauda epididymis of the guinea-pig. (a) The responses to phenylephrine only (○) and the effects of chloroethylclonidine pre-incubation (●). (b) The effect of 5-methylurapidil on the phenylephrine (10 μM)-stimulated accumulation of [^{3}H]-inositol phosphates. Each symbol represents the mean and vertical lines s.e.mean of 7–8 replicate experiments. In some cases error bars have been omitted for clarity.

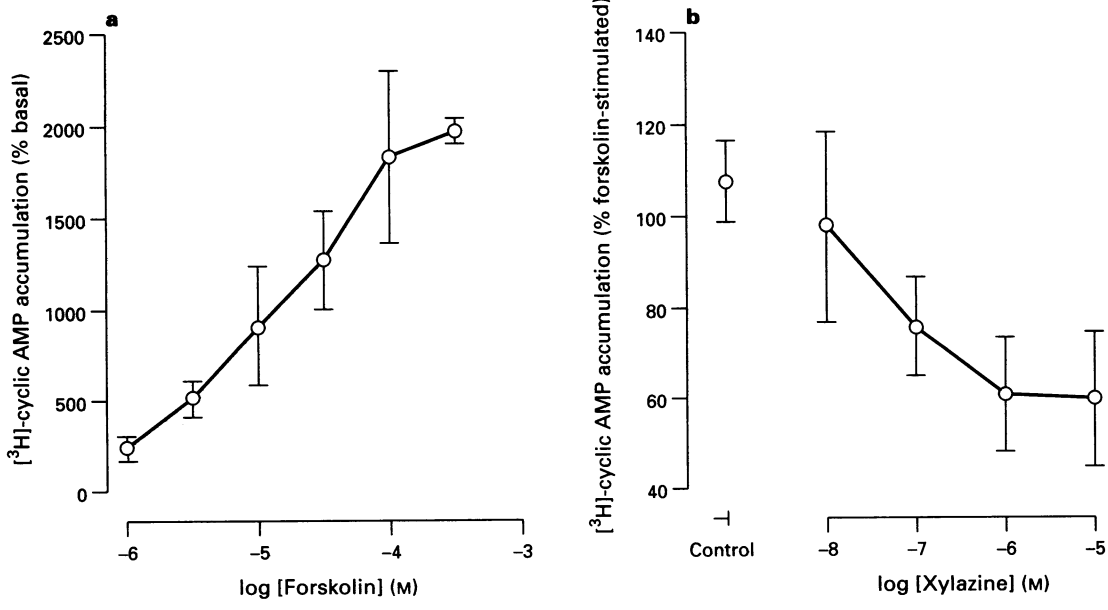

Figure 6 The effects of forskolin and xylazine on [^{3}H]-cyclic AMP accumulation in preparations of cauda epididymis. (a) Forskolin-stimulated accumulation of [^{3}H]-cyclic AMP and (b) inhibition of forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP by xylazine. Each symbol represents the mean and vertical lines s.e.mean of 7–8 replicate experiments.

Table 2 The effects of forskolin (30 μM) on the basal accumulation of [^{3}H]-cyclic AMP in preparations of cauda epididymis

	[^{3}H]-cyclic AMP accumulation (d.p.m. mg^{-1} tissue)		
	Fresh tissue control	*24 h in vehicle*	*24 h in pertussis toxin*
Basal	308±30	996±77	1196±134
Forskolin-stimulated	3892±534	2495±310	2340±417

Preparations were used fresh or were incubated for 24 h in Dulbecco's modified Eagles medium containing either pertussis toxin (200 ng ml^{-1}) or pertussis toxin vehicle. Preparations were then incubated in [^{3}H]-adenine (2 h) before the addition of forskolin or vehicle control (10 min).

incubation of the tissues with pertussis toxin. The latter finding indicates α_2-adrenoceptor coupling through a $G_{i/o}$ protein. Additionally, we have shown that the α_2-adrenoceptors of this tissue couple to adenylate cyclase since xylazine reversed the forskolin-stimulated accumulation of [^{3}H]-cyclic AMP.

Our finding that the incubation (24 h) of tissues with pertussis toxin elevated basal levels of [^{3}H]-cyclic AMP (3 fold) was surprising, particularly since forskolin became less effective at elevating [^{3}H]-cyclic AMP in these preparations. The reduced effectiveness of forskolin in these preparations may account for the finding that xyalzine no longer significantly reduced forskolin-mediated [^{3}H]-cyclic AMP accumulation but still potentiated phenylephrine concentration-response curves. The mechanism of the incubation-induced change in tissue adenylyl cyclase activity is unknown; one hypothesis may be that the tissues become anoxic during the incubation period resulting in elevated adenylyl cyclase activity, as has been shown in cultured vascular smooth muscle cells (Marti *et al.*, 1994).

The finding that xylazine did not potentiate responses to phenylephrine in tissues pre-incubated with chloroethylclonidine may be consistent with the agonist action of chloroethylclonidine obtained at the prejunctional α_2-adrenoceptors of the rat vas deferens (Bultmann & Starke, 1993). To preclude this possible action of chloroethylclonidine, we used idazoxan to protect the postjunctional α_2-adrenoceptors. In these preparations xylazine again potentiated the contractile responses elicited by phenylephrine, indicating that the binding of chloroethylclonidine to α_2-adrenoceptors prevented the action of xylazine. Since the potentiation of phenylephrine concentration-response curves, by xylazine was abolished in the presence of nifedipine, but not following chloroethylclonidine (with idazoxan) pretreatment, it is likely that the activation of postjunctional α_2-adrenoceptor facilitates the α_1-adrenoceptor-stimulated influx of extracellular Ca^{2+}. This suggestion is consistent both with our finding, that the addition of xylazine did not potentiate the phenylephrine-stimulated accumulation of total [^{3}H]-inositol phosphates, and the findings of Xiao & Rand (1989a,b) who proposed an α_2-adrenoceptor-mediated potentiation of Ca^{2+} influx in preparations of rat tail artery. However, other studies have demonstrated that α_2-adrenoceptor stimulation or adenylate cyclase activation can inhibit the phenylephrine-stimulated accumulation of [^{3}H]-inositol phosphates (Manopoulos *et al.*, 1991; Haynes *et al.*, 1993).

Our results indicating that the α_1-adrenoceptor subtypes of the cauda epididymis of the guinea-pig mediated both the influx of Ca^{2+} from extracellular stores and the activation of phospholipase C are consistent with the Ca^{2+} utilization in the smooth muscle of guinea-pig vas deferens described previously (Suzuki & Gomi, 1989; Drescher *et al.*, 1993). We have also shown that activation of α_2-adrenoceptors selectively potentiates responses to the α_{1A}-like (high affinity for 5-methylurapidil, insensitive to chloroethylclonidine), but not to the $\alpha_{1B/D}$-like (low affinity for 5-methylurapidil, sensitive to chloroethylclonidine) adrenoceptor. The mechanism underlying this phenomenon is currently under investigation.

This research was funded by the MRC (Ref. G940858).

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(Received July 24, 1996
Revised August 10, 1996
Accepted August 21, 1996)