British Journal of Pharmacology (2007) 150, 112–120
 0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

α_{1A}-Adrenoceptors mediate contractions to phenylephrine in rabbit penile arteries

JS Morton[1], CJ Daly[1], VM Jackson[2] and JC McGrath[1]

[1]*Autonomic Physiology Unit, Institute of Biomedical and Life Sciences, West Medical Building, University of Glasgow, Glasgow, UK and* [2]*Pfizer Global Research & Development, Sandwich, Kent, UK*

Background and purpose: Maintained penile erection depends on the absence of α-adrenoceptor (α-AR) activation and so can be facilitated by α-blockers. This study seeks the α_1-AR subtypes involved in order to inform the pro-erectile consequences of subtype selective blockade.
Experimental approach: Wire myography was used with dorsal (nutritional supply) and cavernous (erectile inflow) penile arteries; standard α-AR-selective agonists and antagonists were employed to classify responses.
Key results: In both penile arteries noradrenaline (NA) and phenylephrine (PE, α_1-AR agonist) caused concentration-dependent contractions. Sensitivity to NA was increased by NA uptake blockers, cocaine (3 μM) and corticosterone (30 μM). PE responses were antagonised by phentolamine (non-selective α-AR: dorsal pK_B 8.00, cavernous 8.33), prazosin (non-subtype-selective α_1-AR: dorsal 8.60, cavernous 8.41) and RS100329 (α_{1A}-AR selective: dorsal 9.03, cavernous 8.80) but not by BMY7378 (α_{1D}-AR selective: no effect at 1-100 nM) or Rec15/2615 (α_{1B}-AR selective: no effect at 1-100 nM). Schild analysis was straightforward in cavernous artery, indicating that PE activates only α_{1A}-AR. In dorsal artery Schild slopes were low, though α_{1A}-AR was still indicated. Analysis using UK 14,304 and rauwolscine indicated an α_2-AR component in dorsal artery that may account for low slopes to α_1-AR antagonists.
Conclusions and implications: Penile arteries have a predominant, functional α_{1A}-AR population with little evidence of other α_1-AR subtypes. Dorsal arteries (nutritional supply) also have α_2-ARs. Thus, α-AR blockers with affinity for α_{1A}-AR or α_2-AR would potentially have pro-erectile properties; the combination of these perhaps being most effective. This should inform the design of drugs to assist/avoid penile erection.
British Journal of Pharmacology (2007) **150,** 112–120. doi:10.1038/sj.bjp.0706956; published online 20 November 2006

Keywords: penile; dorsal artery; cavernous artery; adrenergic; α_1-adrenoceptor subtypes

Abbreviations: α-AR, α-adrenoceptor; ACh, acetylcholine; CRC, concentration–response curve; CI, confidence interval; NA, noradrenaline; PE, phenylephrine

Introduction

Penile tumescence is brought about by engorgement of the erectile tissues with blood, and this is caused by vasodilatation of the inflow arteries coupled with relative vasoconstriction of outflow veins. Pharmacological interest has centred on vasodilators and vasoconstrictors for their respective effects to improve tumescence, where this is desired to improve sexual performance, or decrease tumescence, where the erection is in a pathologically prolonged state known as priapism. Drugs to treat this condition have been targeted at manipulation of the physiological regulation of penile arterial tone. The concept is to modify the vasodilator nitrergic and vasoconstrictor adrenergic mechanisms that respectively cause and terminate tumescence. On the adrenergic side, α-blockers such as phentolamine have long been known to cause priapism as a side effect when used for other therapeutic purposes, and this has been exploited to treat erectile dysfunction by intra-penile injection (Chin *et al.*, 1998). Conversely, vasoconstrictor α-adrenoceptor (α-AR) agonists such as phenylephrine (PE) can be used to treat priapism, including that to an overdose of phentolamine (Dougherty *et al.*, 2006). The concept of vasoconstrictor α_2-ARs has also been mooted as an explanation for the pro-erectile effects of yohimbine originating from traditional medicine, and yohimbine is still marketed (non-medically) for this effect.

There are three subtypes of α_1-ARs, and α-blockers that are in clinical use (to treat benign prostatic hyperplasia, hypertension and, although currently discontinued, heart

Correspondence: Professor JC McGrath, Autonomic Physiology Unit, Institute of Biomedical and Life Sciences, West Medical Building, University of Glasgow, Glasgow G12 8QQ, UK.
E-mail: i.mcgrath@bio.gla.ac.uk
Received 29 June 2006; revised 6 September 2006; accepted 4 October 2006; published online 20 November 2006

failure) vary in their subtype selectivity. It is therefore of relevance to know which subtypes are involved in vasoconstriction of penile blood vessels in order to avoid priapism, or to cause penile erection according to the desired effect. However, there are currently different opinions on the matter. Two contributing factors are the poor selectivity of antagonists used to define functional responses and the use of strips of erectile tissue rather than preparations of arteries *per se*.

Penile vasculature and erectile tissue are densely innervated by adrenergic nerves (Tamura *et al*., 1995), which cause contraction of the smooth muscle of corpus cavernosum trabecular tissue and penile arteries to initiate and maintain detumescence. Contraction of human and animal erectile tissue and vasculature has been shown to be predominantly mediated by α_1-ARs (Saenz de Tejada *et al*., 1989; Traish *et al*., 1995; Recio *et al*., 1997; Sato and Kawatani, 2002). Expression of mRNA for all three α_1-AR subtypes, α_{1A}, α_{1B} and α_{1D}, has been demonstrated in human (Dausse *et al*., 1998; Goepel *et al*., 1999), rabbit (Peng *et al*., 1998) and rat (Veronneau-Longueville *et al*., 1998) corpus cavernosum tissue. Few studies, however, have attempted to identify α_1-AR subtypes involved in adrenergic contractions of the erectile tissue and the results, so far, are contradictory. One of the main events leading to the initiation of an erection is an increase in arterial inflow to the erectile tissue. This relies greatly on vascular control of the main inflow arteries, the dorsal and cavernous penile arteries. Most studies so far have concentrated on the investigation of contractile responses of strips of penile corpus cavernosum, which contain a variety of cell and tissue types that incorporate many different and potentially conflicting pathways, and so may not faithfully represent the properties of inflow arteries. However, this is the only evidence available on α_1-AR subtypes in the erectile tissues. In summary, in rabbit and rat, major mRNA expression is suggested to be α_{1B}-AR (Peng *et al*., 1998), but functional receptors to be either the α_{1B}-AR (Furukawa *et al*., 1996) or the α_{1A}-AR (Furukawa *et al*., 1996; Tong and Cheng, 1997; Peng *et al*., 1998).

The aim of the present study was to characterize α_1-ARs underlying PE-induced contractions in rabbit isolated genital arteries. To clarify the mechanisms of adrenergic contraction important to normal sexual function, arteries central to the erectile response were investigated. Dorsal arteries primarily supply the glans penis and prepuce while having an additional role in the supply of blood to the corpus cavernosum. The dorsal artery functions to maintain an uninterrupted supply of nutrients and oxygen to the penile tissue. Meanwhile, cavernous arteries comprise the main arterial inflow to cavernous tissue, inflow that is greatly increased during sexual arousal. During the flaccid state, an increased level of sympathetic nerve activity maintains contraction of the cavernous arteries and trabecular tissue and prevents onset of the erectile response (reviewed by Andersson and Wagner, 1995). Therefore, an understanding of the vascular function of both dorsal and cavernous arteries is critical to our understanding of sexual function in general. Both dorsal and cavernous artery preparations were studied to gain an insight into functional mechanisms of the penile tissue.

Methods

Tissues

Penile tissue was obtained from male New Zealand White rabbits (2.5–3.5 kg) killed by an overdose of pentobarbitone (Euthatal, Rhône Merieux, UK) injected into the ear marginal vein. Experiments were carried out in compliance with UK legislation. Further tissue dissections were carried out in ice-cold physiological saline solution, composition (mM) NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.1, EDTA 0.023 and pH 7.3. Dorsal and cavernous penile arteries, internal lumen diameter $181 \pm 1\,\mu$m ($n = 33$) and $150 \pm 2\,\mu$m ($n = 35$), respectively (measured under conditions of zero pressure), were isolated from the erectile tissue. Arterial rings, approximately 2 mm in length, were mounted on two 40 μm wires attached to a wire myograph (DMT, Copenhagen, SV, Denmark) to allow isometric tension recordings. The optimal isometric tension for maximal contraction in response to PE (10 μM) was predetermined using a range of resting tensions from 0 to 1 g and set at 0.25 g for all experiments. Vessels were bathed in physiological saline solution at 37°C and oxygenated with 95% O_2, 5% CO_2.

Protocols

Vessels were allowed to equilibrate under resting tension for 30 min following which a reproducible maximal contraction to noradrenaline (NA) (10 μM) was determined before experimental protocols were begun. The mean NA maximal contraction for each vessel was used to compare experimental responses. Endothelium integrity was confirmed by assessing the ability of acetylcholine (ACh) (3 μM) to relax NA (10 μM) pre-contracted vessels.

During the evaluation of antagonists, first curves to PE were performed with an absence of antagonist in all preparations. PE was added cumulatively in half-log increments from 1 nM to 300 μM. Vessels were incubated with an antagonist for 30 min before the second PE concentration–response curves (CRCs). The antagonists prazosin, RS 100329, Rec 15/2615, BMY 7378 and rauwolscine were tested at concentrations of 1–100 nM, concentrations known to be selective for the appropriate adrenoceptor subtypes and in line with published data. Owing to a lack of effect at concentrations of 1 and 10 nM in the dorsal arteries, phentolamine was tested at an additional concentration of 1 μM. All protocols included a parallel control vessel that was studied in the absence of antagonist during both the first and second curves.

Drugs

All drugs used were of analytical grade and were purchased from Sigma-Aldrich, ACh, NA, PE, prazosin, phentolamine, 5-methylurapidil, BMY 7378 (8-(2-[4-{2-methoxyphenyl}-1-piperazinyl]ethyl)8-azaspiro[4,5]decane-7,9-dione dihydrochloride), corticosterone; Roche Bioscience (Palo Alto, CA, USA), RS 100329 (*N*-[(2-trifluoroethoxy)phenyl],*N*′-(3-thyminylpropyl) piperazine hydrochloride) and L-NAME (N^{ω}-nitro-L-arginine methyl ester hydrochloride); Tocris

(Bristol, UK), rauwolscine and UK 14 304 (5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline); or manufactured in house, Rec 15/2615 (1-(4-amino-6,7-dimethoxy-2-guinazolinil)-4-[2-[2-metoxy-6-(1-methylethyl)phenoxy]acetyl]piperazine hydrochloride). Stock solutions were made from powder form using de-ionized H_2O with the exception of NA, which was dissolved in 23 μM EDTA, and all further dilutions were made in de-ionized H_2O, and UK 14 304 and Rec 15/2615, which were made up as stock solutions in dimethylsulphoxide.

Data analysis

Data, acquired using Chart v4.1.2 (PowerLab, ADInstruments Ltd, Chalgrove, Oxfordshire, UK) were analysed using Prism v3.0 software (GraphPad Software Inc., San Diego, CA, USA) and expressed as mean $\pm$ s.e.m. Where shown, n indicates the number of animals used. Contractions are expressed either as force in g, a percentage of the vessel response to NA (10 μM), or a percentage of the maximum contraction (E_{max}) achieved during a CRC. Agonist responses were expressed using pEC_{50} values; the inverse log of the effective concentration producing 50% of the maximum response. In cases where an antagonist caused a parallel shift of an agonist curve, a Schild plot of log(concentration ratio – 1) versus log(antagonist concentration) was constructed, and a pA_2 was calculated from the x-intercept of this plot. If the slope of the Schild plot was equal to 1, then $pA_2 = pK_B$ and was indicative of competitive binding. Where a pA_2 could not be calculated, a pK_B was calculated instead using the equation

$$pK_B = \log(\text{concentration-ratio} - 1) - \log[B],$$

where pK_B is the negative logarithm of the dissociation constant K_B and $[B]$ is the concentration of antagonist. Individual pK_B values were determined for each experiment at each antagonist concentration. Mean $\pm$ s.e.m. of total data is quoted to provide a pK_B value for a specific tissue.

Hill slopes, pEC_{50} values and maximal responses were compared using a one-way analysis of variance with a Bonferroni post-test. Statistical significance was taken as $P<0.05$.

Results

Agonist profiles

The α_1-AR agonist PE and the non-selective α-AR agonist NA both caused concentration-dependent contractions in dorsal and cavernous arteries from rabbit penile tissue (Table 1). In dorsal arteries, rhythmic activity was often observed in response to agonist stimulation (Figure 1). Where rhythmic activity occurred, the mean tone of the vasoconstriction response was calculated, using Chart v4.1.2 software, over a period of activity where a stable maximum and minimum tone was evident.

Maximal contractions to NA in dorsal arteries exceeded 2 g force with responses to NA > PE ($P<0.01$). In the dorsal artery, responses to NA (10 μM) demonstrated an initial

Table 1 Agonist profile in the dorsal and cavernous arteries

	Dorsal artery			*Cavernous artery*		
	N	E_{max} *(g) (s.e.m.)*	pEC_{50} *(s.e.m.)*	n	E_{max} *(g) (s.e.m.)*	pEC_{50} *(s.e.m.)*
NA	6	2.01 (0.04)	5.85 (0.06)	6	0.96[g] (0.03)	6.14[f] (0.08)
NA plus L-NAME	6	2.47[c] (0.08)	6.57[c] (0.08)	5	1.06 (0.07)	6.28 (0.15)
NA plus uptake blockers	6	1.84 (0.11)	6.75[c] (0.19)	5	1.15 (0.01)	6.84[a] (0.04)
PE	6	1.56[b] (0.03)	5.70 (0.03)	6	0.93[g] (0.06)	6.12[g] (0.01)
PE plus L-NAME	6	2.02[d] (0.08)	6.20[d] (0.09)	5	1.14 (0.08)	6.18 (0.22)
UK 14 304	6	0.81[c] (0.18)	8.14[c] (0.10)	7	0.44[c] (0.07)	8.04[c] (0.06)
UK 14 304 plus L-NAME	6	1.78[e] (0.18)	8.01 (0.13)	5	0.53 (0.13)	8.00 (0.09)

Abbreviations: L-NAME, N^{ω}-nitro-L-arginine methyl ester hydrochloride; NA, noradrenaline; PE, phenylphrine.
Summary of E_{max} and pEC_{50} of NA, PE and UK 14 304 (controls and in the presence of L-NAME or NA uptake blockers).
[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ versus NA control, [d]$P<0.001$ versus PE control, [e]$P<0.01$, versus UK 14 304 control, [f]$P<0.05$, [g]$P<0.001$ versus dorsal artery.

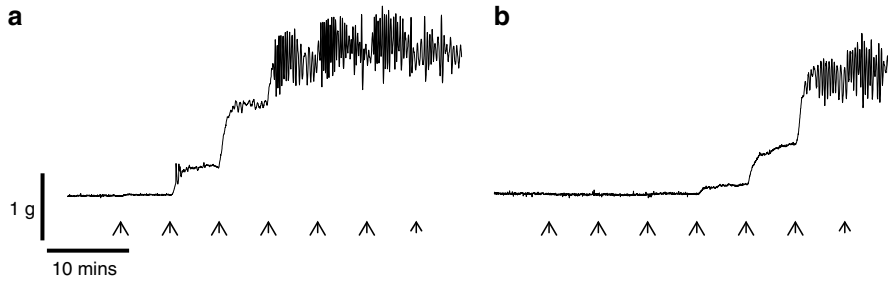

Figure 1 Non-equilibrium activity observed during a CRC in dorsal penile arteries. (**a**) Agonist-induced, rhythmic activity was observed more often in dorsal arteries; in this example, the agonist was PE (0.3–300 μM, arrows). (**b**) Rhythmic activity remained in the presence of prazosin (0.1 μM).

transient contraction before decreasing to a sustained plateau. This fall was unaffected by the inclusion of NA uptake blockers, cocaine (3 μM, uptake 1 blocker) and corticosterone (30 μM, uptake 2 blocker) in the bathing medium. Uptake blockers did, however, increase the sensitivity, but not the E_{max}, of NA responses in both dorsal ($P<0.001$) and cavernous ($P<0.05$) arteries. In the presence of L-NAME (100 μM), maximal contractions to NA and PE were significantly increased in dorsal arteries ($P<0.001$).

In cavernous arteries, maximal contractions to NA and PE were of a similar magnitude and were approximately 50% of NA-induced responses in dorsal arteries ($P<0.001$). Responses were generally well maintained over a 6-min period. In cavernous arteries, maximal contractions to NA and PE were unaffected ($P>0.05$) by incubation with L-NAME (100 μM).

α_1-AR subtypes in the dorsal artery

Two sequential control curves with no antagonist were created in each individual experiment (i.e. time controls). In dorsal arteries, no significant change in sensitivity to PE from first (pEC_{50} 5.74 $\pm$ 0.06) to second (pEC_{50} 5.65 $\pm$ 0.09) CRC was observed ($P>0.05$, $n=27$). The mean PE E_{max} decreased 18% from 1.86 $\pm$ 0.02 *g* to 1.52 $\pm$ 0.01 *g* ($P<0.001$, $n=27$). This decrease occurred uniformly across all second curves, with or without antagonist pre-incubation, regardless of concentration, and so it was not considered indicative of antagonism. However, because of this decrease in E_{max}, the effect of antagonists was investigated by comparison of second phenylephrine concentration–response curves (PE CRCs) using paired preparations with an absence or presence of antagonist.

To determine the α_1-AR subtype/s mediating vasoconstriction in dorsal arteries, non-subtype- and subtype-selective α_1-AR antagonists were tested against responses to the α_1-AR selective agonist PE (Table 2). Phentolamine, a non-selective α-AR antagonist, caused a parallel rightward shift of the PE CRC with pK_B of 8.00 $\pm$ 0.16 ($n=10$). Schild plot analysis for phentolamine gave a pA_2 of 8.32 (95% confidence interval (CI) 7.34 to 10.31) with a slope not significantly different from unity (0.72 $\pm$ 0.19).

The non-subtype-selective α_1-AR antagonist prazosin caused a parallel rightward shift of the PE CRC (Figure 2). Schild analysis of the data demonstrated a shallow regression slope of 0.41 $\pm$ 0.16 while pK_B calculation demonstrated a progressive decrease in potency with increasing concentrations: 9.34 $\pm$ 0.29 at 1 nM ($n=4$), 8.64 $\pm$ 0.23 at 10 nM ($n=7$) and 8.14 $\pm$ 0.17 at 100 nM ($n=7$).

An α_{1A}-AR selective antagonist RS 100329 (Williams *et al*., 1999) caused a parallel rightward shift of the PE curve at concentrations of 1 and 10 nM (Figure 2). RS 100329 has been shown to be a potent antagonist at α_{1A}-ARs with a pA_2/pK_B of between 9.2 and 9.6 (Williams *et al*., 1999; Choppin *et al*., 2001; Cleary *et al*., 2003). Pre-incubation with 100 nM RS 100329 caused no further rightward movement of the PE response in dorsal arteries, and a pK_B of 9.03 $\pm$ 0.07 ($n=6$) was determined from 1 and 10 nM antagonist data. A Schild plot of the total data demonstrated a relationship with a shallow slope, 0.43 $\pm$ 0.11, which was skewed by data obtained using 100 nM RS 100329. Excluding these data gave a slope not significantly different from unity, 0.74 $\pm$ 0.11, with an apparent pA_2 of 9.22 (95% CI 8.82 – 9.84).

The α_{1B}-AR selective antagonist Rec 15/2615 (Testa *et al*., 1997) caused no shift of the PE CRC at concentrations of 1 to 100 nM (Figure 2). Concentration ratios were between 1.01 $\pm$ 0.19 and 1.50 $\pm$ 1.18 ($n=4$) demonstrating that the antagonist was ineffective at the concentrations tested.

The α_{1D}-AR selective antagonist BMY 7378 produced no shift of the PE CRC in the dorsal artery at concentrations of 1 to 100 nM (Figure 2). Concentration ratios lay between 1.02 $\pm$ 0.38 and 1.91 $\pm$ 0.67 ($n=6$). BMY 7378 is a well-characterized α_{1D}-AR selective compound that has been shown to be potent at α_{1D}-ARs in various tissues with pA_2/pK_B's of 8.3 to 9.6 (Kenny *et al*., 1995; Satoh *et al*., 1999; Daly *et al*., 2002; Tanoue *et al*., 2002; Deighan *et al*., 2005). Therefore, 100 nM should be expected to produce a significant shift of approximately 50-fold of an α_{1D}-AR-mediated response even at the insensitive end of this spectrum.

α_1-AR subtypes in the cavernous artery

As in dorsal arteries, no significant change in sensitivity to PE between the first (pEC_{50} 6.25 $\pm$ 0.01) and second (pEC_{50} 6.21 $\pm$ 0.02) CRCs was observed in cavernous arteries ($P>0.05$, $n=26$). A significant decrease in mean E_{max} ($P<0.001$, $n=26$) of 6% from 1.02 $\pm$ 0.01 to 0.96 $\pm$ 0.01 *g* occurred to a uniform extent across all second curves, including those with antagonist pre-incubation. Again, the

Table 2 Antagonist profile in the dorsal artery

Antagonist	n	*pK_B (s.e.m.)*	*pA_2 (95% CI)*	*Schild slope (s.e.m.)*	*Concentration ratio (s.e.m.)*			
					1 nM	*10 nM*	*100 nM*	*1 μM*
Phentolamine	10	8.00 (0.16)	8.32 (7.34–10.31)	0.72 (0.19)	0.80 (0.18)	4.75 (3.75)	21.97 (14.56)	191.39 (161.27)
Prazosin	7	8.60 (0.16)	9.71 (8.13–19.94)	0.41 (0.16)	2.79 (0.97)	8.63 (2.39)	21.84 (7.42)	ND
RS 100329	6	9.03 (0.07)	9.22 (8.82–9.84)	0.43 (0.11)	2.44 (0.04)	9.79 (2.48)	14.94 (6.21)	ND
Rec 15/2615	4	ND	ND	ND	1.03 (0.28)	1.50 (1.18)	1.01 (0.19)	ND
BMY 7378	6	ND	ND	ND	1.91 (0.67)	1.02 (0.38)	1.47 (0.50)	ND
Rauwolscine	4	ND	ND	ND	2.86 (1.49)	3.53 (1.47)	3.18 (1.34)	ND

Abbreviations: CI, confidence interval; ND, not determined; PE CRC, phenylephrine concentration–response curves.
Summary of pK_B's, pA_2's, Schild slopes and concentration ratios of α_1- and α_2-AR antagonists versus PE. Where antagonists did not cause a rightward shift of the PE CRC, pK_B, pA_2 and Schild plots could not be determined.

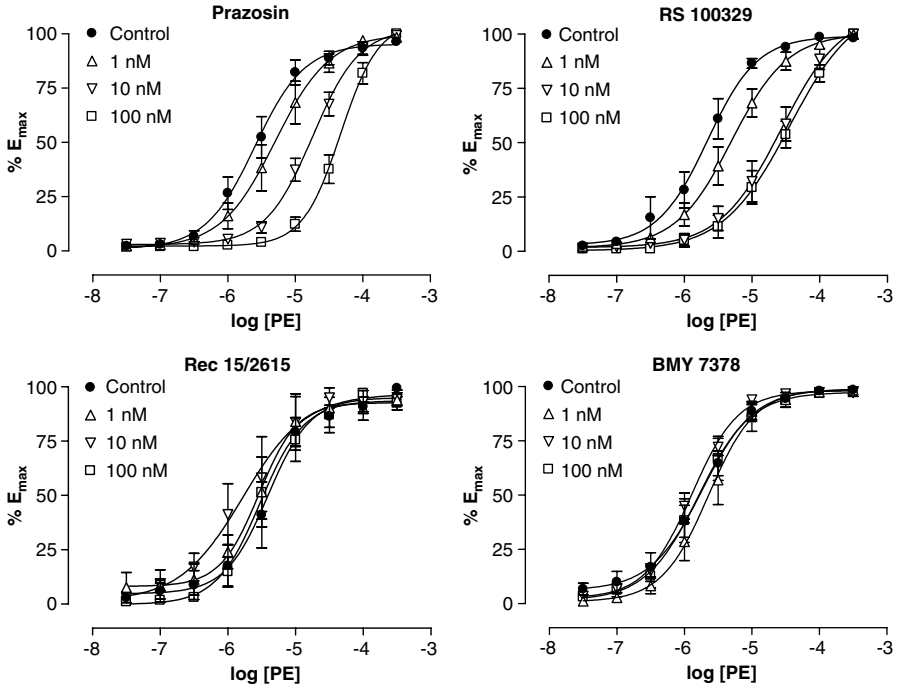

Figure 2 PE-induced vasoconstriction in the dorsal artery in the absence or presence of 1, 10 or 100 nM prazosin (non-selective antagonist, $n=7$), RS 100329 (α_{1A}-AR antagonist, $n=6$), Rec 15/2615 (α_{1B}-AR antagonist, $n=4$) or BMY 7378 (α_{1D}-AR antagonist, $n=6$). A rightward shift of the PE CRC was observed at 1 and 10 nM RS 100329, but no further rightward shift occurred at 100 nM. All PE response data were taken from second CRCs in the absence or presence of antagonist.

Table 3 Antagonist profile in the cavernous artery

Antagonist	n	*p*K$_B$ *(s.e.m.)*	*pA$_2$ (95% CI)*	*Schild slope (s.e.m.)*	*Concentration ratio (s.e.m.)*			
					1 nM	*10 nM*	*100 nM*	*1 μM*
Phentolamine	8	8.33 (0.09)	8.55 (8.09–9.12)	0.81 (0.08)	1.35 (0.09)	5.48 (1.42)	22.21 (6.23)	170.78 (83.18)
Prazosin	8	8.41 (0.15)	8.42 (7.66–9.85)	0.99 (0.25)	0.65 (0.16)	6.76 (3.11)	52.77 (19.21)	ND
RS 100329	7	8.80 (0.09)	8.98 (8.5–9.61)	0.83 (0.10)	2.13 (0.33)	10.22 (3.14)	45.85 (6.70)	ND
Rec 15/2615	4	ND	ND	ND	1.06 (0.47)	2.14 (0.72)	3.00 (2.06)	ND
BMY 7378	8	ND	ND	ND	0.86 (0.33)	1.83 (0.69)	1.30 (0.53)	ND
Rauwolscine	4	ND	ND	ND	1.13 (0.22)	1.17 (0.08)	1.36 (0.45)	ND

Abbreviations: CI, confidence interval; ND, not determined; PE, phenylphrine; PE CRC, phenylephrine concentration–response curves.
Summary of pK_B's, pA_2's, Schild slopes and concentration ratios of α_1- and α_2-AR antagonists versus PE. Where antagonists did not cause a rightward shift of the PE CRC, pK_B, pA_2 and Schild plots could not be determined.

effect of antagonist incubation on PE CRCs was determined by a comparison of second CRCs in the absence or presence of antagonist.

Phentolamine caused a parallel rightward shift of the PE curve in cavernous arteries with a pK_B of 8.33 ± 0.09 ($n=8$, Table 3). Schild analysis of the data gave a pA_2 of 8.55 (95% CI 8.09–9.12) with a slope close to unity of 0.81 ± 0.08. This indicates an effect at α-ARs.

The non-subtype selective α_1-AR antagonist prazosin was shown to cause a parallel rightward shift of the PE CRC with a pK_B of 8.41 ± 0.15 ($n=8$, Figure 3). The corresponding Schild plot gave a pA_2 of 8.42 (95% CI 7.66–9.85) with a slope that was close to unity, 0.99 ± 0.25, confirming the competitive nature of prazosin in this tissue.

The α_{1A}-AR selective antagonist RS 100329 caused a parallel rightward shift of the PE curve with a pK_B of 8.80 ± 0.09 ($n=7$, Figure 3). A Schild plot of the data demonstrated a relationship with a slope close to unity, 0.83 ± 0.10, giving a pA_2 of 8.98 (95% CI 8.51–9.61).

As with the dorsal arteries, the α_{1B}-AR selective antagonist Rec 15/2615 ($n=4$) and the α_{1D}-AR selective antagonist BMY 7378 ($n=8$) caused no shift of the PE curve in cavernous arteries (Figure 3). Concentration ratios ranged from 1.06 ± 0.47 to 3.00 ± 2.06 for Rec 15/2615 and from 0.86 ± 0.33 to 1.83 ± 0.69 for BMY 7378.

α_2-AR responses in the dorsal artery

In a follow-up to the observed shallow Schild slopes of PE responses in the dorsal arteries, investigations were made into the involvement of α_2-ARs. Rauwolscine produced small rightward shifts of the PE CRC in dorsal arteries but the change in EC_{50} was not significant ($P>0.05$, Figure 4). There was no shift in cavernous arteries (Figure 4).

Both dorsal and cavernous arteries demonstrated vasoconstrictor responses to the α_2-AR agonist UK 14 304 that were smaller in magnitude and greater in potency than responses to NA ($P<0.001$, $n=6$–7, Table 1). Similar to previous results using NA and PE, incubation with L-NAME (100 μM) significantly increased maximal responses to UK 14 304 in the dorsal ($P<0.01$, $n=6$), but not cavernous ($P>0.05$, $n=5$) arteries.

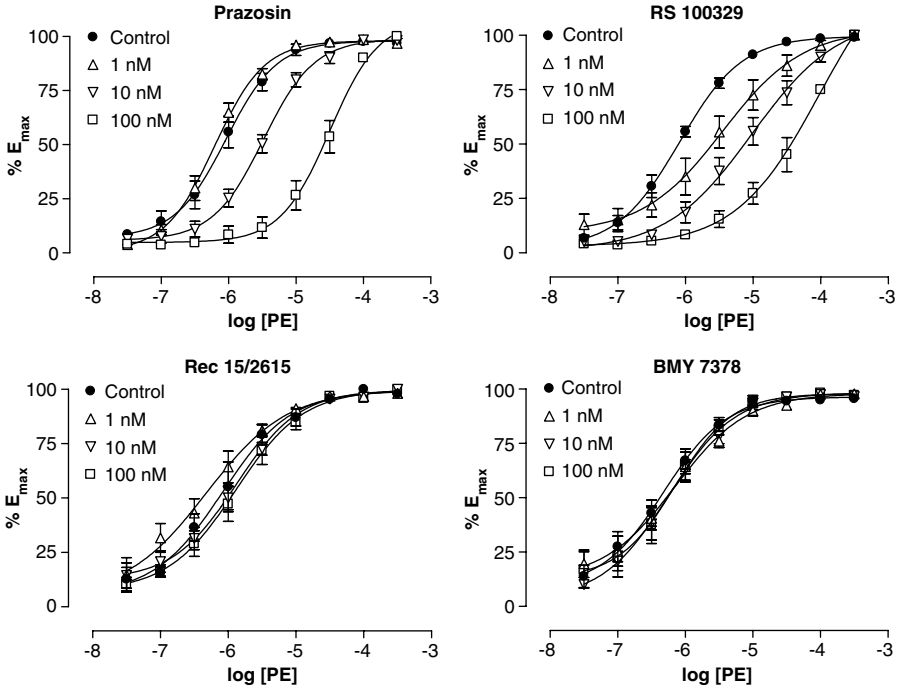

Figure 3 PE-induced vasoconstriction in the cavernous artery in the absence or presence of 1, 10 or 100 nM prazosin (non-selective antagonist, $n=7$–8), RS 100329 (α_{1A}-AR antagonist, $n=5$–6), Rec 15/2615 (α_{1B}-AR antagonist, $n=3$–4) or BMY 7378 (α_{1D}-AR antagonist, $n=6$). All PE response data were taken from second CRCs in the absence or presence of antagonist.

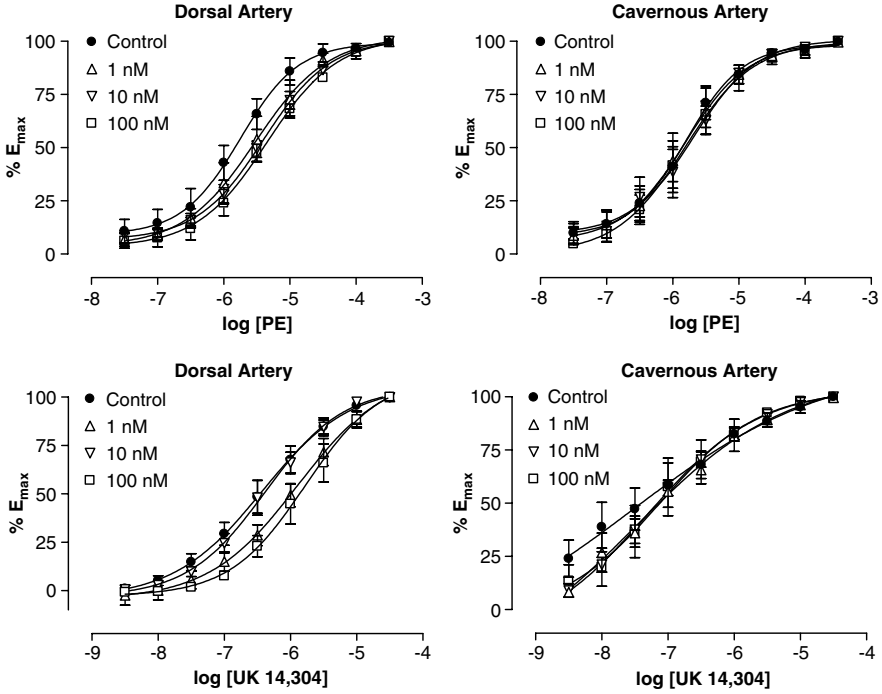

Figure 4 PE (top) or UK 14 304-induced (bottom) vasoconstriction in the dorsal ($n=4$–7) and cavernous ($n=4$) arteries in the absence or presence of 1, 10 or 100 nM rauwolscine (non-selective α_2-AR antagonist). All agonist response data were taken from second CRCs in the absence or presence of antagonist.

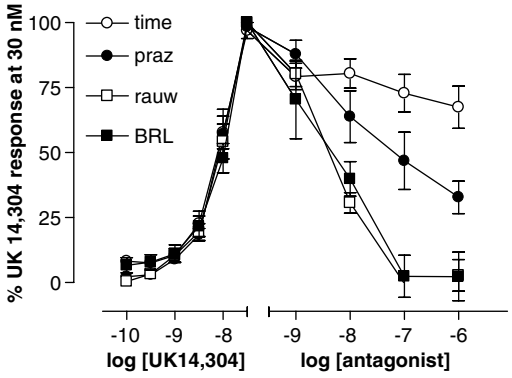

Figure 5 UK 14 304-induced (0.1–30 nM) vasoconstriction in the dorsal artery followed by addition of 1–1000 nM rauwolscine (rauw, non-selective α_2-AR, $n=5$), prazosin (praz, non-selective α_1-AR, $n=5$) or BRL 44408 (BRL, α_{2A}-AR selective, $n=6$) compared to a decrease in tone with the time control (time, $n=4$).

CRCs to UK 14 304 were not significantly shifted at the EC_{50} by rauwolscine (1 to 100 nM) in either penile artery ($P>0.05$, Figure 4). However, responses were smaller in the presence of 1 and 100 nM rauwolscine at some concentrations of agonist, particularly in the dorsal artery (0.1–1 μM, $P<0.05$). This trend was investigated further by assessing the ability of antagonists to decrease UK 14 304-induced vasoconstriction. Following the establishment of UK 14 304-induced tone during a partial CRC (0.1–30 nM), antagonists were added cumulatively in log steps from 1 nM to 1 μM (Figure 5). Rauwolscine significantly reversed UK 14 304-induced tone, causing a maximal reduction in tone of $97\pm6\%$ ($n=5$) compared to a $37\pm5\%$ ($n=4$) loss of tone with time ($P<0.001$). In addition, the α_{2A}-AR antagonist BRL 44408 was demonstrated to be equipotent to rauwolscine at causing a maximal, $97\pm7\%$ ($n=8$), reduction in UK 14 304-induced tone ($P<0.01$). The non-selective α_1-AR antagonist prazosin demonstrated a non-significant trend in decreasing UK 14 304-induced tone by $67\pm6\%$ ($P>0.05$, $n=5$). These results indicate that an α_2-AR-mediated response can be demonstrated in the dorsal artery. A similar analysis applied to cavernous artery showed the same trends but did not reach statistical significance (data not shown).

Discussion

Dorsal and cavernous arteries demonstrated contractile responses to both NA and PE, an α_1-AR selective agonist, with sensitivities in the normal range for adrenergically innervated rabbit arteries (Dunn *et al*., 1991; Naghadeh, 1996). Sensitivity to standard antagonists phentolamine and prazosin was also within the normal range for rabbit arteries (Naghadeh, 1996). However, there are some detailed pharmacological issues that need to be explained. In the dorsal artery, prazosin did not show straightforward characteristics of competitive antagonism of PE responses; affinity estimates decreased with increasing concentrations of prazosin and the corresponding Schild plot had a shallow slope. In addition, pK_B estimates for prazosin in both dorsal and cavernous arteries were lower than 9; considered to be an indicator of a low-affinity α_1-AR (Flavahan and Vanhoutte, 1986; Ford *et al*., 1997). However, low-affinity estimates for prazosin versus PE are not unusual in rabbit vasculature: estimated pA_2's of 8 in the lateral saphenous vein (Naghadeh, 1996), 8.7 in the mesenteric and carotid arteries, 8.8 in the thoracic aorta (Muramatsu *et al*., 1990) and 8.7 in the pulmonary artery (Docherty, 1988) have been demonstrated previously. Therefore, quantitatively, the α_1-AR/α_2-AR agonist and antagonist pharmacology of these arteries is consistent with that of other rabbit arteries.

To characterize α_1-AR subtype/s involved in PE responses, three α_1-AR-subtype selective antagonists were tested. In the cavernous artery, pharmacological analysis of these antagonists was clearcut. RS 100329, selective for the α_{1A}-AR subtype (Williams *et al*., 1999), inhibited PE responses potently and competitively. The high potency and Schild compliance of RS 100329 indicated that there is no need to invoke participation from other α_1-AR subtypes. In addition, antagonists selective for the α_{1B}-AR subtype (Rec 15/2615) and the α_{1D}-AR subtype (BMY 7378) were ineffective when tested against PE. Rec 15/2615 has been previously shown to be selective for the α_{1B}-AR subtype (Testa *et al*., 1997) and has been used in two recent *in vivo* studies of adrenoceptor pharmacology of genital tissues (Sironi *et al*., 2000; Kim *et al*., 2002) in rabbits, rats and dogs. Its lack of effect in the cavernous arteries argues against an involvement of the α_{1B}-AR subtype. BMY 7378 is a tried and tested α_{1D}-AR antagonist and was used at concentrations of 1–100 nM, covering its known affinity for the α_{1D}-AR ($pK_B \sim 8.7$, Kenny *et al*., 1995; Satoh *et al*., 1999; Daly *et al*., 2002; Tanoue *et al*., 2002; Deighan *et al*., 2005) while avoiding its affinity at the α_{1A}-AR ($pK_B \sim 6.6$; Lachnit *et al*., 1997; Zacharia *et al*., 2004; Deighan *et al*., 2005). As no effect on the PE response was observed over this range of concentrations, it would be reasonable to conclude that the α_{1D}-AR subtype is not the predominant functional subtype in cavernous penile arteries.

Pharmacological analysis of the dorsal artery was a little more complicated. Similar to the cavernous artery, the potency order of antagonists pointed to α_{1A}-AR, but the low Schild slopes did not confirm competitive antagonism. Both NA, and to a lesser extent, PE (Brown *et al*., 1988), at high concentrations can act at α_2-ARs. Rabbit penile tissue, specifically corpus cavernosum strips (Gupta *et al*., 1998), has been demonstrated to possess functional post-junctional α_2-ARs in addition to the predominant α_1-ARs. In the present study, analysis with the α_2-AR agonist UK 14 304 and the α_2-AR antagonist rauwolscine demonstrated the presence of α_2-ARs in the dorsal arteries. Additional support for the hypothesis of a mixed α_1- and α_2-AR population in the dorsal arteries comes from a greater maximum response to NA, a non-selective adrenoceptor agonist, than to the α_1-AR selective agonist PE; suggesting an additive effect of responses mediated by α_2-ARs. Determining subtypes of α_2-ARs using selective antagonists is more difficult than for α_1-ARs. However, one compound, BRL 44408, believed to be a relatively selective antagonist for the α_{2A}-AR (Uhlen *et al*., 1995) was as potent as rauwolscine against UK 14 304, favouring the presence of α_{2A}-AR in the dorsal artery.

A further complicating factor in the dorsal, but not cavernous, artery was that blockade of nitric oxide synthase by L-NAME potentiated responses to NA, PE and UK 14 304. This indicated either constitutive or agonist-induced release of endothelial nitric oxide in the course of the construction of agonist CRCs; further suggesting that the conditions for equilibrium responses to PE were compromised in this artery. In functional terms, this is an interesting issue as nitrergic and adrenergic influences are considered to be physiologically antagonistic in erectile arteries, and both are areas of therapeutic interest. The present data show that the dorsal artery is more subject to nitrergic influence than is the cavernous artery, even in the absence of specific nitrergic activation. In a comparison with cavernous artery and arteries from female erectile tissue, dorsal artery showed endothelium-mediated vasodilatation that was almost exclusively mediated by nitric oxide whereas the other vessels had a substantial endothelial-derived hyperpolarizing factor (EDHF) component. The interaction between nitric oxide metabolism and adrenergic mechanisms may, therefore, be of particular importance in regulating the nutritional supply to erectile tissue (Morton *et al*., 2007).

The physiological significance of these findings would be to suggest that the predominant functional receptor in the penile arteries is the α_{1A}-AR. The presence of α_{1A}-ARs in erectile tissues is supported by two studies in cavernous tissue strips (Tong and Cheng, 1997; Peng *et al*., 1998) and by an *in vivo* study (Sironi *et al*., 2000). However, this is the first study to demonstrate the presence of functional α_{1A}-ARs in isolated genital arteries. Increased sympathetic activity and concomitant release of NA during detumescence and flaccidity could lead to activation of α_{1A}-ARs and vasoconstriction of cavernous arteries thereby decreasing arterial inflow. While this study has concentrated on responses to exogenous agonists, the physiological validity of our conclusions could be further investigated using a study of nerve-induced responses. Responses to NA (10 μM) demonstrated that over a 6-min period, vasoconstriction was well maintained in cavernous but not dorsal arteries. In addition, dorsal arteries had a lower sensitivity to NA ($P<0.05$) and PE ($P<0.001$) than cavernous arteries. Less complete vasoconstriction of dorsal arteries, because of lower sensitivity to agonists and less well-maintained vasoconstriction, would allow a residual blood flow for nutritional and oxygenation purposes without initiation of an erectile response.

NA responses were significantly potentiated by the combination of uptake blockers, cocaine and corticosterone in both dorsal and cavernous arteries; demonstrating that NA uptake mechanisms were active in these tissues. This would be particularly relevant when in relation to erectile dysfunction brought about by illegal drug abuse with pro-adrenergic compounds, such as cocaine and indirect sympathomimetics related to amphetamine. It would be expected that acute potentiation of transmission by cocaine would lead to erectile dysfunction owing to an increased sensitivity of tissues to NA. However, long-term cocaine use has been reported to cause priapism (Dougherty *et al*., 2006). Chronic dosage of cocaine may lead to failure of adrenergic transmission either through depletion of NA stores in adrenergic neurons or downregulation of α_{1A}-ARs, each compromising the ability of the erectile tissues to oppose vasodilatation with consequent erection.

Pharmacological antagonism of penile α_{1A}-ARs could be utilized to aid the erectile response in male erectile dysfunction sufferers by reducing vasoconstriction of the penile arteries, in particular, the cavernous artery. Dorsal and cavernous arteries are central to normal sexual function. Maintained vasoconstriction of the cavernous artery during the flaccid state is vital to prevent initiation of an erectile response. Consequently, attenuation of this vasoconstriction is equally important when an erection is required. A more subtle control of the vasoconstrictor/vasodilator state of the dorsal arteries is fundamental in preserving the viability of erectile tissues. Hence, knowledge of the contractile pathways affecting the function of these arteries is vital to allow the selection of potential pharmacological targets for therapies aimed at the treatment of sexual dysfunction. Regarding selectivity of antagonists for facilitating erection, the ideal combination indicated by the current data would be a combination of α_{1A}- and α_2-AR antagonism, a selectivity profile for which we know of no compound. However, the non-selective α-blocker phentolamine would achieve both actions, perhaps explaining why it remains the drug of choice for intracavernous injection, despite the availability of more selective compounds. The same argument would apply to yohimbine, which is only marginally selective for α_2- over α_1-AR and would be likely to block both at an effective dose. Of course, the similarity of the pharmacological profile of the penile arteries to those of all other arteries carries the risk of cardiovascular and other autonomic side effects on systemic administration.

In conclusion, this study demonstrated that the predominant α_1-AR subtype involved in vasoconstriction of both dorsal and cavernous penile arteries to exogenous PE was the α_{1A}-AR. No evidence was found for the involvement of α_{1B}- or α_{1D}-AR subtypes; clarifying an area of investigation previously complicated by conflicting evidence for all three α-AR subtypes and improving the basis from which to study pharmacological targets in the treatment of erectile dysfunction. The effects of the selective α_1-AR antagonists, taken together with the clear effect of the non-selective (between α_1-AR and α_2-AR) antagonist phentolamine and the antagonism of UK 14 304 by rauwolscine and BRL 44408, suggest that dorsal arteries have a mixed receptor population composed of α_{1A} and α_2 (potentially the α_{2A}-AR subtype)-ARs.

Acknowledgements

This work was supported by the Biotechnology and Biological Sciences Research Council and Pfizer Global Research and Development. JSM was funded by a CASE Postgraduate Scholarship.

Conflict of interest

The authors state no conflict of interest.

References

Andersson K-E, Wagner G (1995). Physiology of penile erection. *Physiol Rev* **75**: 191–236.

Brown CM, McGrath JC, Midgley JM, Muir AG, O'Brien JW, Thonoor CM *et al*. (1988). Activities of octopamine and synephrine stereoisomers on alpha-adrenoceptors. *Br J Pharmacol* **93**: 417–429.

Chin MTM, Lee SM, Dinsmore WW (1998). Priapism induced by intracavernosal vasoactive intestinal polypeptide and phentolamine mesylate. *Sexual Dysfunction* **1**: 49–51.

Choppin A, Blue Jr DR, Hegde SS, Gennevois D, McKinnon SA, Mokatrin A *et al*. (2001). Evaluation of oral Ro70-0004/003, an alpha$_{1A}$-adrenoceptor antagonist, in the treatment of male erectile dysfunction. *Int J Impot Res* **13**: 157–161.

Cleary L, Vandeputte C, Docherty JR (2003). Investigation of postjunctional α_1- and α_2-adrenoceptor subtypes in vas deferens from wild-type and $\alpha_{2A/D}$-adrenoceptor knockout mice. *Br J Pharmacol* **138**: 1069–1076.

Daly CJ, Deighan C, McGee A, Mennie D, Ali Z, McBride M *et al*. (2002). A knockout approach indicates a minor vasoconstrictor role for vascular α_{1B}-adrenoceptor in mouse. *Physiol Genomics* **9**: 85–91.

Dausse J-P, Leriche A., Yablonsky F (1998). Patterns of messenger RNA expression for alpha$_1$ adrenoceptor subtypes in human corpus cavernosum. *J Urol* **160**: 597–600.

Deighan C, Methven L, Naghadeh MM, Wokoma A, Macmillan J, Daly CJ *et al*. (2005). Insights into the functional roles of α_1-adrenoceptor subtypes in mouse carotid arteries using knockout mice. *Br J Pharmacol* **144**: 558–565.

Docherty JR (1988). No evidence for more than one type of alpha 1-adrenoceptor in rabbit pulmonary artery. *J Auton Pharmacol* **8**: 327–332.

Dougherty CM, Richard AJ, Carey MJ (2006). Priapism. Kreplick LW, Talavera F, Sinert R, Halamka J and O'Connor RE (eds) http://www.emedicine.com/EMERG/topic486.htm. eMedicine.

Dunn WR, Daly CJ, McGrath JC, Wilson VG (1991). The effects of nifedipine on α_2-adrenoceptor-mediated contractions in several isolated blood vessels from the rabbit. *Br J Pharmacol* **103**: 1493–1499.

Flavahan NA, Vanhoutte PM (1986). α_1-Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol Sci* **7**: 347–349.

Ford APDW, Daniels DV, Chang C-H, Gever JR, Jasper JR, Lesnick JD *et al*. (1997). Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α_1-adrenoceptor classification. *Br J Pharmacol* **121**: 1127–1135.

Furukawa K, Chess-Williams R, Uchiyama T (1996). Alpha 1B adrenoceptor subtype mediating the phenylephrine-induced contractile response in rabbit corpus cavernosum penis. *Jpn J Pharmacol* **71**: 325–331.

Goepel M, Krege S, Price DT, Michelotti GA, Schwinn DA, Michel MC (1999). Characterisation of alpha-adrenoceptor subtypes in the corpus cavernosum of patients undergoing sex change surgery. *J Urol* **162**: 1793.

Gupta S, Moreland RB, Yang S (1998). The expression of functional postsynaptic α_2-adrenoceptors in the corpus cavernosum smooth muscle. *Br J Pharmacol* **123**: 1237–1245.

Kenny BA, Chalmers DH, Philpott PC, Naylor AM (1995). Characterisation of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol* **115**: 981–986.

Kim NN, Min K, Huang Y-H, Goldstein I, Traish A (2002). Biochemical and functional characterisation of alpha-adrenergic receptors in the rabbit vagina. *Life Sci* **71**: 2909–2920.

Lachnit WG, Tran AM, Clarke DE, Ford APDW (1997). Pharmacological characterisation of an α_{1A}-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. *Br J Pharmacol* **120**: 819–826.

Morton JS, Jackson VM, Daly CJ, McGrath JC (2007). Endothelium-dependent relaxation in rabbit genital resistance arteries is predominantly mediated by EDHF in females and nitric oxide in males. *J Urol* in press.

Muramatsu I, Ohmura T, Kigoshi S, Hashimoto S, Oshita M (1990). Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. *Br J Pharmacol* **99**: 197–201.

Naghadeh MM (1996). Vasomodulator mechanisms in rat carotid artery and in vessels from an experimental model of heart failure, PhD Thesis, University of Glasgow.

Peng M, Zhang Y, Xue Z, Han Q (1998). Alpha 1-adrenoceptor subtypes in rabbit penile corpus cavernosum. *Zhonghua Wai Ke Za Zhi* **36**: 497–499.

Recio P, Lopez PG, Fernandez JL, Garcia-Sacristan A (1997). Pharmacological characterisation of adrenoceptors in horse corpus cavernosum penis. *J Auton Pharmacol* **17**: 191–198.

Saenz De Tejada I, Kim N, Lagan I, Krane RJ, Goldstein I (1989). Regulation of adrenergic activity in penile corpus cavernosum. *J Urol* **142**: 1117–1121.

Sato M, Kawatani M (2002). Effects of noradrenaline on cytosolic concentrations of Ca^{2+} in cultured corpus cavernosum smooth muscle cells of the rabbit. *Neurosci Lett* **324**: 89–92.

Satoh M, Enomoto K, Takayanagi I, Koike K (1999). Analysis of α_1-adrenoceptor subtypes in rabbit aorta and arteries: regional difference and co-existence. *Eur J Pharmacol* **374**: 229–240.

Sironi G, Columbo D, Poggesi E, Leonardi A, Testa R, Rampin O *et al*. (2000). Effects of intracavernous administration of selective antagonists of α_1-adrenoceptor subtypes on erection in anaesthetised rats and dogs. *J Pharmacol Exp Ther* **292**: 974–981.

Tamura M, Kagawa S, Kimura K, Kawanishi Y, Tsuruo Y, Ishimura K (1995). Coexistence of nitric oxide synthase, tyrosine hydroxylase and vasoactive intestinal polypeptide in human penile tissue – a triple histochemical and immunohistochemical study. *J Urol* **153**: 530–534.

Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T *et al*. (2002). The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J Clin Invest* **109**: 765–775.

Testa R, Guarneri L, Angelico P, Poggesi E, Taddei C, Sironi G *et al*. (1997). Pharmacological characterisation of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity, Part II. *J Pharmacol Exp Ther* **281**: 1284–1293.

Tong YC, Cheng JT (1997). Subtyping of α_1-adrenoceptors responsible for the contractile response in the rat corpus cavernosum. *Neurosci Lett* **228**: 159–162.

Traish AM, Netsuwan N, Daley J, Padma-Nathan H, Goldstein I, Saenz De Tejada I (1995). A heterogeneous population of alpha$_1$ adrenergic receptors mediates contraction of human corpus cavernosum smooth muscle to norepinephrine. *J Urol* **153**: 222–227.

Uhlen S, Mucenience R, Rangel N, Tiger G, Wikberg JE (1995). Comparison of the binding activities of some drugs on alpha 2A, alpha 2B and alpha 2C-adrenoceptors and non-adrenergic imidazoline sites in the guinea pig. *Pharmacol Toxicol* **76**: 353–364.

Veronneau-Longueville F, Rampin O, Jardin A, Benoit G, Giuliano F (1998). Expression of alpha 1 adrenoceptor subtypes in rat corpus cavernosum. *Int J Impot Res* **10**: 187–194.

Williams TJ, Blue DR, Daniels DV, Davis B, Elworthy T, Gever JR *et al*. (1999). *In vitro* α_1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel α_{1A}-adrenoceptor selective antagonists. *Br J Pharmacol* **127**: 252–258.

Zacharia J, Hillier C, MacDonald A (2004). Pharmacological characterisation of α_1-adrenoceptors in mouse isolated femoral small arteries. *Eur J Pharmacol* **503**: 155–163.