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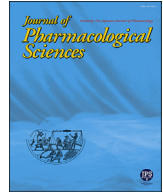
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Full paper

 α_{1L} -adrenoceptors mediate contraction of human erectile tissue

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ABSTRACT

α_1 -adrenoceptor antagonists can impact upon sexual function and have potential in the treatment of erectile dysfunction. Human erectile tissue contains predominantly α_{1A} -adrenoceptors, and here we examined whether contractions of this tissue are mediated by the functional phenotype, the α_{1L} -adrenoceptor. Functional experiments using subtype selective agonists and antagonists, along with radioligand ($[^3H]$ tamsulosin) binding assays, were used to determine the α_1 -adrenoceptor population. A61603, a α_{1A} -adrenoceptor agonist, was a full agonist with a potency 21-fold greater than that of noradrenaline. The α_{1A} - and α_{1D} -adrenoceptor antagonist tamsulosin antagonized noradrenaline responses with high affinity ($pK_D = 9.7 \pm 0.3$), whilst BMY7378 (100 nM) (α_{1D} -adrenoceptor antagonist) failed to antagonize responses. In contrast, relatively low affinity estimates were obtained for both prazosin ($pK_D = 8.2 \pm 0.1$) and RS17053 ($pK_D = 6.9 \pm 0.2$), antagonists which discriminate between the α_{1A} - and α_{1L} -adrenoceptors. $[^3H]$ Tamsulosin bound with high affinity to the receptors of human erectile tissue ($pK_D = 10.3 \pm 0.1$) with a receptor density of 28.1 ± 1.4 fmol mg^{-1} protein. Prazosin displacement of $[^3H]$ tamsulosin binding revealed a single homogenous population of binding sites with a relatively low affinity for prazosin ($pK_i = 8.9$). Taken together these data confirm that the receptor mediating contraction in human erectile tissue has the pharmacological properties of the α_{1L} -adrenoceptor.

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1. Introduction

Penile erection (tumescence) is achieved when the erectile tissue relaxes and becomes engorged with blood, caused by the vasodilation of inflow arteries and compression of the outflow veins. In contrast, activation of the sympathetic nervous system causes contraction of the erectile tissue, mediated by postsynaptic α_1 -adrenoceptors, and leads to flaccidity and detumescence.¹ Thus α_1 -adrenoceptor antagonists have been investigated for their potential therapeutic effect in the treatment of erectile dysfunction and have been shown to impact upon sexual function. Intra-cavernosal injection of α -adrenoceptor antagonists can relax erectile smooth muscle and induce an erection,^{2,3} and positive effects of

oral treatment of erectile dysfunction with α_1 -adrenoceptor antagonists have been reported both experimentally^{4,5} and clinically.^{6,7} These agents can also have adverse effects on erectile dysfunction, with some of the newer generation α_1 -adrenoceptor antagonists such as silodosin, used in the treatment of benign prostatic hyperplasia, known to cause anejaculation.^{8–10} However, the incidence of anejaculation is variable between α_1 -adrenoceptor antagonists, and it remains unknown whether this effect is mediated via genitourinary α_1 -adrenoceptors or centrally via dopamine or serotonin receptors.¹¹

Three α_1 -adrenoceptor subtypes (α_{1A} , α_{1B} and α_{1D}) have been classified via functional and molecular techniques.¹² There is also functional evidence for a fourth subtype, the α_{1L} -adrenoceptor, also known as the $\alpha_{1A/L}$ -adrenoceptor, which represents a functional phenotype of the α_{1A} -adrenoceptor that is pharmacologically distinct and has a low affinity for prazosin.^{13–15} Human erectile tissue expresses mRNA coding for all three cloned α_1 -adrenoceptor subtypes, although it is the α_{1A} -adrenoceptor subtype that predominates, with lower levels of α_{1D} - and α_{1B} -adrenoceptor mRNA.¹⁶

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A heterogeneous α_1 -adrenoceptor population has also been demonstrated in receptor binding experiments, but there is disagreement regarding which subtype predominates at the protein level. The α_{1A} -receptor has been reported as the major subtype,¹⁶ whilst Goepel et al. (1999) reported both α_{1A} - and α_{1B} -adrenoceptors at the protein level, with no evidence for the α_{1D} -receptor.¹⁷ Functionally, a more recent study concluded that the α_{1A} -adrenoceptor was the main subtype mediating contraction of the human cavernosum.¹⁸ However, this was a study of human cavernosal tissue from patients undergoing gender-re-assignment, in which the men had received long-term estrogen therapy (2–5 years). Whilst the effects of long term estrogen therapy on erectile tissue are unknown, this therapy has been shown to affect adrenoceptors elsewhere in body.^{19,20} In addition, the study did not examine the two antagonists that are able to discriminate between the α_{1A} - and α_{1L} -adrenoceptor isoforms, prazosin and RS17053,^{13,21,22} which we have previously used to identify that α_{1L} -adrenoceptors mediate contraction elsewhere in the male lower urinary, specifically the human prostate²³ and vas deferens.²⁴ A small clinical trial of a selective α_{1A} -adrenoceptor antagonist also failed to show any improvement in erectile dysfunction,²⁵ leading to speculation that the α_{1L} -adrenoceptor may be important functionally in human penile erectile tissue.

Thus, the aim of this study was to determine whether the α_{1A} - or the α_{1L} -adrenoceptor subtype mediates contraction of human erectile tissue.

2. Materials and methods

Erectile tissue was obtained as remnants of surgery from consenting patients undergoing urethroplasty (mean age = 38.7 ± 5.4 years). All tissues were obtained with full informed consent and with approval from the appropriate local ethics committee, South Sheffield Hospitals Ethics Committee (Sheffield, UK), Greenslopes Private Hospital Research and Ethics Committee and Bond University Human Research Ethics Committee (Queensland, Australia).

2.1. Functional experiments

Strips of erectile tissue ($10 \times 3 \times 5$ mm) were mounted in organ baths at 37 °C containing Krebs-bicarbonate solution (composition: in mM: NaCl 118.4, KCl 4.7, CaCl₂ 1.9, NaHCO₃ 25.0, MgSO₄ 1.2, KH₂PO₄ 1.2 and glucose 11.7) and gassed with 5% CO₂ in oxygen. Tissues were set-up under a resting tension of 1.5 g and isometric contractions recorded via UF1 transducers linked to a PC via a Cambridge Electronic Design 1401 analogue to digital interface, using LabChart software (ADInstruments).

Tissues were equilibrated for 60 min, with several changes of bathing solution. Following equilibration cumulative concentration–response curves to noradrenaline were obtained in the absence or presence of BMY7378, which is 30–100-fold selective for the α_{1D} -adrenoceptor over the α_{1A} -adrenoceptor²⁶ and was used at 100 nM to determine involvement of the α_{1D} -adrenoceptor. Cumulative concentration–response curves to noradrenaline were also obtained in the absence and presence of the α_{1A} -, α_{1L} - and α_{1D} -adrenoceptor antagonist tamsulosin (3 & 10 nM), the α_{1A} -adrenoceptor antagonist 5-Methylurapidil (30 nM), the α_{1A} -adrenoceptor antagonist RS17053 (30 & 300 nM) (discriminates between α_{1A} and α_{1L}), and the α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor antagonist prazosin (30–300 nM) (also discriminates between α_{1A} and α_{1L}). Tissues were pre-incubated with antagonists for 60 min. Control experiments (without the addition of antagonist) were performed in parallel and used to correct for any time-dependent changes in tissue sensitivity. Contractions to the α_{1A} -adrenoceptor selective agonist A61603 were also obtained. All experiments were performed in the presence of cocaine (10 μ M) and corticosterone

(10 μ M) to prevent amine uptake and propranolol (1 μ M) and yohimbine (0.5 μ M) to antagonize β - and α_2 -adrenoceptors respectively.

2.2. Radioligand binding experiments

For radioligand binding experiments tissues were homogenized in 40 volumes (w/v) of ice cold Tris buffer (50 mM at pH 7.4) using an Ultra-Turrax homogenizer for 30 s, followed by five strokes of a glass-Teflon homogenizer. The homogenate was filtered through muslin, re-homogenized with ten strokes, and then centrifuged twice at 45 000 g for 10 min. The final pellet was resuspended in Tris buffer for use in the binding assay.

Each binding assay was performed in duplicate using 100 μ l of membranes in a final volume of 250 μ l. Phentolamine was used to displace the radioligand [³H]tamsulosin and determine non-specific binding, at a concentration of 10 μ M, since this is 100–1000-fold greater than its dissociation constant at the α_1 -subtypes.²⁷ Incubations were performed at 37 °C for 30 min and the reaction stopped by vacuum filtration through glass-fiber filters (Whatman GF/C) pre-soaked in 0.3% polyethylenimine. The filters were washed twice with 5 ml of ice-cold Tris buffer and the radioactivity remaining on the filters was determined using standard scintillation counting methods with Packard 'Emulsifier safe' scintillation fluid. In saturation binding experiments nine concentrations of [³H]tamsulosin (20 pM–5 nM) were examined. Protein content of the membrane solution was determined by the method of Lowry et al., 1951, using bovine serum albumin (BSA) as standard.²⁸ Competition binding was performed in duplicate using [³H]tamsulosin (700 pM) and a range of concentrations of prazosin (1 pM–500 nM).

2.3. Data analysis

Increases in developed tension in response to the agonists were plotted as a percentage of the maximum increase for each concentration–response curve and expressed as mean \pm SEM. Individual EC₅₀ values (concentration producing a half-maximal response) were determined by non-linear regression of sigmoidal dose–response curves (variable slope) using GraphPad Prism software (La Jolla, CA, USA). Geometric mean EC₅₀ values with 95% confidence limits were also calculated. Where several concentrations of antagonist were examined, Schild plots were constructed and slopes calculated for evidence of a competitive mechanism of action. Apparent pK_D values (–log dissociation constant) were determined from individual shifts of concentration–response curves using the equation:

$$pK_D = \log(CR - 1) - \log[B]$$

where CR is the concentration-ratio (ratio of the EC₅₀ values in the presence and absence of antagonist) obtained with a concentration [B] of antagonist.²⁹

Statistical significance was determined using a two-tailed paired Student's *t*-test for log EC₅₀ values, maximum responses and Hill slopes, and using a two-tailed Student's *t*-test for Schild slopes.

GraphPad Prism software was also used to analyze the radioligand binding data after correcting the free radioligand concentration for any membrane bound radioligand. The density of binding sites and radioligand affinity were calculated using non-linear curve fitting, whilst competition data were fitted to a one- or two-site model, and the IC₅₀ value converted to a K_D value.³⁰ The suitability of a two-site fit was assessed by an *F*-test. A two-tailed unpaired Student's *t*-test was applied to assess whether Hill slopes differed from unity.

2.4. Drugs and chemicals

(±)-Noradrenaline, cocaine, (±)propranolol, yohimbine (all hydrochloride salts) and corticosterone 21-acetate were obtained from Sigma-Aldrich (Poole, UK). (-)-Tamsulosin hydrochloride (YM617) was a gift from Yamanouchi Europe B.V. (Leiderdorp, Netherlands). 5-Methylurapidil, BMY7378 dihydrochloride and prazosin were obtained from Research Biochemicals Inc (Natick, MA, USA). RS17053 hydrochloride and A61603 hydrobromide were obtained from Tocris Cookson Ltd (Bristol, UK). All other chemicals were of reagent grade. Corticosterone was dissolved in 70% ethanol. RS17053 was dissolved in 100% DMSO and diluted in 5 mM phosphoric acid. Prazosin was dissolved in distilled water with a drop of glacial acetic acid and diluted in distilled water. All other drugs were dissolved in distilled water and diluted in Krebs solution. [³H]Tamsulosin ([p-phenoxy-³H]-YM617, specific activity 1117.4 Gbq mol⁻¹) was obtained from NEN (Boston, MA). All reagents were Analar grade.

3. Results

3.1. Responses to agonists

Both noradrenaline and the α_{1A} -adrenoceptor selective agonist A61603 caused concentration-dependent contractions in isolated strips of human erectile tissue (Fig. 1). A61603 was 21-fold more potent than noradrenaline, having a significantly lower ($P < 0.05$, $n = 8$) geometric mean EC₅₀ value of 0.13 (0.04–0.39) μ M compared with 2.70 (1.47–4.96) μ M for noradrenaline. A61603 acted as a full agonist, eliciting a maximum contraction of 108.31 \pm 19.40% relative to noradrenaline (Fig. 1).

3.2. Effect of antagonists on noradrenaline contractions

5-Methylurapidil (30 nM) and tamsulosin (3 & 10 nM) caused rightward shifts of noradrenaline concentration–response curves without affecting maximum contractions (Fig. 2A & B), although the Hill slope for noradrenaline in the presence 10 nM tamsulosin (0.78 \pm 0.14) was significantly lower ($P < 0.05$) than the Hill slope in the absence of this antagonist (1.05 \pm 0.06). Both 5-methylurapidil and tamsulosin had relatively high affinities for the α_1 -adrenoceptors in human erectile tissue, with apparent pK_D values of 8.1 \pm 0.20 ($n = 5$) and 9.7 \pm 0.3 ($n = 8$) respectively.

In contrast, the α_{1D} -adrenoceptor selective antagonist BMY7378 at a concentration of 100 nM failed to significantly affect noradrenaline concentration–response curves (shift = 1.18 \pm 0.46, $n = 5$) (Fig. 2C). The α_{1A} -adrenoceptor selective antagonist RS17053 (low affinity for the α_{1L} -adrenoceptor), at 30 nM, also failed to shift

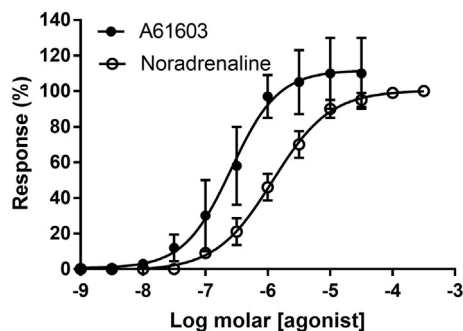


Fig. 1. Mean concentration–response curves of human erectile tissue to noradrenaline and A61603. Responses are plotted as a percentage of the maximum response to noradrenaline ($n = 8$). All experiments were performed in the presence of cocaine (10 μ M), corticosterone (10 μ M), propranolol (1 μ M) and yohimbine (0.5 μ M) to inhibit amine uptake, β -adrenoceptors and α_2 -adrenoceptors, respectively.

noradrenaline concentration–response curves (Fig. 2D), although at a higher concentration of 300 nM it did shift noradrenaline curves to the right, with a significant increase in noradrenaline EC₅₀ values ($P < 0.05$) and yielding an apparent pK_D value of 6.9 \pm 0.2 ($n = 5$). The antagonism appeared to be competitive since maximum responses to noradrenaline were not depressed and the Hill slopes in the presence of RS17053 were not significantly different from those in the absence of antagonist.

Prazosin (30–300 nM) produced concentration-related dextral shifts of noradrenaline concentration–response curves without any change in maximum response or in the Hill slopes (Fig. 3), with a mean pK_D value of 8.2 \pm 0.1 ($n = 14$). Three concentrations of prazosin were examined and the slope of the Schild plot was not significantly different from unity (slope 0.92 \pm 0.21, $P = 0.71$) and had an intercept on the abscissa of 8.4 (Fig. 3).

3.3. Radioligand binding experiments

[³H]Tamsulosin bound to human erectile tissue membranes with a high affinity (mean pK_D of 10.3 \pm 0.1). The binding was saturable, and the density of binding sites was 28.1 \pm 1.4 fmol mg⁻¹ protein (Fig. 4). In a single competition experiment performed in duplicate and using tissue pooled from 4 patients, prazosin displaced [³H]tamsulosin from a single population of binding sites with a relatively low affinity for prazosin (pK_i of 8.9, Fig. 4).

4. Discussion

The role of adrenoceptors in human penile erection is still not fully understood. While stimulation of postsynaptic α_1 -adrenoceptors is known to mediate contraction of the erectile smooth muscle, leading to flaccidity and detumescence,¹ there is still controversy regarding the predominant α_1 -adrenoceptor subtype mediating this contraction in humans. In the present study we used a range of subtype selective agonists and antagonists to determine whether the α_{1L} -adrenoceptor subtype is predominant in human erectile tissue, as we have shown previously for the human prostate and vas deferens.^{23,24}

A61603, an agonist that is highly selective for α_{1A} -adrenoceptors,³¹ was 21-fold more potent in causing contraction of erectile tissue than the endogenous agonist noradrenaline. This value is comparable to the values we have obtained previously for human prostate, under identical conditions, where A61603 was found to be 13-times more potent than noradrenaline (Chess-Williams, unpublished observations). Thus, the agonist data suggest the presence of the α_{1A} -adrenoceptor subtype in human erectile tissue.

Tamsulosin is a competitive antagonist with a very high affinity (>10) at α_{1A} - and α_{1D} -adrenoceptors.³² Both concentrations of tamsulosin used in this study (3 and 10 nM) caused rightward shifts of the noradrenaline concentration–response curves and the high mean affinity estimate (apparent pK_D of 9.7) is consistent with the involvement of α_{1A} and/or α_{1D} -adrenoceptors, and not α_{1B} -adrenoceptors. At 3 nM tamsulosin did not affect the Hill slope for noradrenaline, and (presumably the more accurate) affinity estimate was even higher, at 9.9, and identical to that we showed at the $\alpha_{1A/L}$ -adrenoceptors of the human prostate.³² At the higher concentration (10 nM) tamsulosin reduced the Hill slope for noradrenaline. This is not surprising since tamsulosin has been reported to significantly affect maximum contractions (i.e. have an unsurmountable action) in a number of smooth muscle preparations, including in our previous studies of human prostate²⁷ and human vas deferens.³³ The fact that tamsulosin is a competitive antagonist with a very high affinity (>10) at α_{1A} - and α_{1D} -adrenoceptors,³² and may bind irreversibly to these receptors, may explain this unsurmountable action.

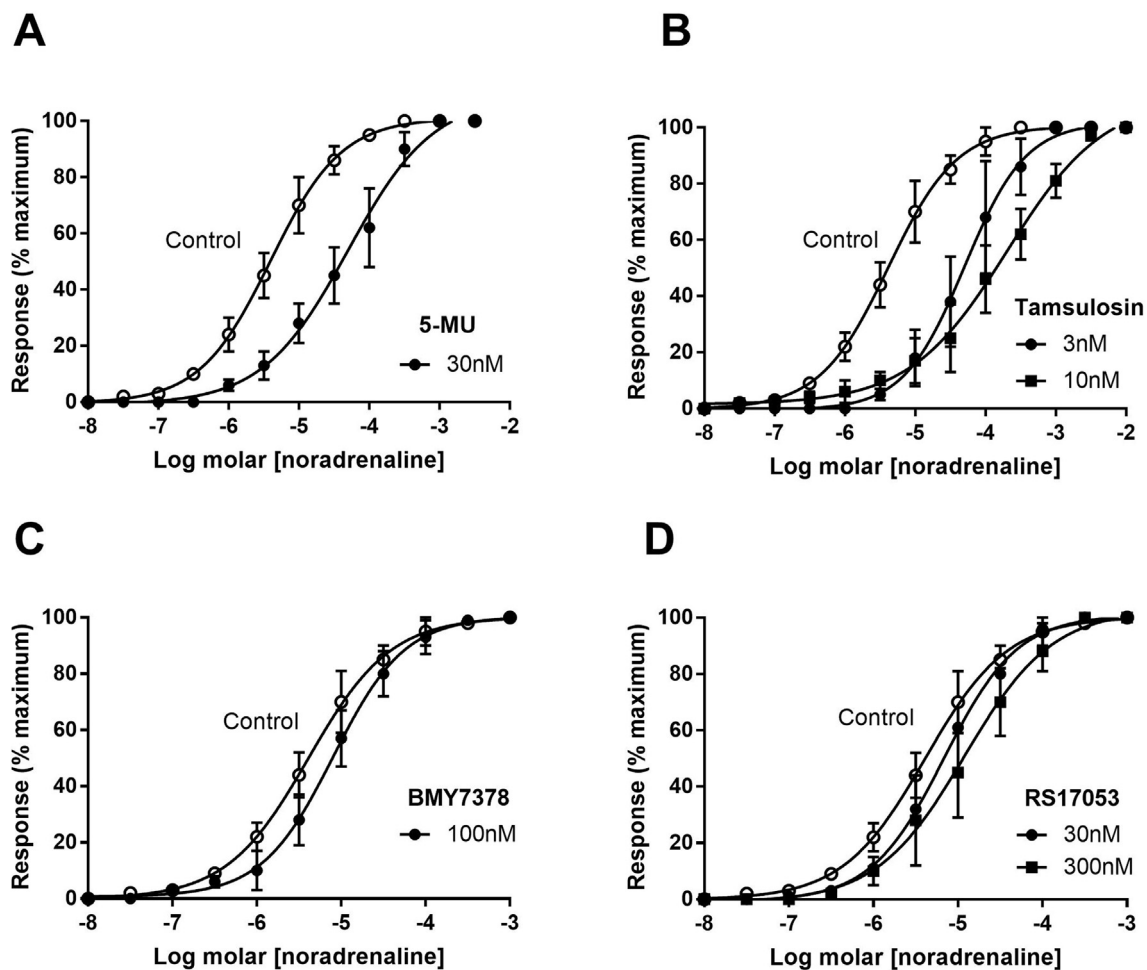


Fig. 2. Mean concentration–response curves of human erectile tissue to noradrenaline in the absence and presence of (A) 5-methylurapidil (5-MU) ($n = 5$), (B) tamsulosin ($n = 3–5$), (C) BMY7378 ($n = 5$), and (D) RS17053 ($n = 4–6$).

The involvement of α_{1A} - or α_{1D} -adrenoceptors was further supported by the relatively high affinity estimate (pK_D 8.1) obtained for the antagonist 5-methylurapidil. This antagonist has a lower affinity at α_{1B} -adrenoceptors ($pK_D = 7.2$),³⁴ and thus the affinity estimate obtained in the present study is higher than would be expected if α_{1B} -adrenoceptors were mediating the contractions to noradrenaline in the human erectile tissue. An affinity of 7.8 has been reported for 5-methylurapidil at α_{1D} -adrenoceptors.³⁴ To rule out the involvement of α_{1D} -adrenoceptors, experiments were performed in the presence of BMY7378, which has a high affinity for α_{1D} -adrenoceptors (pK_i 8.2)²⁶ and is 100-fold selective for this subtype over the α_{1B} - and α_{1A} -receptor subtypes. If the α_{1D} -receptor was involved in contractions, the concentration of BMY7378 used (100 nM) should have produced at least a 10-fold shift. The lack of shift of the noradrenaline concentration–response curves, and the lack of effect on the Hill slopes, indicates that α_{1D} -adrenoceptors are not involved in smooth muscle contraction in this tissue.

These results indicate that responses of human erectile tissue are mediated via α_{1A} -adrenoceptors. However, α_{1A} -adrenoceptors can exist as the distinct functional phenotype α_{1L} -adrenoceptor, and many α_{1A} -selective antagonists, including tamsulosin and 5-methylurapidil, have a high affinity for α_{1L} -adrenoceptors in addition to α_{1A} -adrenoceptors. Prazosin and RS17053 are two antagonists that can distinguish between α_{1A} - and α_{1L} -adrenoceptors. Prazosin has a high affinity at all cloned α_1 -adrenoceptors ($pK_D > 9.5$), but a low affinity ($pK_D < 9$) at the α_{1L} -adrenoceptor.^{13,21}

In the present study the affinity estimate for prazosin in human erectile tissue (pK_D value of 8.2) was low, too low to indicate an action at one of the cloned α_1 -adrenoceptors, and instead suggesting the involvement of α_{1L} -adrenoceptors. Three concentrations of prazosin were examined (30–300 nM) and the resulting Schild plot had a slope that was not significantly different to unity. Furthermore, even the highest concentration of prazosin did not affect the maximum response or Hill slope obtained to noradrenaline. Thus, prazosin appeared to be acting at a single receptor population, as a purely competitive antagonist, with a low affinity for the α_1 -adrenoceptor of human erectile tissue (i.e. α_{1L}).

This is supported by the effects of RS17053, which has a high affinity at cloned and native α_{1A} -adrenoceptors ($pK_D = 9.1–9.9$), but a low affinity at the α_{1L} -adrenoceptor ($pK_D = 7.3$).²² At a concentration of 30 nM, RS17053 failed to significantly affect responses to noradrenaline, and only at the higher concentration of 300 nM did it cause a small dextral shift of noradrenaline concentration–response curves, yielding a low affinity estimate of 6.9, similar to that previously reported at the α_{1L} -adrenoceptor.

The α_{1L} -adrenoceptor has been shown to originate from the α_{1A} -adrenoceptor gene,^{35,36} but is functionally and pharmacologically unique compared to the classical α_1 -adrenoceptor subtypes. α_{1L} -adrenoceptor pharmacology can be induced in cells expressing the α_{1A} -adrenoceptor subtype by manipulation of the experimental conditions, and it is possible that the α_{1L} -adrenoceptor represents an alternative affinity state of the α_{1A} -adrenoceptor.¹⁴ The proposed

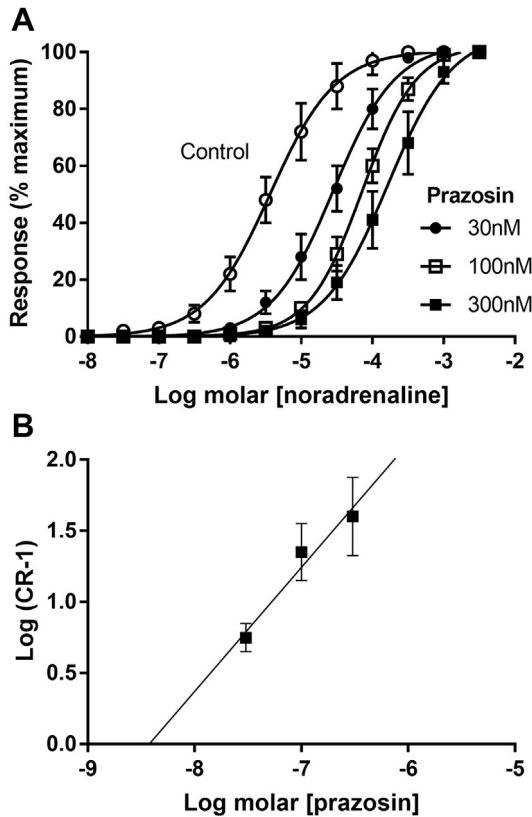


Fig. 3. (A) Mean concentration–response curves of human erectile tissue to noradrenaline in the absence and presence of prazosin (30–300 nM, $n = 4–5$). (B) Schild plot for the antagonism of responses to noradrenaline by prazosin.

molecular mechanisms underlying this change in phenotype remain controversial, but may involve receptor-interacting proteins.³⁷ In cell lines expressing α_{1A} -adrenoceptors, the α_{1A} -adrenoceptor phenotype was shown to be downregulated by the presence of CRELD1 α , a cysteine-rich epidermal growth factor-like protein, which interacts with the α_{1A} -adrenoceptor to alter binding profile.³⁸ α_{1A} -adrenoceptors have also been shown to form specific receptor heteromers with chemokine-receptors, which changes their pharmacological profile.³⁹

Since interacting proteins such as CRELD1 α would be expected to be removed during the normal homogenization of tissues for radioligand binding experiments, it has been proposed that in order to detect the α_{1L} -adrenoceptor phenotype via radioligand binding assays, whole cells or intact tissues segments must be used,⁴⁰ and that the α_{1L} -adrenoceptor phenotype is not demonstrable at the protein level using such a technique. However, this was not the case in the present study. Using [³H]tamsulosin, a radioligand with high affinity for the α_{1L} -adrenoceptor, we found that radioligand binding data supported our functional observations. [³H]tamsulosin bound to erectile tissue with high affinity (>10), as we have shown previously at the α_{1L} -adrenoceptor of the human prostate.²³ Whereas in the human prostate we identified two binding sites for prazosin, the high affinity site (α_{1A}) with an affinity (pK_i) of 10.3 and the low affinity site (α_{1L}) with an affinity (pK_i) of 8.9, in the present study in erectile tissue a single binding site was identified by prazosin, with an affinity (pK_i) of 8.9. This is identical to that of the low affinity site identified for prazosin in the human prostate using this radioligand and suggests a homogeneous population of α_{1L} -adrenoceptors in human erectile tissue.

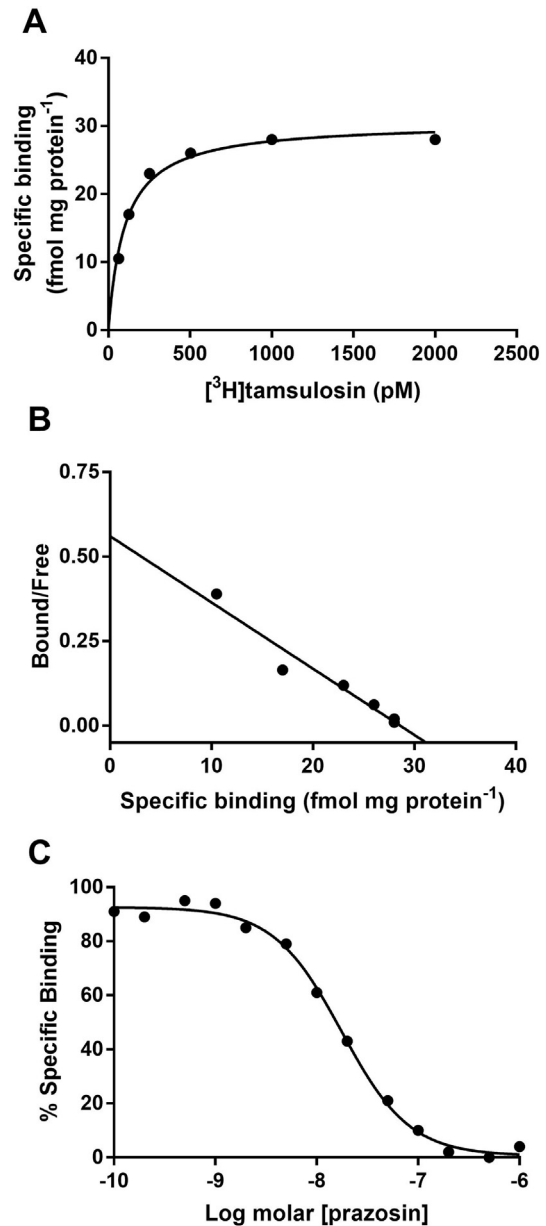


Fig. 4. (A) Representative saturation curve and (B) Scatchard plot for [³H]tamsulosin binding to membranes prepared from human erectile tissue. Experiments were performed in duplicate with tissues from 4 patients. (C) Competition binding curve for prazosin displacement of [³H]tamsulosin binding.

In conclusion, this study demonstrates the α_{1L} -adrenoceptor subtype at both the functional and protein level in human erectile tissue and confirms that erectile smooth muscle contraction is mediated via the α_{1L} -adrenoceptor. The α_{1L} -adrenoceptor is also found in the human prostate and is the target of current $\alpha_{1A/L}$ -adrenoceptor antagonists used to treat benign prostatic hyperplasia, and so this may explain the improvement in sexual function reported in patients receiving these agents. Since the α_{1L} -adrenoceptor is also predominant in the human vas deferens, any future drug development aimed at targeting α_{1L} -adrenoceptors for erectile dysfunction would need to consider the potential for anejaculatory effects, which vary between the current α_{1A} -adrenoceptor antagonists and may or may not be mediated via α_{1} -adrenoceptors.¹¹

Conflicts of interest

C.R.C. has received consultancy, research, and speaker fees from Allergan, Astellas, Medtronic, and Recordati (Milan, Italy); consultancy and speaker fees from Lilly (Indiana, USA); research and speaker fees from ONO (Osaka, Japan) and Pfizer, speaker fees from Ranbaxy (Haryana, India) and has received personal fees and nonfinancial support from Allergan and Pfizer, and grants, personal fees, and nonfinancial support from Astellas. All other authors declare no conflicts of interest.

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