α1L-adrenoceptors mediate contraction of human erectile tissue

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ABSTRACT

α1-adrenoceptor antagonists can impact upon sexual function and have potential in the treatment of erectile dysfunction. Human erectile tissue contains predominantly α1A-adrenoceptors, and here we examined whether contractions of this tissue are mediated by the functional phenotype, the α1L-adrenoceptor. Functional experiments using subtype selective agonists and antagonists, along with radioligand [3H]tamsulosin binding assays, were used to determine the α1-adrenoceptor population. A61603, a α1A-adrenoceptor agonist, was a full agonist with a potency 21-fold greater than that of noradrenaline. The α1A- and α1D-adrenoceptor antagonist tamsulosin antagonized noradrenaline responses with high affinity (pKᵦ = 9.7 ± 0.3), whilst BMY7378 (100 nM) (α1D-adrenoceptor antagonist) failed to antagonize responses. In contrast, relatively low affinity estimates were obtained for both prazosin (pKᵦ = 8.2 ± 0.1) and RS17053 (pKᵦ = 6.9 ± 0.2), antagonists which discriminate between the α1A- and α1L-adrenoceptors. [3H]Tamsulosin bound with high affinity to the receptors of human erectile tissue (pKᵦ = 10.3 ± 0.1) with a receptor density of 28.1 ± 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 (pKᵦ = 8.9). Taken together these data confirm that the receptor mediating contraction in human erectile tissue has the pharmacological properties of the α1L-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 α1-adrenoceptors, and leads to flaccidity and detumescence. Thus α1-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. Intracavernosal injection of α-adrenoceptor antagonists can relax erectile smooth muscle and induce an erection, and positive effects of oral treatment of erectile dysfunction with α1-adrenoceptor antagonists have been reported both experimentally and clinically.6,7 These agents can also have adverse effects on erectile dysfunction, with some of the newer generation α1-adrenoceptor antagonists such as silodosin, used in the treatment of benign prostatic hyperplasia, known to cause anejaculation.8 However, the incidence of anejaculation is variable between patients.10 Three α1-adrenoceptor subtypes (α1A, α1B and α1D) have been classified via functional and molecular techniques.12 There is also functional evidence for a fourth subtype, the α1L-adrenoceptor, also known as the α1A/L-adrenoceptor, which represents a functional phenotype of the α1A-adrenoceptor that is pharmacologically distinct and has a low affinity for prazosin.13–15 Human erectile tissue expresses mRNA coding for all three cloned α1-adrenoceptor subtypes, although it is the α1A-adrenoceptor subtype that predominates, with lower levels of α1D- and α1B-adrenoceptor mRNAs.16
A heterogenous α1A-adrenoceptor population has also been demonstrated in receptor binding experiments, but there is disagreement regarding which subtype predominates at the protein level. The α1A receptor has been reported as the major subtype, whilst Goepel et al. (1999) reported both α1A- and α1B-adrenoceptors at the protein level, with no evidence for the α1D-receptor. Functionally, a more recent study concluded that the α1A-adrenoceptor was the main subtype mediating contraction of the human cavernous. 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. In addition, the study did not examine the two antagonists that are able to discriminate between the α1A- and α1L-adrenoceptor isoforms, prazosin and RS17053,13,21,22 which we have previously used to identify that α1L-adrenoceptors mediate contraction elsewhere in the male lower urinary, specifically the human prostate and vas deferens. A small clinical trial of a selective α1A-adrenoceptor antagonist also failed to show any improvement in erectile dysfunction, leading to speculation that the α1L-adrenoceptor may be important functionally in human penile erectile tissue. Thus, the aim of this study was to determine whether the α1A- or the α1L-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 × 3 × 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 LabChart software (ADInstruments).

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 α1A-adrenoceptor selective agonist A61603 were also obtained. All experiments were performed in the presence of cocaine (10 μM) and corticosterone (10 μM) to prevent amine uptake and propranolol (1 μM) and yohimbine (0.5 μM) to antagonize β- and α2-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. 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 μl of membranes in a final volume of 250 μl. Phentolamine was used to displace the radioligand [3H]tamsulosin and determine non-specific binding, at a concentration of 10 μM, since this is 100–1000-fold greater than its dissociation constant at the α1-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% polyethyleneimine. 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 (20pM–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 ± 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.

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 pKd values (−log dissociation constant) were determined from individual shifts of concentration–response curves using the equation:

\[ \text{pKd} = \log(CR - 1) - \log[B] \]

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 Kd 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.
3.2. Effect of antagonists on noradrenaline contractions

ceptor in human erectile tissue, with apparent pKD values of (\(\text{[phenoxy-3H]}\)-YM617, specific activity 1117.4 GBq mol\(^{-1}\)) was obtained from NEN (Boston, MA). All reagents were Analar grade.

3.3. Radioligand binding experiments

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

4. Discussion

The role of adrenoceptors in human penile erection is still not fully understood. While stimulation of postsynaptic \(\alpha_1\)-adrenoceptors is known to mediate contraction of the erectile smooth muscle, leading to faciidity and detumescence,\textsuperscript{1} there is still controversy regarding the predominant \(\alpha_1\)-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 \(\alpha_{1L}\)-adrenoceptor subtype is predominant in human erectile tissue, as we have shown previously for the human prostate and vas deferens.\textsuperscript{2,3,4}

A61603, an agonist that is highly selective for \(\alpha_{1A}\)-adrenoceptors,\textsuperscript{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 \(\alpha_{1A}\)-adrenoceptor subtype in human erectile tissue. Tamsulosin is a competitive antagonist with a very high affinity (>10) at \(\alpha_{1A}\) and \(\alpha_{1D}\)-adrenoceptors.\textsuperscript{2,4} 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 pKD of 9.7) is consistent with the involvement of \(\alpha_{1A}\) and/or \(\alpha_{1D}\)-adrenoceptors, and not \(\alpha_{1L}\)-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 \(\alpha_{1A}\)-adrenoceptors of the human prostate.\textsuperscript{2,4} 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 prostate\textsuperscript{2,4} and human vas deferens.\textsuperscript{2} The fact that tamsulosin is a competitive antagonist with a very high affinity (>10) at \(\alpha_{1A}\) and \(\alpha_{1D}\)-adrenoceptors,\textsuperscript{2,4} and may bind irreversibly to these receptors, may explain this unsurmountable action.
The involvement of $\alpha_{1A}$- or $\alpha_{1D}$-adrenoceptors was further supported by the relatively high affinity estimate ($pKD = 8.1$) obtained for the antagonist 5-methylurapidil. This antagonist has a lower affinity at $\alpha_{1B}$-adrenoceptors ($pKD = 7.2$), and thus the affinity estimate obtained in the present study is higher than would be expected if $\alpha_{1B}$-adrenoceptors were mediating the contractions to noradrenaline in the human erectile tissue. An affinity of 7.8 has been reported for 5-methylurapidil at $\alpha_{1D}$-adrenoceptors. To rule out the involvement of $\alpha_{1D}$-adrenoceptors, experiments were performed in the presence of BMY7378, which has a high affinity for $\alpha_{1D}$-adrenoceptors ($pKi = 8.2$) and is 100-fold selective for this subtype over the $\alpha_{1B}$- and $\alpha_{1A}$-receptor subtypes. If the $\alpha_{1D}$-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 $\alpha_{1D}$-adrenoceptors are not involved in smooth muscle contraction in this tissue.

These results indicate that responses of human erectile tissue are mediated via $\alpha_{1A}$-adrenoceptors. However, $\alpha_{1A}$-adrenoceptors can exist as the distinct functional phenotype $\alpha_{1L}$-adrenoceptor, and many $\alpha_{1A}$-selective antagonists, including tamsulosin and 5-methylurapidil, have a high affinity for $\alpha_{1A}$-adrenoceptors in addition to $\alpha_{1A}$-adrenoceptors. Prazosin and RS17053 are two antagonists that can distinguish between $\alpha_{1A}$ and $\alpha_{1L}$-adrenoceptors. Prazosin has a high affinity at all cloned $\alpha_{1A}$-adrenoceptors ($pK_D > 9.5$), but a low affinity ($pK_D < 9$) at the $\alpha_{1L}$-adrenoceptor. 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 $\alpha_1$-adrenoceptors, and instead suggesting the involvement of $\alpha_{1L}$-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 $\alpha_{1L}$-adrenoceptor of human erectile tissue (i.e. $\alpha_{1L}$).

This is supported by the effects of RS17053, which has a high affinity at cloned and native $\alpha_{1A}$-adrenoceptors ($pK_D = 9.1–9.9$), but a low affinity at the $\alpha_{1L}$-adrenoceptor ($pK_D = 7.3$). 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 $\alpha_{1L}$-adrenoceptor.

The $\alpha_{1L}$-adrenoceptor has been shown to originate from the $\alpha_{1A}$-adrenoceptor gene, but is functionally and pharmacologically unique compared to the classical $\alpha_1$-adrenoceptor subtypes. $\alpha_{1L}$-adrenoceptor pharmacology can be induced in cells expressing the $\alpha_{1A}$-adrenoceptor subtype by manipulation of the experimental conditions, and it is possible that the $\alpha_{1L}$-adrenoceptor represents an alternative affinity state of the $\alpha_{1A}$-adrenoceptor. The proposed
molecular mechanisms underlying this change in phenotype remain controversial, but may involve receptor-interacting proteins. In cell lines expressing α₁A-adrenoceptors, the α₁A-adrenoceptor phenotype was shown to be downregulated by the presence of CRELD1, a cysteine-rich epidermal growth factor-like protein, which interacts with the α₁A-adrenoceptor to alter binding profile. α₁A-adrenoceptors have also been shown to form specific receptor heteromers with chemokine-receptors, which changes their pharmacological profile.

Since interacting proteins such as CRELD1 might 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 α₁L-adrenoceptor phenotype via radioligand binding assays, whole cells or intact tissues segments must be used, and that the α₁L-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 α₁L-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 α₁L-adrenoceptor of the human prostate. Whereas in the human prostate we identified two binding sites for prazosin, the high affinity site (α₁A) with an affinity (pKᵦ) of 10.3 and the low affinity site (α₁L) with an affinity (pKᵦ) of 8.9, in the present study in erectile tissue a single binding site was identified by prazosin, with an affinity (pKᵦ) 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 α₁L-adrenoceptors in human erectile tissue.

In conclusion, this study demonstrates the α₁L-adrenoceptor subtype at both the functional and protein level in human erectile tissue and confirms that erectile smooth muscle contraction is mediated via the α₁L-adrenoceptor. The α₁L-adrenoceptor is also found in the human prostate and is the target of current α₁A/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 α₁L-adrenoceptor is also predominant in the human vas deferens, any future drug development aimed at targeting α₁L-adrenoceptors for erectile dysfunction would need to consider the potential for anejaculatory effects, which vary between the current α₁A/L-adrenoceptor antagonists and may or may not be mediated via α₁-adrenoceptors.
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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