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In vitro α_1-adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel α_{1A}-adrenoceptor selective antagonists

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1 It has been hypothesized that in patients with benign prostatic hyperplasia, selective antagonism of the α_{1A}-adrenoceptor-mediated contraction of lower urinary tract tissues may, *via* a selective relief of outlet obstruction, lead to an improvement in symptoms.

2 The present study describes the α_1-adrenoceptor (α_1-AR) subtype selectivities of two novel α_1-AR antagonists, Ro 70-0004 (aka RS-100975) and a structurally-related compound RS-100329, and compares them with those of prazosin and tamsulosin. Radioligand binding and second-messenger studies in intact CHO-K1 cells expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-AR showed nanomolar affinity and significant α_{1A}-AR subtype selectivity for both Ro 70-0004 (pK_i 8.9: 60 and 50 fold selectivity) and RS-100329 (pK_i 9.6: 126 and 50 fold selectivity) over the α_{1B}- and α_{1D}-AR subtypes respectively. In contrast, prazosin and tamsulosin showed little subtype selectivity.

3 Noradrenaline-induced contractions of human lower urinary tract (LUT) tissues or rabbit bladder neck were competitively antagonized by Ro 70-0004 (pA_2 8.8 and 8.9), RS-100329 (pA_2 9.2 and 9.2), tamsulosin (pA_2 10.4 and 9.8) and prazosin (pA_2 8.7 and 8.3 respectively). Affinity estimates for tamsulosin and prazosin in antagonizing α_1-AR-mediated contractions of human renal artery (HRA) and rat aorta (RA) were similar to those observed in LUT tissues, whereas Ro 70-0004 and RS-100329 were approximately 100 fold less potent (pA_2 values of 6.8/6.8 and 7.3/7.9 in HRA/RA respectively).

4 The α_{1A}-AR subtype selectivity of Ro 70-0004 and RS-100329, demonstrated in both cloned and native systems, should allow for an evaluation of the clinical utility of a 'uroselective' agent for the treatment of symptoms associated with benign prostatic hyperplasia.

Keywords: α_1-adrenoceptors; Ro 70-0004; RS-100329; prazosin; tamsulosin; prostate; rat aorta; human renal artery; radioligand binding; inositol phosphates

Abbreviations: AR, adrenoceptors; BPH, benign prostatic hyperplasia; CHO, Chinese hamster ovary; HRA, human renal artery; LUT, lower urinary tract; NA, noradrenaline; RA, rat aorta; RBN, rabbit bladder neck

Introduction

Molecular and pharmacological studies have led to the division of α_1-adrenoceptors into three subtypes: α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (see Heible *et al*., 1995). An alternative subdivision has been proposed (Flavahan & VanHoutte, 1986; Muramatsu, 1992) based on α_1-adrenoceptor affinities for prazosin in functional studies: i.e. α_{1H}-adrenoceptors (high, subnanomolar affinity for prazosin; α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors) and α_{1L}-adrenoceptors (low, suprananomolar affinity for prazosin). Other antagonists, including RS-17053 (Ford *et al*., 1996) and WB4101, are also reported to distinguish between the 'classical' α_{1A}-adrenoceptor and the α_{1L}-adrenoceptor, displaying significantly lower affinity at the latter. However, the proposal of further subtypes has yet to be supported by molecular biological evidence. Indeed, it has been suggested that existing cloned subtypes may fully account for observations which led to the α_{1H} versus α_{1L} subdivision, in as much as cells expressing the human cloned α_{1A}-adrenoceptor have been shown to exhibit an α_{1L}-like pharmacology (see Ford *et al*., 1994; 1997; Williams *et al*., 1996).

Following earlier studies with the alkylating agent phenoxybenzamine (Caine *et al*., 1978), competitive α_1-adrenoceptor antagonists such as terazosin (Lepor, 1995), prazosin (Hedlund *et al*., 1983; Chapple *et al*., 1992) and alfuzosin (Buzelin *et al*., 1993) have been shown to be effective in relieving urinary outflow obstruction and reducing symptom scores in patients with benign prostatic hyperplasia (BPH). However, the usefulness of α_1-adrenoceptor antagonists in BPH is offset by their dose-limiting cardiovascular effects, including postural hypotension, particularly with initial dosing. The discovery and definition of α_1-adrenoceptor subtypes offers the potential for more selective agents. Recently, interest has focused on the role of the α_{1A}-adrenoceptor subtype in BPH, as a result of studies demonstrating that this subtype predominates in the urethra and prostate of man (Price *et al*., 1993; Faure *et al*., 1994; Taniguchi *et al*., 1997), and has been claimed to be the receptor mediating noradrenaline (NA) induced smooth muscle contraction in these tissues (Forray *et al*., 1994; Hatano *et al*., 1994; Marshall *et al*., 1995). The resulting tone is believed to contribute substantially to the urinary outflow obstruction observed in patients with BPH (Furuya *et al*., 1982), with the remaining obstruction being attributable to increased prostate mass. These observations have fuelled the hypothesis – yet to be supported by clinical data – that an α_{1A}-

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subtype-selective antagonist may, *via* a selective and significant decrease in outlet resistance, lead to improved pharmacotherapy for BPH. Tamsulosin, recently approved for the treatment of BPH, is claimed to be 'uroselective' based on a degree of α_{1A}-subtype-selectivity, but a recent report by Blue *et al.* (1997a), assessing the *in vitro* and *in vivo* pharmacology of this compound, does not support this proposal.

In this study we report the *in vitro* α_1-adrenoceptor affinity profiles of two novel α_{1A}-adrenoceptor selective antagonists, Ro 70-0004 (RS-100975; 3-(3-{4-[Fluoro-2-(2,2,2-trifluoroethoxy)-phenyl]-piperazin-1-yl}-propyl)-5-methyl-1H-pyrimidine-2,4-dione mono hydrochloride monohydrate, Figure 1) and a structurally-related compound, RS-100329 (Figure 1), in comparison with those of prazosin and tamsulosin.

Methods

Cell culture

Chinese hamster ovary cells (CHO-K1) expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (see Ford *et al.*, 1997) were cultured to confluence in tissue culture flasks (T-162) containing Ham's F-12 nutrient medium supplemented with 10% foetal bovine serum (FBS), geneticin (150 μg ml^{-1}) and penicillin/streptomycin (30 u ml^{-1}, 30 μg ml^{-1}) at 37°C in 7% CO_2.

Radioligand binding studies

Affinity estimates (pK_i) were made from competition curves (using ten concentrations of displacing agent) using intact CHO-K1 cells stably expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors. Cells were grown as described above and harvested by incubating with Dulbecco's phosphate buffered saline (PBS) containing EDTA (30 μM) for 10 min at 37°C. Harvested cells were washed twice by centrifugation and resuspension in Ham's medium, and finally resuspended in Ham's medium at approximately 0.2×10^6 cells ml^{-1}.

[^{3}H]-Prazosin (0.3 – 0.4 nM; specific activity 82 Ci mmole^{-1}) was used as the radioligand and specific binding was defined using 10 μM phentolamine. Assay tubes contained 100 μl competing compound, 100 μl [^{3}H]-prazosin and 300 μl cell suspension. All equilibrations were carried out for 30 min at 37°C in Ham's culture medium (pH 7.4), and were terminated by vacuum filtration through glass fibre filters. Bound radioactivity was determined using liquid scintillation spectroscopy. Concentrations of competing agent producing 50% reduction of specific [^{3}H]-prazosin binding (IC_{50}) were calculated using non-linear iterative curve-fitting methodologies (Kaleidagraph, Synergy Software), and affinity estimates (pK_i) were estimated according to Cheng & Prusoff (1973). Estimates of pK_D were made by saturation analysis using 10 – 12 concentrations of [^{3}H]-prazosin (1 pM – 3 nM).

[^{3}H]-Inositol phosphates accumulation

The method used was a modification of well-established procedures (Brown *et al.*, 1984). Cells were washed with PBS and incubated overnight in 15 ml inositol-free Ham's F-12 medium containing 10% dialyzed FBS and 25 μCi [^{3}H]-*myo*-inositol. Following this, the medium was aspirated and the cells washed with PBS to remove unincorporated [^{3}H]-*myo*-inositol. PBS containing EDTA (30 μM for 5 – 10 min at 37°C) was used to dissociate cells from the flask. The cells were washed three times by centrifugation (5 min at $500 \times g$, 37°C) and resuspension in PBS and were finally resuspended in inositol-free Ham's medium at approximately 5×10^6 cells ml^{-1}.

Reactions were performed in triplicate with a final reaction volume of 300 μl. Two-hundred and forty microlitres of cell suspension was added to 30 μl antagonist or vehicle and incubated at 37°C for 20 min. The reaction was initiated by the addition of 30 μl agonist or vehicle, containing LiCl (final concentration 10 mM). Tubes were then gently mixed and placed in a 37°C bath for 10 min. Reactions were terminated by the addition of 50 μl ice-cold perchloric acid (20%), and the tubes placed on ice for 20 min. Samples were then neutralized with 160 μl 1 M KOH, vortex-mixed and centrifuged at $1000 \times g$ at 4°C for 10 min. Samples were then gently diluted with the addition of 2 ml Tris-HCl (50 mM, pH 7.5) and decanted onto disposable columns containing 1 ml Dowex AG 1X8, chloride form (1 : 1, w v^{-1}) slurry which had been washed with 5 ml distilled H_2O. Columns were then washed with 20 ml distilled H_2O and the eluate discarded. [^{3}H]-Inositol phosphates ([^{3}H]-InsPs) were eluted with 3 ml HCl (1 M) into scintillation vials containing 15 ml Ready-Safe liquid scintillation cocktail. Accumulated [^{3}H]-InsPs were measured by liquid scintillation spectroscopy. Iterative nonlinear curve-fitting methods using Kaleidagraph (Synergy Software) were used to fit data to the general logistic functions in order to determine EC_{50} or IC_{50} values, maxima and Hill slopes for each curve. Affinity values of test substances (pK_B were calculated according to Leff & Dougall (1993), such that $K_B = IC_{50}/((2 + ([A]/EC_{50})^{n_H})^{1/n_H} - 1)$.

In vitro *tissue bath studies*

Tissue bath studies were conducted at 37°C in 10 ml organ baths and used Krebs' buffer (mM: Na^+ 143.5, K^+ 6.0, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 126, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2), pH 7.4, gassed with 95% O_2, 5% CO_2, and supplemented with cocaine (30 μM), corticosterone (30 μM), propranolol (1 μM), idazoxan (0.3 μM), ascorbate (100 μM) and nitrendipine (1 μM: rat aorta only, see Blue *et al.*, 1995) to ensure equilibrium conditions and α_1-adrenoceptor isolation.

Human lower urinary tract tissues (LUT) were obtained from patients undergoing transurethral resection of the prostate (TURP). Samples were kept in cold Krebs' solution until use in functional studies, which were performed later the same day or early the following day. Human renal artery sections (3 mm × 6 mm) were obtained from a transplant

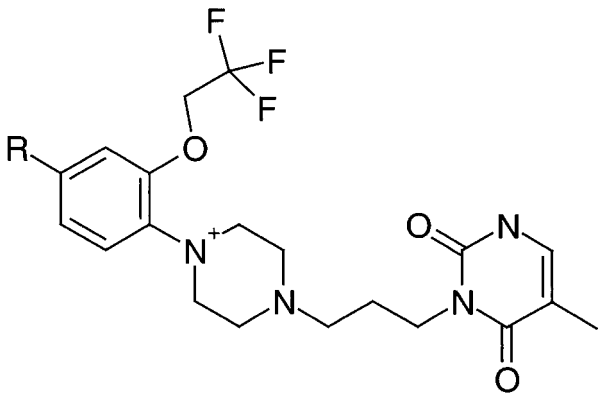

Figure 1 Chemical structures of Ro 70-0004 (RS-100975: R = F) and RS-100329 (R = H).

donor bank, and were usually received within 24–36 h of removal. Rabbit bladder neck strips (2 mm × 6 mm) were from male New Zealand White rabbits (2.5–3.5 kg) and rat aortic rings (2 mm width) were from male Sprague-Dawley rats (350–500 g), both euthanized with carbon dioxide. Vascular tissues were endothelium-denuded prior to study.

Tissues were mounted under 0.5 g (human LUT, renal artery) or 1.0 g (rabbit bladder neck, rat aorta) resting tension and allowed to equilibrate for at least 1 h. Cumulative concentration-response curves to NA were constructed in the absence and presence (following a 1 h equilibration) of various concentrations of antagonist. Responses were measured as changes in isometric tension. Iterative curve fitting (Kaleidagraph, Synergy Software) was used to determine EC_{50} values for the agonist in the absence and presence of various concentrations of each antagonist. Schild plots (Arunlakshana & Schild, 1959) were constructed in order to estimate antagonist affinity estimates.

Materials

Ham's F-12 nutrient media, Dulbecco's phosphate buffered saline, geneticin (G418), foetal bovine serum (qualified and dialyzed), penicillin/streptomycin and versene (EDTA) were obtained from Gibco (Gaithersburg, MD, U.S.A.). *Myo*-[2-^{3}H]-Inositol (10–20 Ci mmol^{-1}) and [^{3}H]-prazosin (82 Ci mmol^{-1}) were obtained from Amersham (Arlington Heights, IL, U.S.A.). Disposable plastic columns (CC-09-M) were obtained from E & K Scientific Products (Saratoga, CA, U.S.A.). Prazosin was obtained from Research Biochemicals International (Natick, MA, U.S.A.). Acidic alumina (activity grade 1, Brockmann) was purchased from ICN Biomedicals GmbH (Eschwege, Geramany). NA and bulk chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). All other compounds were synthesized in the Department of Medicinal Chemistry, Neurobiology Business Unit, Roche Bioscience (Palo Alto, CA, U.S.A.). Ready-Safe liquid scintillation cocktail was purchased from Baxter Scientific (McGraw Park, IL, U.S.A.). Chinese hamster ovary cells (CHO-K1) expressing human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors were prepared by Dr David Chang, Center for Biological Research, Neurobiology Business Unit, Roche Bioscience (Palo Alto, CA, U.S.A.).

Results

Radioligand binding studies

Saturation studies demonstrated high-affinity specific (>90%) binding of [^{3}H]-prazosin to whole CHO-K1 cells expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, yielding affinity estimates (pK_D, mean±s.e.mean, $n \geqslant 3$) of 9.12±0.07, 10.08±0.10 and 9.51±0.11, respectively. Estimated B_{max} values were 1.64±0.20, 1.22±0.13 and 1.17±0.05 pmoles mg protein^{-1} respectively.

In competition studies Ro 70-0004, RS-100329, prazosin and tamsulosin all competed to the same extent for [^{3}H]-prazosin binding: calculated affinity estimates (pK_i) are shown in Table 1. Prazosin and tamsulosin demonstrated limited α_1-AR subtype selectivity (<10 fold), whereas significant selectivities for the α_{1A}- over the α_{1B}- and α_{1D}-AR subtypes were evident for Ro 70-0004 (60 and 50 fold respectively) and RS-100329 (126 and 50 fold respectively).

Radioligand binding studies were also used to determine the affinity of Ro 70-0004 at more than 30 other neurotransmitter receptors (data not shown). Those receptors where the affinity (pK_i) of Ro 70-0004 was found to be 7.0 or greater were: α_{2B}-adrenoceptors (pK_i 7.0: rat kidney), human recombinant D_{2L} receptors (pK_i 7.2), human recombinant D_3 receptors (pK_i 7.7) and 5-HT_{2A} receptors (pK_i 7.4: rat cortex).

[^{3}H]-Inositol phosphates accumulation

Accumulation of [^{3}H]-inositol phosphates in CHO-K1 cells expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors was stimulated by NA (approximately 10, 4 and 4 fold over basal) in a concentration-dependent manner, with respective pEC_{50} values of 6.6±0.1, 6.5±0.1 and 7.7±0.1 (mean±s.e.mean, $n \geqslant 3$). Pre-incubation of cells with test antagonists inhibited the stimulation by NA (2 μM for α_{1A}-, α_{1B}-adrenoceptors, 0.2 μM for α_{1D}-adrenoceptors) in a concentration-dependent manner. Resultant affinity estimates (pK_B) are shown in Table 1. Note that for tamsulosin, a NA concentration of 100 μM was used to stimulate inositol phosphates accumulation (α_{1A}-adrenoceptors only). The rationale for this is discussed in Ford *et al*. (1997): briefly, a higher concentration of agonist was required in order to compensate for the significant loss of tamsulosin which occurred at low concentrations of the antagonist. Under these conditions none of the antagonists tested produced inhibition curves with slopes significantly different from unity.

Prazosin showed some selectivity for the α_{1B}- and α_{1D}-AR subtypes over the α_{1A}-AR subtype, whereas tamsulosin showed approximately 10 fold selectivity for the α_{1A}-AR subtype over the α_{1B}-, but not the α_{1D}-AR subtype. Ro 70-0004 (Figure 2a) was 30 and 80 fold selective for the α_{1A}-subtype over the α_{1B}- and α_{1D}-subtypes respectively, and RS-100329 (Figure 2b) also showed significant α_{1A}-adrenoceptor selectivity (60 and 50 fold respectively).

In vitro *tissue bath studies*

In all tissues assayed, NA caused concentration-dependent contractions which were well maintained, allowing construction of cumulative concentration-response curves. Affinity

Table 1 Antagonist affinity estimates at human cloned expressed α_1-AR subtypes

	α_{1A}-AR		α_{1B}-AR		α_{1D}-AR	
Antagonist	pK_i	pK_B	pK_i	pK_B	pK_i	pK_B
Ro 70-0004	8.9±0.1	8.6±0.1	7.1±0.1	6.7±0.1	7.2±0.1	7.1±0.1
RS-100329	9.6±0.1	9.6±0.1	7.5±0.1	7.8±0.2	7.9±0.1	7.9±0.1
Prazosin	9.0±0.1	8.7±0.1	9.9±0.1	9.6±0.1	9.5±0.1	9.6±0.3
Tamsulosin	10.0±0.1	10.5±0.1	9.7±0.1	9.4±0.2	9.8±0.1	9.8±0.3

Affinity estimates (mean±s.e.mean, $n \geqslant 3$) for antagonist inhibition of [^{3}H]-prazosin binding (pK_i) or NA-stimulated [^{3}H]-inositol phosphates accumulation (pK_B) in intact CHO-K1 cells expressing human cloned α_{1A}-, α_{1B}- and α_{1D}-AR subtypes.

estimates calculated for the four antagonists studied are shown in Table 2.

In human LUT tissues, contractions to NA were antagonized in a surmountable and concentration-dependent manner by Ro 70-0004, RS-100329 and prazosin with similar affinity ($pA_2 \sim 9$): tamsulosin was approximately 10 fold more potent (Table 2). Figure 3 shows the antagonism of the prostatic smooth muscle response by various concentrations of Ro 70-0004 and the resultant Schild plot. The data show a small decrease in the maximum response obtained to NA in the presence of Ro 70-0004: however, the unity slope of the Schild plot is consistent with competitive antagonism at an homogeneous population of receptors. Affinity estimates from rabbit bladder neck studies were similar to those obtained using human lower urinary tract tissues (Table 2).

In contrast, both Ro 70-0004 and RS-100329 were approximately 100 fold weaker in antagonizing the contractile response to NA in human renal artery. The affinities of prazosin and tamsulosin did not differ from those observed in studies using human LUT tissues (Table 2). In studies using rat

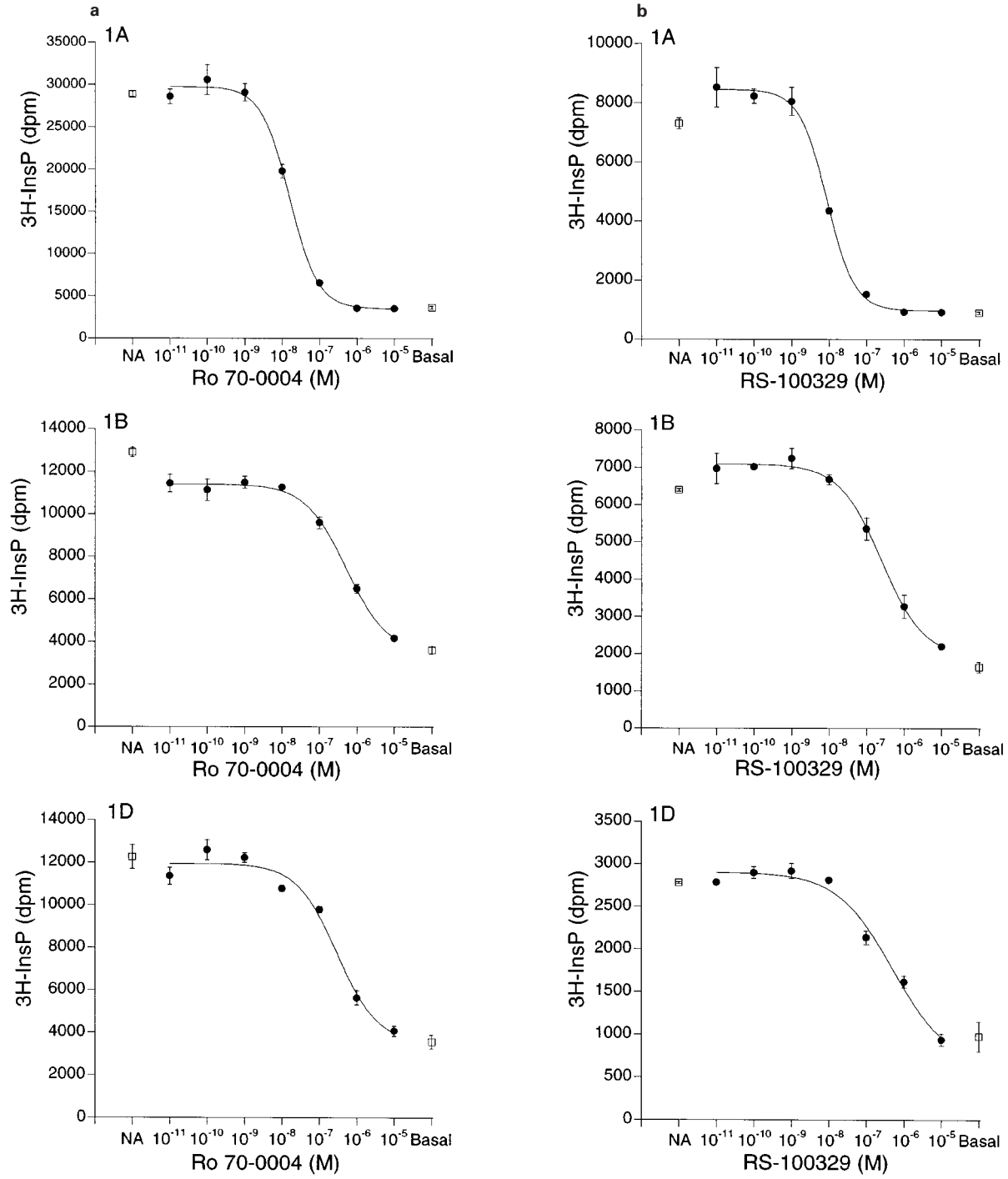

Figure 2 Antagonism by (a) Ro 70-0004 and (b) RS-100329 of NA-stimulated [^{3}H]-inositol phosphates accumulation in CHO-K1 cells expressing human recombinant α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes. Cells were stimulated with either 2 μM (α_{1A}- and α_{1B}-AR subtypes) or 0.2 μM (α_{1D}-AR) NA ('NA'). 'Basal': no NA added. Data are means$\pm$s.e.mean of triplicate determinations in single representative experiments.

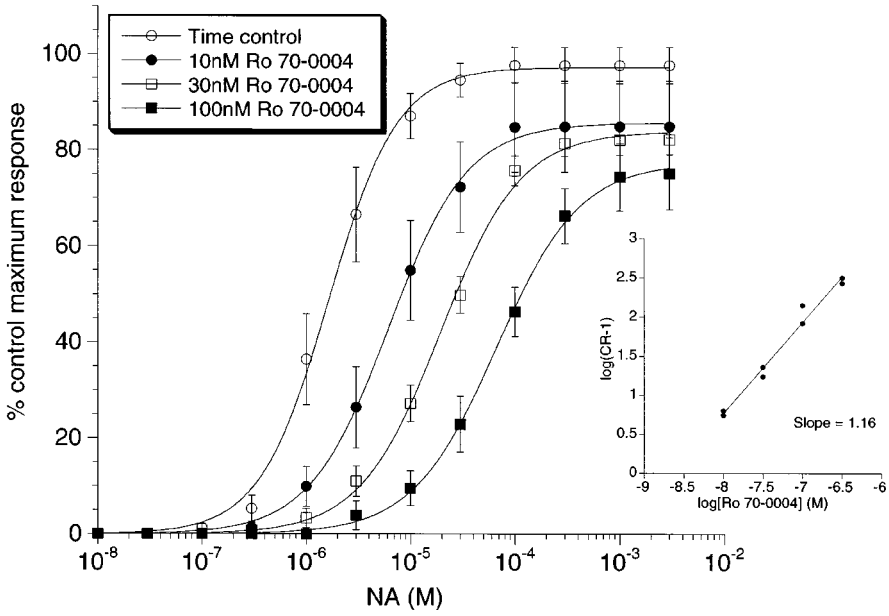

Figure 3 Antagonism of NA-induced contractions of human isolated lower urinary tract tissues *in vitro* by various concentrations of Ro 70-0004. Inset: resultant Schild plot. Data are means ± s.e.mean of at least six determinations.

Table 2 Affinity estimates for antagonist inhibition of *in vitro* NA-stimulated contractions of isolated smooth muscle preparations

Antagonist	*Human LUT (α_{1A})*	*Human renal artery ($\alpha_{1?}$)*	*RBN (α_{1A})*	*Rat aorta (α_{1D})*
Ro 70-0004	8.8 ± 0.1	6.8 ± 0.1	8.9 ± 0.1	6.8 ± 0.1
RS-100329	9.2 ± 0.9	7.3 ± 0.2	9.2 ± 0.1	7.9 ± 0.2
Prazosin	8.7 ± 0.1	8.7 ± 0.1	8.3 ± 0.1	9.6 ± 0.1
Tamsulosin	10.4 ± 0.1	10.5 ± 0.1	9.8 ± 0.1	9.7 ± 0.2

Affinity estimates (pA_2, mean ± s.e.mean, $n \geqslant 3$) for antagonist inhibition of *in vitro* NA-stimulated contractions of human lower urinary tract (LUT) tissues, human renal artery, rabbit bladder neck (RBN) and rat aortic rings.

aortic rings, Ro 70-0004, RS-100329 and tamsulosin antagonized contractile responses to NA with affinities close to those observed for human renal artery. Prazosin however, was approximately 10 fold more potent in rat aorta when compared to human renal artery.

Discussion

The present study has compared the α_1-AR subtype affinities of two novel subtype-selective antagonists, Ro 70-0004 and RS-100329, with those of prazosin and tamsulosin, two compounds currently used in the symptomatic treatment of BPH. In addition to the use of human recombinant α_1-adrenoceptor subtypes, studies were also performed on native α_1-adrenoceptors in LUT and vascular tissues of animals and man.

In CHO-K1 cells expressing the human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes, both Ro 70-0004 and RS-100329 showed high affinity and clear selectivity (in excess of 50 fold) for the α_{1A}-subtype over the α_{1B}- and α_{1D}-subtypes in intact cell radioligand binding studies using [^{3}H]-prazosin. Functional studies (antagonism of NA-stimulated [^{3}H]-inositol phosphates accumulation) in the same cells produced similar results. In binding studies tamsulosin showed no subtype selectivity, whereas in functional studies the compound was slightly (approximately 10 fold) selective for the α_{1A}-subtype over the α_{1B}- but not the α_{1D}-adrenoceptor subtype. In both binding and functional studies prazosin showed lower, suprananomolar affinity at the α_{1A}-adrenoceptor than at the other two subtypes (reminiscent of the so-called 'α_{1L}-adrenoceptor'). In a recent study Williams *et al.* (1996) reported that in radioligand binding studies performed under suitable assay conditions (whole cells, 37°C), the pharmacological profile of the human recombinant α_{1A}-adrenoceptor expressed in CHO-K1 cells more closely resembled that of the proposed 'α_{1L}'-adrenoceptor (see Ford *et al.*, 1997) than that found in membrane homogenates (at 20°C). The latter resembled the classically-defined α_{1A}-adrenoceptor. Specifically, with whole cells at 37°C some antagonists, including prazosin, WB4101 and RS-17053 but not tamsulosin or indoramin showed significantly lower affinities than those measured in membranes and also reported in 'classical' α_{1A}-adrenoceptor preparations. This 'α_{IL}-like' pharmacological profile for the recombinant receptor is not confined to binding studies, since it is also observed in functional studies measuring antagonism of NA-induced [^{3}H]-inositol phosphates accumulation (Ford *et al.*, 1997), [^{3}H]-cAMP accumulation (Gever *et al.*, 1997) or intracellular Ca^{2+} elevation (unpublished observations) in the same cells. Although the 'classical' α_{1A}-adrenoceptor was first described in rat tissues (Morrow & Creese, 1986), studies have shown that recombinant α_{1A}-adrenoceptors from rat, rabbit and man exhibit similar pharmacological profiles ('α_{IL}-like') in functional studies (Daniels *et al.*, 1996), so species variation does not seem to account for α_{1A}/α_{IL}-AR differences. The mechanisms underlying the different pharmacological profiles exhibited by the α_{1A}-adrenoceptor are yet to be determined. Although the possibility remains that further α_1-adrenoceptor subtypes may be cloned, to date there is no compelling evidence to suggest that a novel subtype is required to account for the pharmacological observations of the α_{1L}-AR.

Notwithstanding the above, one of the proposed therapeutic advantages of an α_{1A}-adrenoceptor-selective antagonist is a selective action on lower urinary tract tissues and minimal cardiovascular side effects. In accordance with their high affinities at recombinant α_{1A}-adrenoceptors, both Ro 70-0004 and RS-100329 showed nanomolar affinities in functional studies assessing their ability to antagonize NA-induced contractions of human lower urinary tract tissues and rabbit bladder neck *in vitro*. The latter tissue contracts in response to α_1-adrenoceptor agonism *via* a receptor with a pharmacological profile which correlates closely with that observed in human LUT tissues (Kava *et al*., 1998). In contrast to their affinities in LUT tissues, Ro 70-0004 and RS-100329 (but not prazosin or tamsulosin) were found to be significantly less potent when tested as antagonists of NA-induced contractions in human renal artery or rat aorta. The α_1-adrenoceptor subtype(s) mediating contraction of human renal artery to NA are not yet determined, but those mediating contraction of rat aorta appear to be predominantly of the α_{1D}-subtype (Buckner *et al*., 1996; Saussy *et al*., 1996; but see also Van der Graaf *et al*., 1996). The results of the present study would support this hypothesis: affinities from functional studies using rat aorta closely match those from binding and functional studies using the cloned expressed α_{1D}-adrenoceptor.

Clearly, pharmacological data from *in vitro* studies using isolated vascular tissues cannot necessarily be extrapolated to exclude significant *in vivo* cardiovascular effects of α_{1A}-adrenoceptor-selective antagonists. The extent to which larger conductance vessels, rather than smaller arterioles, contribute to total peripheral resistance is thought to be minor. At least in rat, *in vitro* pressor responses to α_1-adrenoceptor agonists in perfused mesentery (Williams & Clarke, 1995) and perfused kidney (Blue *et al*., 1995) both seem to be mediated by α_{1A}-adrenoceptors. To address this question Blue *et al*. (1997b) have described a 'reflex-compromised' model for the assessment of the hypotensive potential of α_1-adrenoceptor antagonists in conscious rats. In this model, rats are pretreated with a β_1-adrenoceptor antagonist, an AT_1 angiotensin receptor antagonist, and a non-subtype-selective α_2-adrenoceptor antagonist in order to maximize the hypotensive potential of α_1-adrenoceptor antagonism, by blunting compensatory cardiovascular reflexes. In this model, Ro 70-0004 was observed to be approximately 200 fold less potent than prazosin at lowering resting blood pressure (Blue *et al*., 1997b). In addition, Ro 70-0004 was found to be approximately 100 fold less potent than prazosin at producing postural hypotension during head-up tilt (Blue *et al*., 1996). Similar results were seen in tilt studies using conscious dogs. Other *in vivo* studies with Ro 70-0004 also support a selective action on LUT tissues. Blue *et al*. (1996) report that in anaesthetized mongrel dogs, Ro 70-0004 was 76 fold more potent at inhibiting hypogastric nerve stimulation-induced rises in intraurethral pressure versus phenylephrine-induced rises in diastolic blood pressure. In the same study, neither prazosin nor tamsulosin – despite its claimed α_{1A}-selectivity – distinguished between urethral and diastolic blood pressure responses. Similar results with tamsulosin have also been observed by other authors (Kenny *et al*., 1996). *In vivo* studies performed using RS-100329 in rats and dogs (unpublished data) confirmed selectivity properties similar to those of Ro 70-0004, but with greater potency, commensurate with its higher affinity for the α_{1A}-AR. In studies with Rec 15/2739 (SB 216469), which is also selective for the α_{1A}-subtype, the compound was also reported to be 'uroselective' in dogs (Testa *et al*., 1997). Thus, there is significant data supporting the hypothesis that *in vitro* selectivity for the α_{1A}-adrenoceptor can translate into a selective action on LUT tissues *in vivo*.

In summary, the present study has shown Ro 70-0004, and a structurally-related compound RS-100329, to be high affinity antagonists at the α_{1A}-adrenoceptor, with considerable selectivity over the α_{1B}- and α_{1D}-subtypes. The compounds potently inhibit *in vitro* and *in vivo* α_1-adrenoceptor-mediated contractions of lower urinary tract tissues and appear to exhibit only weak cardiovascular effects when compared to standard non-subtype-selective α_1-adrenoceptor antagonists. The clinical utility of Ro 70-0004 in the symptomatic treatment of BPH is currently being assessed.

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