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ACCELERATED COMMUNICATION

RS-17053 (*N*-[2-(2-Cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), a Selective α_{1A}-Adrenoceptor Antagonist, Displays Low Affinity for Functional α_1-Adrenoceptors in Human Prostate: Implications for Adrenoceptor Classification

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SUMMARY

Norepinephrine (NE) contracts smooth muscle cells within the human lower urinary tract (LUT) (bladder neck, prostate, and urethra). Receptor distribution and pharmacological evidence have implicated activation of α_{1A}-adrenoceptors. We disclose the pharmacological properties of the novel, selective α_{1A}-adrenoceptor antagonist *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (RS-17053) and examine critically the pharmacological identity of the α_1-adrenoceptor mediating contractions to NE in human LUT tissues. In several tissues from rat and cloned adrenoceptors, RS-17053 displayed high affinity for the α_{1A}-adrenoceptor (pK_i and pA_2 estimates of 9.1–9.9) and a 30–100-fold selectivity over the α_{1B}- and the α_{1D}-adrenoceptor subtypes (pK_i and pA_2 estimates of 7.7–7.8). However, in isolated smooth muscle preparations from human LUT tissues, RS-17053 antagonized responses to NE only at high concentrations. Estimates of affinity (pA_2) at α_1-adrenoceptors mediating NE-induced contractions were 7.5 in prostatic periurethral longitudinal smooth muscle (compared with 8.6 for prazosin), 6.9 in anterior fibromuscular stroma (prazosin, 8.9), and 7.1 in bladder neck (prazosin, 8.5). These findings indicate that contractile responses to NE in human LUT tissues are mediated by a receptor displaying pharmacological properties that are clearly different from those of the defined α_{1A}-adrenoceptor and raise the possibility that multiple forms of the α_{1A}-adrenoceptor may exist in human LUT that are discriminated by RS-17053. In this regard, the affinity estimates obtained with RS-17053 and other α_1-adrenoceptor antagonists in human LUT tissues are identical to those described for the putative α_{1L}-adrenoceptor.

Three subtypes of α_1-adrenoceptor have been characterized fully using operational (pharmacological), structural, and transductional criteria, i.e., α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors (1, 2). An additional subtype, the α_{1L}-adrenoceptor, has been proposed, but evidence for this receptor rests on functional, pharmacological studies alone (3–5).

The α_{1A}-adrenoceptor (known previously as the α_{1C}-adrenoceptor) has been shown in radioligand binding and molecular studies to be the predominant α_1-adrenoceptor subtype in human LUT tissues (6–8) and is proposed by some to be the receptor through which NE mediates contraction of pros-

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ABBREVIATIONS: NE, norepinephrine; WB 4101, (2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride; BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)-ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride; HV 723, α-ethyl-3,4,5-trimethoxy-α-(3-(2-(2-methoxyphenoxyethyl)-amino)-propyl)-benzene-acetonitrile fumarate; RS-17053, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride; SNAP 5089, 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid-*N*[3-(4,4-diphenylpiperidin-1-yl)propyl]amide methyl ester; LUT, lower urinary tract.

tatic smooth muscle (7, 9–11). Accordingly, a selective α_{1A}-adrenoceptor antagonist may be therapeutically advantageous in the treatment of urinary tract obstruction (e.g., in benign prostatic hyperplasia). In contrast, Muramatsu *et al.* (12) proposed that the α_{1L}-adrenoceptor mediates prostatic contraction to NE, based principally on the key pharmacological characteristic of a low affinity for prazosin compared with that determined for the α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors.

A major problem hindering resolution of this issue is the lack of subtype-selective α_1-adrenoceptor antagonists (2). We describe *in vitro* pharmacological properties of a novel, selective α_{1A}-adrenoceptor antagonist, RS-17053 (Fig. 1), with particular emphasis on its effect on human LUT tissues. In addition, a novel analogue of *S*-(+)-niguldipine (SNAP 5089; Ref. 13) has been studied in selected assays. Both *S*-(+)-niguldipine and SNAP 5089 exhibit high affinity for α_{1A}-adrenoceptors, but SNAP 5089 lacks the complication of Ca^{2+}-channel blocking activity, which is a characteristic of *S*-(+)-niguldipine (1, 14). Data obtained with these agents and other competitive antagonists for α_1-adrenoceptors indicate that the pharmacologically defined α_{1A}-adrenoceptor is not responsible for NE-induced contraction of the human prostate, prostatic urethra, and bladder neck. Instead, a novel pharmacological recognition site appears to mediate responses to NE in these tissues, and this site displays an antagonist affinity profile similar to the putative α_{1L}-adrenoceptor, as proposed by Muramatsu (12). A preliminary account of this work has been presented previously (15).

Materials and Methods

Radioligand binding studies. Estimates of affinity (pK_i) at α_1-adrenoceptors for RS-17053 and other competitive α_1-adrenoceptor antagonists were made from displacement curves (using 10 concentrations of displacing agent) in membrane homogenates of native tissues or heterologous expression systems (cloned bovine or human α_{1a}-, hamster α_{1b}-, or rat α_{1d}-adrenoceptors), as described previously (16). [^{3}H]Prazosin (0.1 nM; specific activity, 82 Ci/mmol) was used to label α_1-adrenoceptors, and phentolamine (10 μM) was used to determine specific binding. All equilibrations were carried out for 60 min at 23°. Concentrations of displacing agent producing 50% displacement of [^{3}H]prazosin (IC_{50}) were interpolated with the use of nonlinear iterative curve-fitting methodologies and converted into pK_i with the equation of Cheng and Prusoff (17).

***In vitro* functional pharmacological studies.** Estimates of antagonist affinity (pA_2) from functional studies were made on isolated perfused kidney and isolated perfused mesenteric arteries (both are α_{1A}-adrenoceptor preparations) and isolated aortic rings (an α_{1D}-adrenoceptor preparation) from rat (male Sprague-Dawley, 350–500 g) and on portions of human LUT tissue (periurethral longitudinal smooth muscle and anterior fibromuscular stroma of prostate and bladder neck sphincteric muscle).

Fresh specimens of LUT tissues were obtained immediately after excision from 29 men (mean age, 63.8 ± 1.36 years; range, 49–80 years) at radical prostatectomy (24 patients) or cystoprostatectomy (5 patients). Samples were obtained from surgery and kept in cold Krebs' solution until use in functional studies, which were performed later on the same day or early on the next day. Mean prostate mass was 54.2 ± 3.7 g (range, 24–90 g). A vertical midline incision was made into the prostate anteriorly and extending into the urethra along its entire intraprostatic extent. When the incised specimen was opened, the urethral mucosa was exposed as two lateral surfaces. The mucosa was stripped from one side, exposing the longitudinal periurethral muscle layer, which was then undermined by blunt dissection and excised proximally and distally. A second incision was made transversely, encircling the bladder neck at the proximal end of the urethra and penetrating 1–2 mm through the superficial tissue. Beginning at the bottom of the incision, the location of the sphincter was identified, and a horizontal strip of bladder neck sphincteric muscle was excised that exhibited an approximately triangular cross section. Last, the anterior midline surface of the prostate, along the initial incision, was stabilized and retracted with forceps, exposing the surface of the anterior fibromuscular stroma; a sample was taken as a broad vertical strip adjacent to the midline.

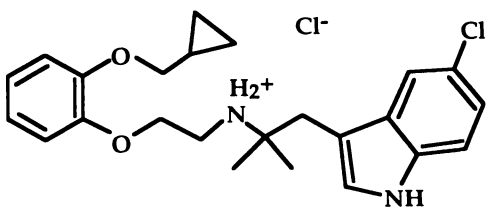

Fig. 1. Chemical structure of RS-17053.

In kidney and mesentery, noncumulative concentration/effect (E/[A]) curves to NE-induced increases in perfusion pressure were constructed as described previously (16, 18). Antagonists were equilibrated with the tissue for 60 min before and during construction of the second E/[A] curve to NE. Human LUT tissues (1–3-mm-wide, 6–10-mm-long strips) and rings (3–4-mm wide) of endothelium-denuded thoracic aorta from rat (male Sprague-Dawley, 350–500 g) were mounted in 10-ml tissue baths (resting tensions, 7.5–10 mN at 37°) for isometric measurement of changes in tension. All tissues were primed with a sighting concentration of NE (10 μM) and washed thoroughly to re-establish base-line tension before construction of two cumulative E/[A] curves to NE. Antagonist equilibration (1–2 hr) was allowed between curves.

For all functional studies, we used Krebs' solution composed of 143.5 mM Na^+, 6.0 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 125.8 mM Cl^-, 25 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, and 1.2 mM SO_4^{2-} and supplemented with 30 μM cocaine, 30 μM corticosterone, 110 μM ascorbate, 1 μM propranolol, 0.3 μM idazoxan, and 10 μM indomethacin to establish equilibrium conditions and to prevent the involvement of β-adrenoceptors, α_2-adrenoceptors, and arachidonate metabolites in responses to NE. In addition, for studies in tissues of rat, 1 μM nitrendipine was included in the Krebs' solution to isolate a homogeneous population of α_1-adrenoceptors (16). All studies were performed at 37° with solutions gassed continuously with an O_2/CO_2 mixture (95%:5%).

E/[A] curves were plotted using nonlinear iterative curve-fitting methodologies to a form of the logistic equation for estimation of midpoint location parameter ($[A]_{50}$), such that $E = E_{max} \cdot [NE]^{nH} / ([NE]^{nH} + [A]_{50}^{nH})$

where E_{max} is the magnitude of the upper asymptote, and n_H is the Hill coefficient (defining the slope of the relationship). Antagonist affinity estimates (as pK_B or pA_2) were obtained by construction of Schild regressions in the case of perfused kidney and aorta of rat and by single-concentration analysis otherwise (assuming a Schild regression slope of 1) according to the equation: $pA_2 = -\log[B] + \log(r-1)$, where [B] is molar concentration of antagonist, and r is the concentration ratio of $[A]_{50}$ in the presence of antagonist divided by that obtained in the absence of antagonist. Wherever possible, estimates of r were corrected for variations in tissue sensitivity to agonist over time. Terms and equations used were as recommended by the IUPHAR Committee on Receptor Nomenclature and Drug Classification (19).

Compounds were obtained from the following sources: (±)-NE hydrochloride, (−)-NE hydrochloride, (±)-propranolol hydrochloride, cocaine hydrochloride, corticosterone, indomethacin, phentolamine, and prazosin hydrochloride, Sigma Chemical Co. (St. Louis, MO); WB 4101, idazoxan hydrochloride, *S*-(+)-niguldipine, nitrendipine, BMY 7378, and 5-methylurapidil, Research Biochemicals (Natick,

MA); and HV 723, tamsulosin (YM 617), RS-17053, and SNAP 5089, synthesized in the Chemistry Department, Center for Neurobiology, Roche Bioscience (Palo Alto, CA). Stock solutions were prepared in distilled water or dimethylsulfoxide on the day of study and diluted in appropriate media. In the case of RS-17053, all stock solutions were prepared in dimethylsulfoxide and were diluted serially in phosphoric acid (5 mM). Analysis by high performance liquid chromatography confirmed the accuracy of the dilutions. The addition of small quantities of phosphoric acid (5 mM) was without effect on pH of the Krebs' solution.

Cloned bovine (α_{1a}), hamster (α_{1b}), and rat (α_{1d}) α_1-adrenoceptors, stably expressed in rat-1 fibroblasts, were purchased from Dr. Lee Allen, Duke University (Durham, NC). Human α_{1a}-adrenoceptors were stably expressed in Chinese hamster ovary cells and were obtained from the Department of Molecular Biology, Roche Bioscience.

Results

Radioligand binding studies. Estimates of affinity (pK_i) for RS-17053 and a selection of competitive α_1-adrenoceptor antagonists are shown in Table 1. RS-17053 displayed subnanomolar affinity (pK_i = 9.1) at displacing [^{3}H]prazosin from membranes expressing native α_{1A}-adrenoceptors (rat submaxillary gland) and transfected cells expressing cloned α_{1a}-adrenoceptors (pK_i = 9.5 for bovine α_{1a}-adrenoceptor expressed in rat-1 fibroblasts; pK_i = 9.2 for human α_{1a}-adrenoceptor expressed in Chinese hamster ovary cells). These consistent pK_i estimates for RS-17053 indicate a cell- and species-independent high affinity for the α_{1A}-adrenoceptor. At α_{1B}-adrenoceptors (cloned and native) and α_{1d}-adrenoceptors (cloned), RS-17053 displayed a significantly lower affinity (pK_i = 7.8).

Prazosin did not exhibit selectivity for a particular α_1-adrenoceptor subtype, whereas phentolamine, indoramin, 5-methylurapidil SNAP 5089, and *S*-(+)-niguldipine were, in increasing order, 10–1000-fold selective for α_{1A}-adrenoceptors over α_{1B}- and α_{1D}-adrenoceptors. WB 4101 and tamsulosin displayed high affinity at both α_{1A}-adrenoceptors and α_{1D}-adrenoceptors with moderate selectivity over α_{1B}-adrenoceptors. BMY 7378 displayed considerable selectivity (~100-fold) for the α_{1D}-adrenoceptor over α_{1A}- and α_{1B}-adrenoceptors, as has been described previously (20), and HV 723 displayed ~10-fold lower affinity at α_{1D}-adrenoceptors compared with its affinity at α_{1A}- and α_{1B}-adrenoceptors.

***In vitro* functional pharmacological studies.** Estimates of antagonist affinity in functional studies for RS-17053 and other competitive α_1-adrenoceptor antagonists (expressed as pK_B or pA_2 values) are shown in Table 2. In isolated perfused kidney and mesenteric arteries of rat, RS-17053 antagonized vasoconstrictor responses to NE with high affinity (pA_2 of 9.8 and 9.9, respectively). Fig. 2a shows the effect of increasing concentrations of RS-17053 (0.3–30 nM) on E/[A] curves to NE in isolated perfused kidney of rat. Antagonism was concentration dependent, as can be seen from the Schild regression analysis (Fig. 2b), and a mean pA_2 of 9.78 ± 0.05 (20 experiments) was estimated. The slope of the Schild regression (1.23; 95% confidence limit, 1.14–1.33) was significantly >1, indicating that conditions of equilibrium were not achieved completely. In perfused mesenteric arteries of rat, the pA_2 estimate of 9.9 (Table 2) was determined from a single concentration of RS-17053 (1 nM), as higher concentrations led to insurmountable antagonism of responses to NE, precluding Schild regression analysis.

In rings of thoracic aorta from rat, RS-17053 behaved as a weak antagonist against the contractile response to NE. Schild regression analysis yielded a line with slope not significantly different from 1 and a pK_B estimate of 7.69 ± 0.08 (12 experiments). As can be seen from inspection of the values for other antagonists, affinity estimates from contractile studies in aortic rings of rat bear closest resemblance to estimates of binding affinity at cloned α_{1D}-adrenoceptors (compare Tables 1 and 2). This relationship is in close agreement with the studies of others (21–23). An exception is noted for *S*-(+)-niguldipine, which differs in affinity estimates by 10-fold between aorta and α_{1D}-adrenoceptor. This may be related to other properties of this antagonist (see introductory paragraphs) and highlights further the limitations associated with its use.

In human LUT tissues, RS-17053 produced rightward displacements of E/[A] curves to NE but only at high concentrations (≥0.03 μM; Fig. 3). Affinity estimates for RS-17053 produced by these higher concentrations (0.03–10 μM) were 7.52 ± 0.17 (prostatic periurethral longitudinal smooth muscle), 6.89 ± 0.21 (anterior fibromuscular stroma), and 7.10 ±

TABLE 1

Affinity estimates (mean pK_i) for RS-17053 and selected α_1-adrenoceptor antagonists in cloned and native cell membrane homogenates labeled with [^{3}H]prazosin

Cloned mammalian α_1-adrenoceptors expressed in rat-1 fibroblasts, except human α_{1A}-adrenoceptor expressed in CHO-K1 cells. Values are mean ± standard error for at least 3 determinations. n.d. = not determined.

Antagonist	α_{1A} rat[a] (native)	α_{1a} bovine (clone)	α_{1a} human (clone)	α_{1B} rat[b] (native)	α_{1b} hamster (clone)	α_{1d} rat (clone)
RS-17053	9.1 ± 0.17	9.5 ± 0.13	9.2 ± 0.22	7.8 ± 0.09	7.8 ± 0.03	7.8 ± 0.06
Prazosin	10.1 ± 0.01	9.9 ± 0.01	9.9 ± 0.01	10.2 ± 0.05	10.1 ± 0.01	9.9 ± 0.04
WB 4101	10.1 ± 0.04	10.0 ± 0.03	9.8 ± 0.08	8.8 ± 0.19	8.6 ± 0.03	9.6 ± 0.02
5-Methylurapidil	9.2 ± 0.04	9.4 ± 0.03	9.2 ± 0.04	7.4 ± 0.20	7.5 ± 0.01	8.0 ± 0.02
Indoramin	n.d.	8.4 ± 0.13	n.d.	n.d.	7.4 ± 0.04	7.3 ± 0.02
Tamsulosin	10.2 ± 0.30	10.4 ± 0.11	10.4 ± 0.16	8.9 ± 0.04	9.3 ± 0.07	10.2 ± 0.06
HV 723	n.d.	9.6 ± 0.02	9.2 ± 0.09	n.d.	9.7 ± 0.06	8.5 ± 0.05
BMY 7378[c]	n.d.	6.1 ± 0.02	6.6 ± 0.20	n.d.	6.2 ± 0.03	8.2 ± 0.06
S-(+)- Niguldipine	9.7 ± 0.05	9.8 ± 0.15	9.7 ± 0.09	7.6 ± 0.25	7.0 ± 0.29	7.1 ± 0.07
SNAP 5089	n.d.	8.7 ± 0.18	n.d.	n.d.	6.9 ± 0.10	6.8 ± 0.06
Phentolamine	8.8 ± 0.05	8.9 ± 0.03	8.8 ± 0.09	8.1 ± 0.11	7.9 ± 0.04	8.1 ± 0.10

[a] Rat submaxillary gland membrane homogenate.
[b] Rat liver membrane homogenate.
[c] Data from Ref. 20.

TABLE 2

Affinity estimates (pA_2) for RS-17053 and selected α_1-adrenoceptor antagonists in native tissue functional assays against responses to NE

All values are mean ± standard error for data from at least three determinations.

Antagonist	α_{1A} rat: perfused kidney	α_{1A} rat: perfused mesentery	α_{1D} rat: aortic rings	α_{1L} man: LUT (present study)[a]	α_1 man: LUT (literature)[b]
RS-17053	9.8[c]	9.9	7.7 ± 0.1	7.3 ± 0.1	n.d.
Prazosin	9.5	9.3	9.6 ± 0.1	8.7 ± 0.1	9.0 (8.3)
WB 4101	10.3	9.6	9.0 ± 0.1	8.9 ± 0.1	8.9 (8.4)
5-Methylurapidil	9.2	9.0	7.6 ± 0.1	8.2 ± 0.1	8.5 (8.3)
Indoramin	n.d.	n.d.	7.3 ± 0.2	8.5 ± 0.2	8.4
Tamsulosin	10.2[d]	n.d.	9.7 ± 0.2	10.4 ± 0.1	n.d.
HV 723	9.3	n.d.	8.7 ± 0.1	8.8 ± 0.1	n.d.
BMY 7378	n.d.	n.d.	8.5 ± 0.2	6.4 ± 0.1	n.d.
S-(+)-Niguldipine	10.5[d]	i.s.	8.1 ± 0.3	7.3 ± 0.5	n.d.
SNAP 5089	n.d.	n.d.	<6.5[e]	<6.5[e]	n.d.
Phentolamine	8.6	n.d.	n.d.	n.d.	n.d.

[a] Data pooled from affinity estimates (pA_2) made from this study in three LUT tissues: longitudinal periurethral muscle, anterior fibrous stroma of prostate, and bladder neck.

[b] Mean affinity estimates (pA_2) from published functional data characterizing LUT of man from Refs. 7, 9, 10, and 11; values in parentheses are from Muramatsu (12).

[c] Schild slope significantly >1 ($p < 0.05$).

[d] Displayed insurmountable antagonism at >0.1 nM.

[e] No significant shift observed at 0.3 μM.

n.d. = not determined; i.s., insurmountable antagonism at 0.03–0.3 nM.

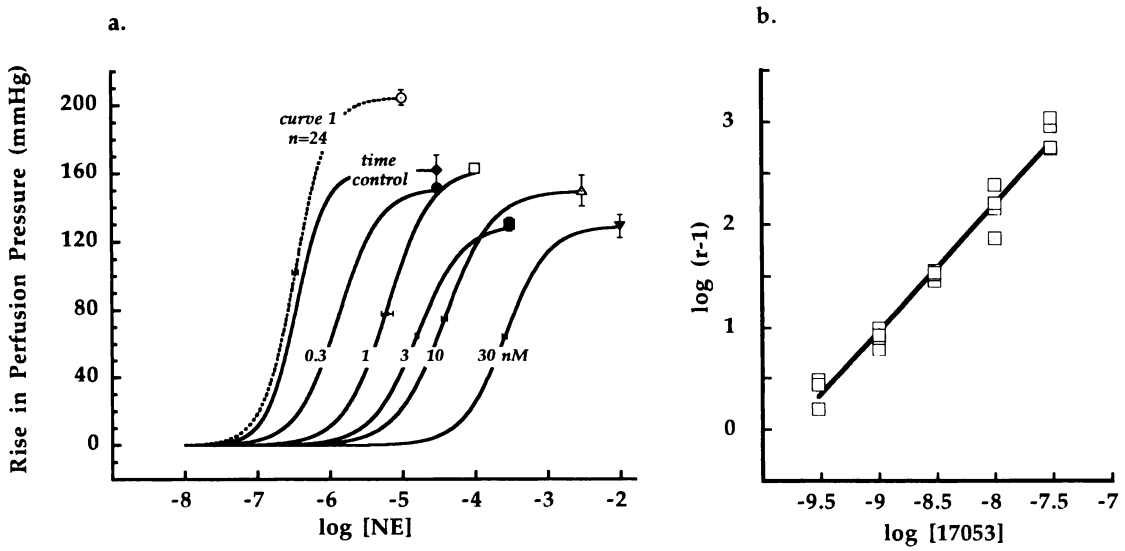

Fig. 2. Effect of RS-17053 on NE-induced vasoconstrictor responses in isolated perfused kidney from rat. a, Rises in perfusion pressure for first noncumulative E/[A] curve (○) and for second E/[A] curve in the absence of RS-17053 (time control; ♦) and in the presence of RS-17053 (60-min equilibration) at 0.3 (●), 1 (□), 3 (■), 10 (△), and 30 nM (▲) (four kidneys per group). Mean parameters for $[A]_{50}$, E_{max}, and n_H are shown with standard error bars. b, Schild regression analysis for RS-17053 using concentrations ratios (r) estimated from individual kidneys. pA_2 = 9.78 ± 0.05 (20 kidneys), Schild slope = 1.23 (95% confidence limit, 1.14–1.33).

0.29 (means bladder neck) (from at least three patients) compared with 8.61 ± 0.07, 8.94 ± 0.33, and 8.54 ± 0.14 for prazosin. Mean estimates of affinity for these and other antagonists (pooled from the three human LUT regions studied) are given in Table 2. For all antagonists, affinity estimates were found to be independent of the particular anatomic region studied. For many antagonists, including RS-17053, prazosin, and tamsulosin, we also observed that larger rightward displacements of E/[A] curves, in particular in bladder neck, were associated with varying degrees of reduction in E_{max} (Fig. 3, c and d).

The pA_2 values for RS-17053, SNAP 5089, and S-(+)-niguldipine obtained in human LUT tissues and, to a lesser extent, those obtained for prazosin, WB 4101, HV 723, and 5-methylurapidil differed from those documented for any fully characterized α_1-adrenoceptor subtype and, in particular, are distinct from the α_{1A}-adrenoceptor (compare the radioligand binding studies in Table 1 with the contractile studies in Table 2). In the case of SNAP 5089, concentrations up to 0.3 μM did not yield reproducible displacement of E/[A] curves to NE. Furthermore, the absence of significant antagonism of NE responses by low concentrations of S-(+)-niguldipine suggests little or no involvement of L-type Ca^{2+} channels in the transduction of the response to NE in human LUT tissues. Tamsulosin was the only antagonist showing comparable high affinity in human LUT and α_{1A}-adrenoceptors in both binding and functional studies.

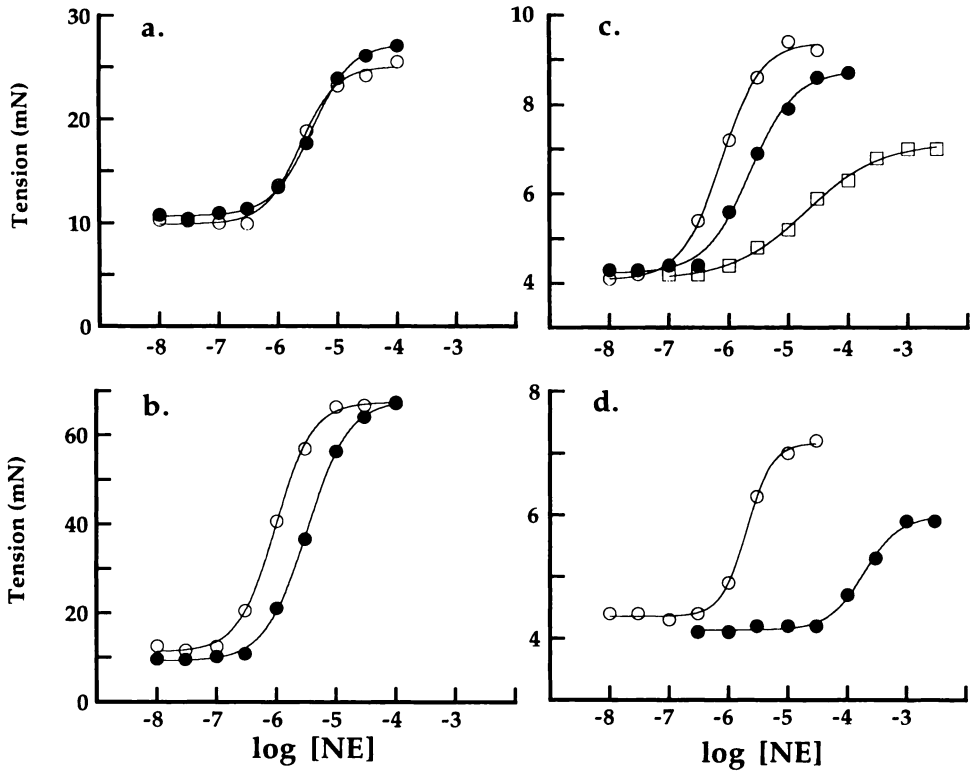

Fig. 3. Effects of RS-17053 and tamsulosin on NE-induced contractile responses in isolated strips of human LUT smooth muscle. a and b, Representative E/[A] curves in response to NE in periurethral longitudinal smooth muscle strips from a single patient showing first curves (○) and the effect of (a) time (●) and (b) RS-17053 (●) at 30 nM (estimated pA_2 = 7.58). c and d, Representative E/[A] curves in response to NE in bladder neck smooth muscle strips from a different patient showing effect of (c) RS-17053 at 30 nM (●) and 1 μM (□) (estimated pA_2 = 7.78, from 30 nM concentration) and (d) tamsulosin at 3 nM (●) (pA_2 = 10.41).

Discussion

The present study shows that RS-17053 displays a high affinity and selectivity for the α_{1A}- (α_{1a}-)adrenoceptor subtype. RS-17053 antagonized α_{1A}-adrenoceptors with subnanomolar affinity (pA_2 = >9.1) and exhibited selectivity for the α_{1A}-adrenoceptor of 30–50-fold over α_{1B}- and α_{1D}-adrenoceptors (ligand binding data) and 100-fold selectivity over α_{1D}-adrenoceptors based on functional studies (perfused kidney versus aortic rings of rat). Consequently, RS-17053 may be a key discriminative pharmacological probe for characterizing α_1-adrenoceptor populations in both functional and radioligand binding assays. Although a degree of insurmountability was observed with high concentrations (≥1 μM) of RS-17053 in certain functional assays (e.g., human bladder neck; see Fig. 3, c and d), this was not unique to RS-17053 as prazosin, tamsulosin, and *S*-(+)-niguldipine induced similar phenomena. We speculate that these observations do not arise from noncompetition but rather relate to both the kinetic properties of certain antagonists (high affinity, slow off-rate) and, in particular, the time course of NE-induced contraction (absence or presence of fade). This speculation is supported by the fact that contractile responses to (+)-*cis*-dioxolane (a muscarinic cholinoceptor agonist) in human bladder neck were not influenced by high concentrations of α_1-adrenoceptor antagonists (data not shown).

RS-17053 proved to be a key ligand in establishing the most important finding from the present study, i.e., the lack of pharmacological resemblance between the α_1-adrenoceptor mediating NE-induced contraction of human LUT tissues and the native or cloned α_{1A}-adrenoceptor. This result contrasts with previous reports claiming a similarity, if not identity, between the α_{1A}-adrenoceptor and the α_1-adrenoceptor mediating human prostatic smooth muscle contraction (7, 9–11). Species differences are evidently not the explanation, as affinity estimates for the cloned human α_{1a}-adrenoceptor were in agreement with those found in the cloned bovine and native rat α_{1A}-adrenoceptors. Furthermore, affinity estimates obtained in human LUT tissue do not align with those defining the α_{1B}- and α_{1D}-adrenoceptors and are not consistent with the presence of a mixed functional population of α_1-adrenoceptor subtypes. It should be noted that the involvement of α_2-adrenoceptors in the response to NE in LUT tissues is unlikely, as all studies were conducted in the presence of the α_2-adrenoceptor antagonist idazoxan. Additional supportive data for the singular involvement of α_1-adrenoceptors have been generated by the use of selective α_2-adrenoceptor agonists, which were ineffective, and selective α_1-adrenoceptor agonists (e.g., methoxamine, SDZ NVI 085), which yielded similar pharmacology to that obtained with NE.

Differences in estimates of antagonist affinity between human LUT and α_{1A}-adrenoceptors were most noticeable for RS-17053 (affinity estimates differed by 100-fold), but SNAP 5089 and *S*-(+)-niguldipine also revealed large differences. These differences must be interpreted with caution, as chemical instability and loss of compound (due to high lipophilicity) may lead to underestimates of affinity for the dihydropyridines (1). However, despite potential physicochemical problems, in a study in which we used isolated strips of caudal artery of rat (24), we reported high affinity estimates for SNAP 5089 (pA_2 = 9.5 ± 0.1), which are fully consistent with α_{1A}-adrenoceptor antagonism. Similarly, high affinity estimates have been obtained for RS-17053 in caudal artery (pA_2 = 9.2 ± 0.2; Ref. 24). In both of these studies, the experimental conditions that were used are identical to those we describe. Finally, to ensure equilibrium conditions with

RS-17053, we increased incubation times (up to 24 hr) and the frequency with which the incubation solution was changed (5-min intervals). These modifications failed to change affinity estimates for RS-17053 in human LUT tissues (data not shown).

Prazosin, WB 4101, HV 723, and 5-methylurapidil also displayed lower affinity (by 6–30-fold) in contractile studies of human LUT compared with estimates of affinity at α_{1A}-adrenoceptors (cloned and native). Tamsulosin and indoramin, in contrast, demonstrated a high affinity in human LUT tissues as well as at α_{1A}-adrenoceptors in both binding and functional studies. This observation negates the suggestion that low affinity estimates in human LUT tissues compared with cloned and native α_{1A}-adrenoceptors result from a frame-shift of estimates. BMY 7378, which is ~100-fold selective for α_{1D}-adrenoceptors over α_{1A}- and α_{1B}-adrenoceptors (20), displayed low affinity in human LUT, providing evidence against the involvement of α_{1D}-adrenoceptors.

As stated above, the notion that α_{1A}-adrenoceptors do not mediate NE-induced contraction of human LUT challenges the assertion made by several groups (7, 9–11). The averaged results from these studies are given in Table 2 and are compared with those from Muramatsu *et al.* (12). It is our contention that previous characterizations of human LUT tissues have been confounded by the use of too few pharmacologically discriminating antagonists or too many nonselective antagonists. In the report by Forray *et al.* (7), evidence for pharmacological identity was based principally on data for indoramin and its analogue SNAP 1069, each of which offer, at best, 10-fold selectivity for α_{1A}-adrenoceptors, whereas other key antagonists, such as WB 4101 and *S*-(+)-niguldipine, were not tested in prostatic tissues, although they were recognized as key pharmacological probes from binding studies. Similarly, in other studies (10, 11), pharmacological analysis was restricted to prazosin, WB 4101, and 5-methylurapidil, which, although providing a profile most akin to that of the α_{1A}-adrenoceptor, is not identical. This can be clearly seen in Table 2, where affinity estimates from the present study are compared with those described in the reports discussed above. Good agreement exists for the limited number of antagonist affinity estimates reported; however, data for selective antagonists, such as RS-17053, clearly pinpoint the discordance between affinity estimates in human LUT and those for the α_{1A}-adrenoceptor.

Pharmacological studies of human prostatic tissues by Muramatsu *et al.* (12) have also disputed the involvement of the α_{1A}-adrenoceptor in contractile responses. With prazosin, WB 4101, 5-methylurapidil, and HV 723, a profile was obtained that differs from that of the α_{1A}-adrenoceptor and equates well with that of the α_{1L}-adrenoceptor. Consequently, the α_{1L}-adrenoceptor, which has been characterized only on the basis of functional pharmacological studies, was proposed to be the sole receptor involved in contraction of prostatic smooth muscle. We now support and extend this characterization.

In previous studies, we observed a similar pharmacological profile by using the same antagonist probes in a study of the responses of rat anococcygeus muscle (25). Estimates of affinity (pA_2) for selected antagonists against contractile responses to NE were as follows: prazosin, 8.9; RS-17053, 7.3; WB 4101, 9.0; and tamsulosin, 10.3. These values are almost identical to estimates of affinity in human LUT tissues (Table 2). The observation of functional α_{1L}-adrenoceptor pharmacology in a rat tissue is important, as it indicates that in different tissues within a single species functional studies can reveal α_{1A}-adrenoceptor (e.g., rat kidney, 16; rat caudal artery, 24) and α_{1L}-adrenoceptor (rat anococcygeus, 25) pharmacology.

Despite the controversial functional data highlighted by the present study, it is not disputed that a high preponderance of α_{1A}-adrenoceptor binding sites and mRNAs exist in the human prostate (6–8). Our investigations (data not shown) support fully these observations. Therefore, several pertinent questions arise. Is the α_{1L}-adrenoceptor a distinct molecular entity? If so, is there a separate, previously unidentified gene that encodes for it? What is the role of the high density of α_{1A}-adrenoceptor binding sites throughout human LUT tissues, if not to participate in contraction? Can radioligand binding studies detect clearly a population α_{1L}-adrenoceptors in human LUT (bearing in mind that by definition the α_{1L}-adrenoceptor displays low affinity for the most commonly used radioligand, [^{3}H]prazosin)? Are there tissues from humans that clearly display the pharmacology of the α_{1A}-adrenoceptor in functional studies? Finally, could the α_{1L}-adrenoceptor pharmacology observed in human LUT reflect involvement of a splice variant of the human α_{1A}-adrenoceptor, for which three isoforms have been reported (26)? None of these questions can be readily answered at this time, and each requires further attention.

Even without clear answers to these questions, evidence is convincing that the antagonist affinity profiles of α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors are not adequate to describe the pharmacological properties of many functional α_1-adrenoceptor populations. The α_{1L}-adrenoceptor is emerging with a unique and clear pharmacology, which would be strengthened further if it could be identified clearly in radioligand binding studies. Studies with radioligands other than prazosin may be of greater value; for example, [^{3}H]tamsulosin could be used, as this agent exhibits a much higher affinity for the α_{1L}-adrenoceptor.

In summary, RS-17053 is a potent and selective α_{1A}-adrenoceptor antagonist. The low affinity of RS-17053 in antagonizing contractions of human LUT tissues to NE provides strong evidence against pharmacological identity of this α_1-adrenoceptor subtype with the cloned and native α_{1A}-adrenoceptor. In this regard, RS-17053 is not singular, as several other α_1-adrenoceptor antagonists (prazosin, WB 4101, *S*-(+)-niguldipine, and SNAP 5089) also distinguish between the two adrenoceptors. It is clear that the three fully defined α_1-adrenoceptors do not describe satisfactorily the functional data in the human LUT, supporting the notion that the α_{1L}-adrenoceptor exists as a distinct pharmacological entity. As a final point, the present study highlights the necessity in drug development of conducting functional pharmacological investigations in target tissues from humans.

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