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The α_1-Adrenergic Receptor that Mediates Smooth Muscle Contraction in Human Prostate Has the Pharmacological Properties of the Cloned Human α_{1c} Subtype

CARLOS FORRAY, JONATHAN A. BARD, JOHN M. WETZEL, GEORGE CHIU, ELLEN SHAPIRO,[1] RUI TANG,[1] HERBERT LEPOR,[1] PAUL R. HARTIG, RICHARD L. WEINSHANK, THERESA A. BRANCHEK, and CHARLES GLUCHOWSKI

Synaptic Pharmaceutical Corporation, Paramus, New Jersey 07652 (C.F., J.A.B., J.M.W., G.C., P.R.H., R.L.W., T.A.B., C.G.), and Departments of Urology, and Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226 (E.S., R.T., H.L.)

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SUMMARY

Molecular cloning studies have revealed the existence of three subtypes of α_1-adrenergic receptors. However, the link between any individual subtype and its functional role in the body has remained elusive. In an effort to bridge the gap between molecular biology and pathophysiology, we have chosen a model smooth muscle system, the human prostate, and investigated the role of α_1 subtypes in this tissue. To determine which α_1-adrenergic receptor subtype mediates the contractile response of the human prostate, we first studied the pharmacological properties of three cloned human α_1 subtypes ($\alpha_{1a/d}$, α_{1b}, and α_{1c}). Prazosin, terazosin, doxazosin, alfuzosin, and abanoquil showed no selectivity for the human α_1 subtypes. WB-4101 and 5-methylurapidil showed a rank order of potency of $\alpha_{1c} > \alpha_{1a/d} \gg \alpha_{1b}$. Indoramin and (+)-niguldipine were selective for the α_{1c}-adrenergic receptor, with at least 10-fold lower affinity at either $\alpha_{1a/d}$ or α_{1b} subtypes. SK&F104856 was found to be 6-fold more potent at the $\alpha_{1a/d}$ receptor subtype than at α_{1b}- or α_{1c}-adrenergic receptors. We next determined the potency of these antagonists to inhibit the phenylephrine-induced contraction of human prostatic tissue *in vitro*. The potencies of indoramin, 5-methylurapidil, and SK&F104856 to inhibit the contractile response and to displace [^{3}H]prazosin from the cloned human α_{1c} subtype were similar. Our data suggest that the α_1 receptor that mediates the contraction of human prostate smooth muscle has the pharmacological properties of the cloned human α_{1c}-adrenergic receptor. The findings of the present study suggest that selective α_{1c}-adrenergic receptor antagonists may be clinically more efficacious and better tolerated agents for the treatment of symptomatic benign prostatic hyperplasia.

Although α_1-ARs were originally subclassified, according to pharmacological criteria, into two receptor subtypes (α_{1A} and α_{1B})[2] (1), recently the genes encoding three different α_1-AR subtypes have been cloned (1–4). In spite of the advances in the molecular cloning of the α_1-ARs, the relationship between cloned and pharmacologically defined subtypes still remains unclear. The evidence gathered so far indicates that the hamster α_{1b}-AR encodes a receptor that has the properties of the pharmacologically defined α_{1B} subtype (2). However, it is not clear whether either of the other two cloned α_1-ARs encodes the α_{1A} subtype (3–5). On one hand, the cloned bovine α_{1c}-AR was found to have high affinity for α_{1A}-selective antagonists but, because it was inactivated by CEC and its mRNA could not be detected in rat tissues, the assumption was that it did not encode the α_{1A} subtype (3). On the other hand, the rat α_{1a}-AR encodes a receptor that initially was found to be resistant to inactivation by CEC and expressed in rat tissues enriched in the α_{1A} subtype (4). However, it was later found that a rat clone encoding a receptor that differed in two amino acids from the original α_{1a}-AR was sensitive to CEC, in sharp contrast to the virtual insensitivity of the α_{1A} subtype to CEC, and it was designated as the α_{1d}-AR (5). It is conceivable that, by studying the pharmacological properties of the three cloned receptors from the same species, a more consistent picture can emerge and some of these differences can be reconciled. The recent cloning of the human homologues of the rat $\alpha_{1a/d}$-, hamster α_{1b}-, and bovine α_{1c}-ARs (6–8) should also facilitate the study of the physiological or physiopathological roles of the α_1-AR subtypes.

The norepinephrine-induced contraction of human prostate smooth muscle is known to be mediated by α_1-ARs (9–11). α_1-AR antagonists are effective drugs for the treatment of symp-

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[1] Present address: Department of Urology, New York University Medical Center, New York, NY 10021.

[2] Throughout this paper the cloned subtypes are designated by lower case subscripts and the pharmacologically defined subtypes are designated by upper case subscripts.

ABBREVIATIONS: AR, adrenergic receptor; BPH, benign prostatic hyperplasia; bp, base pair(s); CHO, Chinese hamster ovary; CEC, chloroethylclonidine.

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tomatic BPH (12–17). The rationale behind this treatment is related to the ability of α_1-AR antagonists to decrease the tone of prostatic smooth muscle, leading to relief of the obstructive and irritative symptoms (18). To identify the α_1-AR subtype that mediates the contraction of human prostate smooth muscle, we have studied the pharmacological profile of the human α_1-AR subtypes, using cell lines expressing the three cloned human α_1-AR genes. Having identified a series of selective antagonists, we correlated their binding affinity with their potency to inhibit the contraction of human prostate smooth muscle *in vitro*. Results from these studies suggest that the contractile response of the human prostate is mediated by a receptor encoded by the α_{1c}-AR gene.

Materials and Methods

Chemicals

The following compounds were obtained from Research Biochemicals International (Natick, MA): CEC, epinephrine bitartrate, (+)-niguldipine, norepinephrine HCl, phenylephrine HCl, phentolamine mesylate, prazosin HCl, rauwolscine HCl, 5-methylurapidil, WB-4101, and yohimbine HCl. The following compounds were prepared by published procedures: terazosin [(±)-4-amino-6,7-dimethoxy-2-[4-(tetrahydro-2-furoyl)piperazin-1-yl]quinazoline hydrochloride], doxazosin [(±)-4-amino-2-[4-(1,4-benzodioxan-2-ylcarbonyl)piperazin-1-yl]-6,7-dimethoxyquinazoline hydrochloride] (19), alfuzosin [(±)-4-amino-6,7-dimethoxy-2-{*N*-methyl-*N*-[3-(tetrahydro-2-furoylamino)propyl]}quinazoline hydrochloride] (20), abanoquil [4-amino-6,7-dimethoxy-2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)quinoline hemifumarate hydrate] (21), indoramin [3-[2-(4-benzamido-1-piperidin)ethyl] indole] (22), SNAP-1069 [1-(3-benzoylpropyl)-4-benzamidopiperidine dihydrate] (23), and SK&F104856 (7-chloro-2-ethenyl-3,4,5,6-tetrahydro-4-methylthieno[4,3,2-*ef*][3]benzazepine hydrochloride) (24).

Cloning of the Human α-AR Subtypes

$\alpha_{1a/d}$. Human $\alpha_{1a/d}$ receptor clones were isolated from both human genomic and cDNA (hippocampal) libraries.[3] The full length coding region, including 150 bp of 5′ untranslated sequence and 300 bp of 3′ untranslated sequence, was constructed by ligation of partially overlapping clones into the *Bam*HI and *Cla*I sites of the polylinker-modified eukaryotic expression vector pcEXV-3 (25).

α_{1b}. The coding region of the human α_{1b}-AR, including 200 bp of 5′ untranslated sequence and 600 bp of 3′ untranslated sequence, was obtained from a human brainstem cDNA library and cloned into the *Eco*RI site of the pcEXV-3 eukaryotic expression vector.

α_{1c}. Human α_{1c}-AR clones were isolated from both human genomic and cDNA (hippocampal) libraries. The coding region of the human α_{1c}-AR, including 400 bp of 5′ untranslated sequence and 200 bp of 3′ untranslated sequence, was constructed by splicing together three overlapping fragments (whereby the *Hinc*II and *Pst*I sites at the 5′ and 3′ ends of a human hippocampal cDNA clone were utilized for ligation to two human genomic DNA clones, a 5′-end 600-bp *Hinc*II genomic clone and a 3′-end 600-bp *Pst*I genomic clone). The complete full length gene was cloned into the *Kpn*I site of the polylinker-modified pcEXV-3 eukaryotic expression vector.

Transfection of the Human Receptor Genes

Stable cell lines were obtained by co-transfection of the expression vector containing the cDNA constructs of each of the human α_1-ARs with the plasmid pGCcos3neo into LM(tk$^-$) or CHO cells, using the calcium phosphate technique. Cells were grown as monolayers in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) containing 25 mM glucose and supplemented with 10% bovine calf serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate. Stable clones were then selected for resistance to G418 (1 mg/ml) as described previously (26). Cells were harvested and membrane preparations were assayed for their ability to bind [^{3}H]prazosin as described below.

Radioligand Binding Assays

Transfected cells from culture flasks were scraped into 5 ml of 5 mM Tris·HCl, 5 mM EDTA, pH 7.5, and lysed by sonication (Branson Sonic Power Co., Danbury, CT). The cell lysates were centrifuged at 1000 rpm for 5 min at 4°, and the supernatant was centrifuged at 30,000 × *g* for 20 min at 4°. The pellet was suspended in 50 mM Tris·HCl, 1 mM $MgCl_2$, 0.1% ascorbic acid, pH 7.5. Binding of 0.5 nM [^{3}H]prazosin (specific activity, 76.2 Ci/mmol; New England Nuclear, Boston, MA) to membrane preparations of transfected LM(tk$^-$) cells was performed in a final volume of 0.25 ml, with incubation at 37° for 30 min. Nonspecific binding was determined in the presence of 10 μM phentolamine, and it accounted for <6% of the radioactivity bound in the absence of phentolamine. The amount of protein added to the binding reaction was adjusted for each receptor subtype, in each experiment, so that the total bound [^{3}H]prazosin did not exceed 10% of the radioactivity added to the reaction mixture. The reaction was stopped by filtration through GF/B filters, using a cell harvester (Tomtec, Orange, CT), and tritium was determined by liquid scintillation counting. Data were analyzed by a computerized nonlinear regression program (INPLOT; GraphPAD Software, San Diego, CA). Protein concentration was determined by a colorimetric assay using a commercial kit, with bovine serum albumin as the standard (Bio-Rad Laboratories, Hercules, CA).

Contractile Studies

Fresh human prostatic tissue was obtained from male patients (between 50 and 80 years of age) undergoing prostatectomy for BPH. Strips of prostatic tissue approximately 15 mm in length and 200 mg in weight were dissected and suspended in organ baths containing Krebs-Ringer buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM glucose, 25 mM $NaHCO_3$). The baths were maintained at 37° and continuously bubbled with 95% O_2/5% CO_2. Isometric tension was measured with force transducers (Grass Instruments, Quincy, MA) coupled to a general purpose amplifier (Stemtec Inc., Houston, TX) that was interfaced to a personal computer (Datag Instruments, Akron, OH). Resting tension was set at 2 g and the tissue strips were equilibrated for 10 min. The contractile response induced by cumulative doses of phenylephrine was determined in the presence or absence of antagonists. Before the addition of phenylephrine, the tissue was equilibrated with antagonist for 20 min. The antagonist dissociation constant (pA_2) was determined by Schild analysis (27).

Results

Cloning of the three human α_1-ARs. From the deduced amino acid sequence, the cloned human $\alpha_{1a/d}$-AR has an overall identity of 83.5% with the rat $\alpha_{1a/d}$-AR. A comparison of the amino acid sequences of only the seven hydrophobic domains that transverse the cell membrane revealed 98% identity between the rat and human α_{1a}-ARs (3, 5). The human $\alpha_{1a/d}$-AR described here exhibited only 86.5% identity with the previously published human H318/3 clone (6). This discrepancy is related, in part, to the presence of an adenosine residue in the carboxyl-terminal region of our gene that is absent from the H318/3 clone and that results in a predicted translational frame shift (6); however, the nucleotide sequence in the carboxyl-terminal region is >99% identical between the two human $\alpha_{1a/d}$-AR clones. Furthermore, the first 178 bp of the H318/3 clone, encompassing the 5′ untranslated region and the 5′ end of the amino-terminal region of the open reading frame, are com-

[3] The sequences of the three human α_1-ARs have been deposited in GenBank (accession numbers: α_{1a}, U03864; α_{1b}, U03865; α_{1c}, U03866).

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pletely divergent from our sequence. Our analysis revealed that nucleotides 1–178 of the published H318/3 are actually the reverse complement of nucleotides 454–630 of H318/3 (6). This indicates that the published amino-terminal end of H318/3 actually contains the reverse complement of the third and fourth transmembrane domains of the same receptor. However, when a comparison was made in the region that lies between the first and seventh transmembrane domains (including intracellular loop 3), the amino acid sequence identity was 100%.

The deduced amino acid sequence of the human α_{1b}-AR is 94.5% and 95.5% identical to the rat (28) and hamster (2) α_{1b}-AR, respectively. In the transmembrane regions the sequence identity of the human α_{1b}-AR is as high as 99%, compared with either rat or hamster α_{1b}-ARs. Moreover, our clone is 99.5% identical from the intron at the sixth transmembrane region to the termination codon and 99.8% identical from the start codon to the intron at the sixth transmembrane region of the previously published human α_{1b}-AR genomic clones (7).

The deduced amino acid sequence of the human α_{1c}-AR showed an overall homology of 92% with the bovine α_{1c}-AR, with 97% identity in the transmembrane regions (4). The deduced amino acid sequence of our α_{1c}-AR clone is 99.6% identical to that recently reported by Hirasawa *et al.* (8), with 99.9% identity at the nucleotide level.

Binding studies with cloned human α_1-ARs. Membrane preparations from LM(tk$^-$) cells stably transfected with the cloned human α_1-AR genes showed saturable binding of [^{3}H]prazosin, with K_d values of 0.21 ± 0.03, 0.3 ± 0.1, and 0.39 ± 0.08 nM at the $\alpha_{1a/d}$-, α_{1b}-, and α_{1c}-ARs, respectively. Consistent with their identity as α_1-ARs, the three cloned α_1-AR subtypes showed an extremely low affinity for the α_2-selective antagonist rauwolscine (Table 1). The endogenous catecholamines norepinephrine and epinephrine and the α-AR agonist phenylephrine were found to be 50-, 9-, and 5-fold more potent, respectively, at the human $\alpha_{1a/d}$-AR than at the α_{1b}-AR subtype. These phenylethylamines were 2–5-fold more potent at the α_{1c}-AR than at the α_{1b}-AR. Prazosin analogues such as terazosin, doxazosin, alfuzosin, and abanoquil showed very small differences in their binding potencies at the different α_1-AR subtypes. However, there were several antagonists that showed marked differences in their potencies to inhibit [^{3}H]prazosin binding to the three cloned human α_1-AR subtypes. Among these were WB-4101 and 5-methylurapidil, which showed high affinity for the human α_{1c} subtype (K_i values of 0.8 and 7 nM, respectively), followed by 2- and 6-fold lower potency, respectively, at the human α_{1a}-AR. However, WB-4101 and 5-methylurapidil were found to be 15- and 36-fold less potent, respectively, at the α_{1b}-AR than at the α_{1c} subtype. Indoramin and its analogue SNAP-1069 were 50- and 10-fold more potent, respectively, at the α_{1c}-AR than at the $\alpha_{1a/d}$ and α_{1b} subtypes, suggesting that these are selective α_{1c}-AR antagonists. The calcium channel antagonist (+)-niguldipine was found to be selective for the receptor encoded by the α_{1c}-AR gene and showed 107- and 48-fold lower potency for the $\alpha_{1a/d}$- and α_{1b}-AR subtypes, respectively. The α_2-AR antagonist SK&F104856 (29) was found to be selective for the human $\alpha_{1a/d}$ subtype, with 10- and 8-fold lower potency at the α_{1b}- and α_{1c}-AR subtypes, respectively (Table 1).

Effects of CEC on CHO cells expressing the cloned human α_1-ARs. As shown in Table 2, when intact LM(tk$^-$) cells transfected with the cloned human $\alpha_{1a/d}$-AR and CHO cells transfected with the α_{1b}-AR were incubated for 20 min

TABLE 1

Potency of αAR agonists and antagonists at the cloned human α_1-ARs

Equilibrium competition binding assays using [^{3}H]prazosin were performed as described in Materials and Methods, in membrane preparations from cultured LM (tk$^-$) cells stably transfected with the cloned human $\alpha_{1a/d}$-, α_{1b}-, and α_{1c}-ARs. Estimates of equilibrium inhibition constants are shown as pK_i (−log K_i) values and were determined by nonlinear regression analysis. n_H, pseudo-Hill coefficient. Values are mean ± standard error of three independent experiments. Statistical significance was assessed by one-way analysis of variance, and p values were corrected for multiple comparisons.

Compound	pK_i (n_H)		
	$\alpha_{1a/d}$	α_{1b}	α_{1c}
Agonists			
Norepinephrine	5.81 ± 0.13 (0.8 ± 0.1)	4.86 ± 0.11 (0.8 ± 0.1)	5.10 ± 0.09 (0.8 ± 0.2)
Epinephrine	6.31 ± 0.06[a,b] (0.8 ± 0.1)	5.36 ± 0.06 (0.9 ± 0.1)	5.59 ± 0.08 (0.8 ± 0.1)
Phenylephrine	5.49 ± 0.13 (0.9 ± 0.03)	4.73 ± 0.11 (0.8 ± 0.1)	5.08 ± 0.13 (0.9 ± 0.1)
Antagonists			
Abanoquil	10.4 ± 0.01 (1.1 ± 0.09)	10.1 ± 0.02 (0.9 ± 0.1)	10.4 ± 0.03 (1.0 ± 0.2)
Alfuzosin	8.40 ± 0.05 (0.9 ± 0.1)	8.53 ± 0.05 (1.0 ± 0.1)	8.20 ± 0.10 (1.0 ± 0.1)
Doxazosin	8.78 ± 0.07 (1.0 ± 0.02)	8.98 ± 0.05 (0.9 ± 0.1)	8.56 ± 0.07 (0.9 ± 0.1)
Indoramin	6.74 ± 0.1 (1.0 ± 0.1)	7.39 ± 0.1 (1.0 ± 0.1)	8.35 ± 0.1[b,c] (1.0 ± 0.1)
5-Methylurapidil	7.91 ± 0.07[b] (1.0 ± 0.1)	6.76 ± 0.17 (1.0 ± 0.04)	8.68 ± 0.09[b,c] (0.9 ± 0.1)
(+)-Niguldipine	6.72 ± 0.08 (0.9 ± 0.1)	7.07 ± 0.12 (1.0 ± 0.1)	8.75 ± 0.10[b,c] (1.1 ± 0.1)
Prazosin	9.48 ± 0.11 (1.0 ± 0.1)	9.26 ± 0.13 (1.0 ± 0.1)	9.23 ± 0.08 (1.0 ± 0.1)
Rauwolscine	5.31 ± 0.03 (1.0 ± 0.1)	5.33 ± 0.34 (1.0 ± 0.1)	5.13 ± 0.43 (0.9 ± 0.1)
SK&F104856	8.48 ± 0.06[a,b] (1.0 ± 0.1)	7.70 ± 0.09 (0.9 ± 0.1)	7.60 ± 0.16 (1.0 ± 0.04)
SNAP-1069	6.12 ± 0.18 (0.9 ± 0.1)	6.76 ± 0.28 (0.9 ± 0.1)	7.83 ± 0.03 (1.0 ± 0.1)
Terazosin	8.46 ± 0.04 (0.9 ± 0.1)	8.71 ± 0.04 (0.9 ± 0.1)	8.16 ± 0.02 (1.0 ± 0.1)
WB-4101	9.04 ± 0.14 (0.9 ± 0.1)	8.34 ± 0.1 (1.0 ± 0.1)	9.41 ± 0.2[b] (0.9 ± 0.1)

[a] $p < 0.05$ versus α_{1c}.
[b] $p < 0.05$ versus α_{1b}.
[c] $p < 0.05$ versus α_{1a}.

TABLE 2

Effects of CEC on the binding of [^{3}H]prazosin to cloned human α_1-ARs

Intact LM (tk$^-$) cells stably transfected with the cloned human $\alpha_{1a/d}$-AR and CHO cells stably transfected with α_{1b}- and α_{1c}-ARs were incubated in phosphate-buffered saline in the absence (control) and presence of 100 μM CEC (CEC) for 20 min at 37°. Cell cultures were washed three times with 3 volumes of phosphate-buffered saline, and membrane proteins were prepared as described in Materials and Methods. To determine the number of receptors, membrane preparations were incubated with [^{3}H]prazosin (from 6×10^{-12} to 6×10^{-9} M), in the absence or presence of 10 μM phentolamine. The reaction mixture was incubated for 30 min at 37°, and bound [^{3}H]prazosin was separated by filtration, as described in Materials and Methods. Data are mean ± standard error of the K_d and B_{max} estimates from three independent experiments.

Subtype	Control		CEC		Inactivation
	K_d	B_{max}	K_d	B_{max}	
	nM	pmol/mg of protein	nM	pmol/mg of protein	%
$\alpha_{1a/d}$-AR	0.21 ± 0.03	0.72 ± 0.04	0.18 ± 0.02	0.19 ± 0.02[a]	74
α_{1b}-AR	0.5 ± 0.049	11 ± 1.5	0.09 ± 0.01[a]	0.95 ± 0.07[a]	91
α_{1c}-AR	0.33 ± 0.03	4.0 ± 0.04	0.31 ± 0.01	3.3 ± 0.15	18

[a] $p < 0.05$ versus control.

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with phosphate-buffered saline containing 100 μM CEC, there was a 74% and 91% reduction, respectively, in the total number of receptors measured by [^{3}H]prazosin binding. However, exposure of CHO cells transfected with the α_{1c}-AR to CEC resulted in only an 18% reduction in the total number of binding sites, with no significant effects on [^{3}H]prazosin affinity. Consistent with these findings, similar treatment with CEC resulted in a 10% reduction of the phosphoinositide response induced by norepinephrine in CHO cells transfected with the α_{1c}-AR, whereas 70% of this response was inhibited in CHO cells transfected with the α_{1b}-AR (data not shown). When membrane preparations from CHO cells transfected with the human α_{1c}-AR were incubated for 20 min with 100 μM CEC in hypotonic buffer (50 mM Tris·HCl, 1 mM $MgCl_2$, pH 7.5), there was a 71% reduction in the number of receptors (from 3.1 ± 0.2 to 0.9 ± 0.08 pmol/mg of protein, three experiments), compared with untreated membranes. These results are similar to those reported recently for the human cloned α_{1c}-AR (8) and are in agreement with earlier observations that when the exposure of α_1-ARs to CEC is performed in hypotonic medium there is an increase in the loss of α_1 binding sites (30).

Agonist-induced contraction of human prostate smooth muscle. To identify which α_1-AR subtype is involved in regulating smooth muscle tone, we assessed the potency of a set of selective or nonselective α_1-AR antagonists to inhibit the phenylephrine-induced contraction of human prostate *in vitro*. Two nonselective α-AR antagonists, prazosin and terazosin, were found to be potent at inhibiting the agonist-induced contraction of human prostate (Fig. 1; Table 3). However, the potencies of 5-methylurapidil, indoramin, and its analogue SNAP-1069 to block the agonist-induced contraction were found to be closest to their affinities at the cloned human α_{1c} subtype. Similarly, the $\alpha_{1a/d}$-selective compound SK&F104856 inhibited the contractile response with a potency similar to that found at the cloned α_{1c} subtype. The comparisons of the binding and functional potencies of these six antagonists indicate that there is a close correlation between the potency to inhibit the α_1-AR-mediated contraction and the potency to inhibit [^{3}H]prazosin binding at the human α_{1c}-AR ($r = 0.94$ for α_{1c} versus 0.34 and 0.58 for $\alpha_{1a/d}$ and α_{1b} subtypes, respectively) (Fig. 2).

Discussion

One of the objectives of the present study was to utilize *in vitro* expression systems for genes encoding G protein-coupled receptors to characterize the pharmacological properties of the human homologues of the three α_1-ARs. To our knowledge, this is the first time that the pharmacological properties of the three α_1-AR subtypes from the same species have been studied simultaneously. The classical α_1-AR antagonist prazosin and the prazosin analogues terazosin, doxazosin, and alfuzosin were found to be potent but nonselective ligands at the three human α_1-AR subtypes. Our data indicate that abanoquil, another prazosin analogue, also has similar potency at the three human α_1-ARs. This observation is at variance with a recent report that found abanoquil to be 70-fold more potent at the cloned rat $\alpha_{1a/d}$-AR than at the cloned hamster α_{1b}-AR or bovine α_{1c}-AR (31). The reasons for this discrepancy are presently unknown, and based on our results the potency of abanoquil to block human prostate contraction is useless for identification of a specific human α_1-AR subtype. The results of the binding profile of the human α_1-ARs using selective α-AR antagonists clearly define three different receptor subtypes. The human α_{1c}-AR is characterized by the following rank order of potencies: WB-4101 > (+)-niguldipine ≥ 5-methylurapidil ≥ S&KF104856 > indoramin > SNAP-1069. Because these antagonists are selective for the α_{1c} subtype, they can be used to differentiate the α_{1c}-AR from the other two receptor subtypes. We found that the rank order of potencies for these antagonists at the α_{1a} subtype is WB-4101 > S&KF104856 > 5-methylurapidil ≫ (+)-niguldipine = indoramin ≥ SNAP-1069. However, WB-4101 and 5-methylurapidil are limited in their ability to differentiate the $\alpha_{1a/d}$ subtype from the α_{1c} subtype. The α_{1b}-AR subtype can be differentiated from the other two subtypes because it shows at least 10-fold lower affinity for both WB-4101 and 5-methylurapidil and close to 50-fold lower affinity for (+)-niguldipine. There is complete agreement between our results and those reported recently for the cloned human

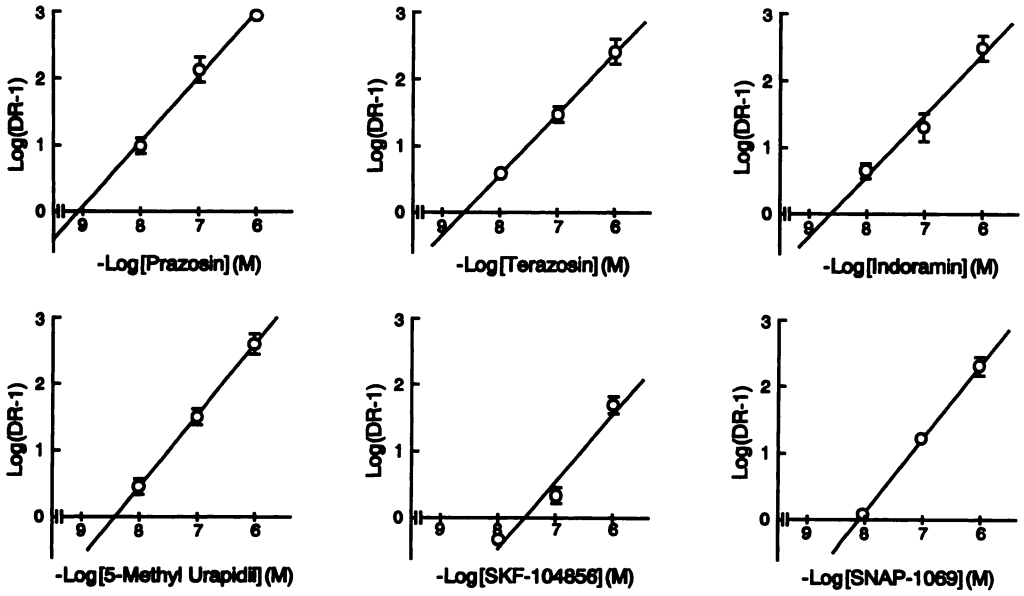

Fig. 1. Schild regressions of the inhibition by α-AR antagonists of the phenylephrine-induced contraction of human prostate. Strips of human prostate were incubated in the absence or presence of antagonist (from 10nM to 1μM), and the contractions induced by cumulative concentrations of phenylephrine (from 10nM to 10mM) were measured as described in Materials and Methods. Dose ratios were calculated from the ratio of the EC_{50} values for phenylephrine in the presence and absence of antagonist. Each *point* represents the mean ± standard error of three independent experiments. The *lines* represent the best fit from linear regression analysis.

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TABLE 3

Potency of α-AR antagonists to inhibit phenylephrine-induced contraction of prostate smooth muscle

Data are shown as the mean ± standard error of the *x*-intercept (pA_2) and slope estimates, from linear regression analysis of the data shown in Fig. 1.

Compound	pA_2	Slope
Indoramin	8.64 ± 0.27	0.9 ± 0.1
5-Methylurapidil	8.55 ± 0.65	1.0 ± 0.2
Prazosin	9.13 ± 0.29	1.0 ± 0.1
SK&F104856	7.51 ± 0.17	1.0 ± 0.2
SNAP-1069	7.93 ± 0.03	1.1 ± 0.03
Terazosin	8.48 ± 0.01	1.0 ± 0.01

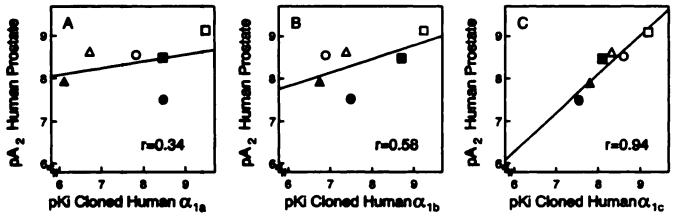

Fig. 2. Correlation plots of the potency of α-AR antagonists to inhibit the contractile response mediated by α_1-ARs in the human prostate versus their binding potency at the cloned human α_1-AR subtypes. The pK_i values from Table 1 were compared with the pA_2 values listed in Table 2 for the following compounds: prazosin (□), terazosin (■), indoramin (▲), SNAP-1069 (△), 5-methylurapidil (○), and SK&F104856 (●). The parameters from the regression lines were correlation coefficient (r) = 0.34 and slope = 0.16 ± 0.21 (A), r = 0.58 and slope = 0.33 ± 0.23 (B), and r = 0.94 and slope = 0.91 ± 0.16 (C).

α_{1c}-AR (8). However, in a comparison of our data with those reported earlier for the rat $\alpha_{1a/d}$-, hamster α_{1b}-, and bovine α_{1c}-ARs, only the rank orders of potencies of selective α_1-AR antagonists are similar (4, 32).

Our results indicate that the cloned human $\alpha_{1a/d}$-AR does not have the pharmacological properties of the α_{1A} subtype, because it has 100-fold lower affinity than the α_{1c}-AR for the α_{1A}-selective antagonist (+)-niguldipine (33). Similar observations were made by others in the case of the rat α_{1a}-AR and led to its designation as the α_{1D}-AR (5) and to the proposal of the existence of a fourth α_1-AR (32). However, the findings of this study indicate that the gene encoding the human α_{1c}-AR has the pharmacological properties predicted for the α_{1A} subtype, i.e., 1) high affinity for 5-methylurapidil, WB-4101, and (+)-niguldipine (33, 34) and 2) resistance to inactivation by CEC (30). In fact, the selectivity of 5-methylurapidil, WB-4101, and (+)-niguldipine for the human α_{1c}-AR is similar to that observed for the cloned bovine α_{1c}-AR, compared with the rat α_{1a} and α_{1b} subtypes (32). At variance with the properties of the bovine α_{1c}-AR (3), the human α_{1c}-AR showed a much higher resistance to inactivation by CEC, suggesting that there is a species-related difference in the sensitivity to CEC. Furthermore, the premise that the α_{1c}-AR has the properties of the α_{1A} subtype is also supported by our findings that the rat homologue of the bovine and human α_{1c}-ARs also exhibits an α_{1A}-like pharmacological profile when expressed in COS-7 cells (35). Thus, the comparison of the three rat and human α_1-ARs indicates that the α_{1c}-AR gene encodes an α_1-AR with the pharmacological attributes of the α_{1A} subtype.

Recent binding studies indicate that there is a mixed population of α_1-ARs in the human prostate, 62% of which showed high affinity for 5-methylurapidil and WB-4101 (36). Moreover, the potency of these two antagonists to inhibit the agonist-induced contraction of human prostate led to the suggestion that the α_{1A} subtype mediated this response. However, given the binding profile obtained for these compounds with the cloned human receptors, the contractile response of the human prostate could be mediated by the α_1-AR encoded by either the $\alpha_{1a/d}$- or α_{1c}-AR genes, or both. As mentioned above, (+)-niguldipine was found to have the greatest separation of binding affinities between the $\alpha_{1a/d}$- and α_{1c}-AR subtypes. Unfortunately, the potent antagonist activity of (+)-niguldipine at L-type calcium channels precluded its inclusion in the contractile studies. However, the potency of the α_{1c}-selective antagonists indoramin, 5-methylurapidil, and SNAP-1069, as well as the potency observed for the $\alpha_{1a/d}$-selective compound SK&F104856 to inhibit the contractile response, is in agreement with the affinities of these compounds at the α_{1c} subtype (Fig. 2C). Thus, the evidence derived from the use of selective α_1-AR antagonists strongly supports the conclusion that the α_1-AR that mediates the contraction of human prostate has the same pharmacological properties as does the cloned human α_{1c}-AR subtype. In support of this conclusion is the evidence that the α_{1c}-AR mRNA represents 70% of the total α_1-AR mRNA of the human prostate (37). The fact that the expression of α_{1c}-AR mRNA is predominantly localized to the stromal compartment of the human prostate, which is rich in smooth muscle, is also in agreement with the involvement of this receptor subtype in the contractile response of prostate smooth muscle. The functions of the other α_1-AR subtypes in human prostate remain to be established, although preliminary evidence indicates that the $\alpha_{1a/d}$ subtype is localized to the glandular component.[4]

It is noteworthy that the current therapies for the treatment of symptomatic BPH include several α_1-AR antagonists, such as terazosin, doxazosin, and alfuzosin, that show no selectivity for the human α_1-AR subtypes. Although their nonselectivity does not preclude their clinical efficacy, it is likely that these drugs block α_1-ARs that are involved in a broad range of physiological actions. In fact, results from clinical studies with terazosin have shown that the clinical response to α_1-AR antagonists is dose dependent and that the development of adverse events limits the potential therapeutic benefit for some patients with BPH (38). Thus, it is conceivable that α_{1c}-selective antagonists might have a more favorable pharmacological profile for the treatment of symptomatic BPH. Recent studies on the expression of α_1-AR mRNA in human tissues suggest that arterial tissue expresses similar levels of $\alpha_{1a/d}$- and α_{1b}-AR mRNA but very low levels of α_{1c}-AR (39). This observation suggests that nonselective α_1-AR antagonists may produce their effects on arterial tone by actions at $\alpha_{1a/d}$- or α_{1b}-ARs. These findings emphasize the potential for α_{1c}-selective antagonists to have minimal effects on the α_1-AR involved in the control of arterial tone.

In summary, as a result of the characterization of the α_1-ARs encoded by the three human α_1-AR genes, we have obtained evidence suggesting that the gene encoding the α_{1c}-AR subtype has the pharmacological properties of the classically defined α_{1A} subtype. Furthermore, by developing an understanding of the pharmacological properties of the cloned human α_1-AR subtypes we have been able to identify the α_{1c}-AR subtype as the AR responsible for the contraction of human prostate smooth muscle. These findings support the hypothesis that the

[4] H. Lepor, personal communication.

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development of an α_{1c}-selective antagonist might result in a therapeutically effective agent with reduced side effects for the treatment of symptomatic BPH.

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Send reprint requests to: Carlos Forray, Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652-1410.

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