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In vitro studies on L-771,688 (SNAP 6383), a new potent and selective α_{1A}-adrenoceptor antagonist

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Abstract

L-771,688 (SNAP 6383, methyl(4*S*)-4-(3,4-difluorophenyl)-6-[(methyloxy)methyl]-2-oxo-3-[({3-[4-(2-pyridinyl)-1-piperidinyl]propyl}amino)carbonyl]-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate) had high affinity ($K_i \leq 1$ nM) for [^{3}H]prazosin binding to cloned human, rat and dog α_{1A}-adrenoceptors and high selectivity (> 500-fold) over α_{1B} and α_{1D}-adrenoceptors. [^{3}H]Prazosin/(±)-β-[^{125}I]-4-hydroxy-phenyl)-ethyl-aminomethyl-teralone([^{125}I]HEAT) binding studies in human and animal tissues known to contain α_{1A} and non-α_{1A}-adrenoceptors further demonstrated the potency and α_{1A}-subtype selectivity of L-771,688. [^{3}H]L-771,688 binding studies at the cloned human α_{1A}-adrenoceptors and in rat tissues indicated that specific [^{3}H]L-771,688 binding was saturable and of high affinity(K_d = 43–90 pM) and represented binding to the pharmacologically relevant α_{1A}-adrenoceptors. L-771,688 antagonized norepinephrine-induced inositol-phosphate responses in cloned human α_{1A}-adrenoceptors, as well as phenylephrine or A-61603 (*N*-[5-4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-terahydro-naphthlen-1-yl] methanesulfonamide hydrobromide) induced contraction in isolated rat, dog and human prostate, human and monkey bladder neck and rat caudal artery with apparent K_b values of 0.02–0.28 nM. In contrast, the contraction of rat aorta induced by norepinephrine was resistant to L-771,688. These data indicate that L-771,688 is a highly selective α_{1A}-adrenoceptor antagonist.

Keywords: α_{1A}-Adrenoceptor; Adrenoceptor; α_{1A}-Adrenoceptor antagonist; Prostate; Adrenoceptor subtype

1. Introduction

Three subtypes of α_1-adrenoceptors, namely, α_{1A}, α_{1B}, and α_{1D} (Bylund et al., 1998) have been cloned over recent years. The discrete distribution of these receptor subtypes in various tissues has been demonstrated using functional, radioligand binding and molecular biological studies. The existence of different α_1-adrenoceptors in diverse tissues may provide therapeutic opportunity by selectively blocking the desirable subtype in target tissue without affecting other tissues having other subtypes, thus minimizing adverse effects. Subtype nonselective α_1-adrenoceptor antagonists such as terazosin and doxazosin which were originally developed for hypertension have been used for the treatment of benign prostate hyperplasia. These agents were shown to be effective but may cause orthostatic hypotension because of the existence of α_1-adrenoceptors in the vasculature (Chapple, 1995). Subsequently, it was shown that α_{1A}-adrenoceptor subtype was predominant in human prostate (Price et al., 1993; Yazawa and Honda, 1993; Laz et al., 1994; Forray et al., 1994; Chang et al., 1996; Moriyama et al., 1997). Moreover, it was demonstrated that selective α_{1A}-adrenoceptor antago-

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nists preferentially lower urethral pressure without affecting blood pressure in rat and dog (Broten et al., 1999; Akiyama et al., 1999; Pulito et al., 2000). These facts indicate the possibility that an α_{1A}-adrenoceptor selective antagonist may be effective for the treatment for benign prostate hyperplasia with fewer side effects. Tamsulosin, which was approved recently for treatment of benign prostate hyperplasia, was claimed to be α_{1A}-adrenoceptor selective though its selectivity was relatively low, approximately only 10–15 × vs. α_{1B}-adrenoceptor and less than 5 × vs. α_{1D}-adrenoceptor (Shibata et al., 1995; Richardson et al., 1997; Pulito et al., 2000). Hence, efforts have been made by us and others to develop α_{1A}-adrenoceptor selective antagonists and several compounds have been recently reported (Wetzel et al., 1995; Shibata et al., 1995; Adkison et al., 1998; Nagarathnam et al., 1998; Williams et al., 1999; Pulito et al., 2000). Here, we report the in vitro pharmacological characterization of L-771,688. L-771,688 is the first truly selective α_{1A}-adrenoceptor antagonist to be reported to have efficacy on urodynamic in BPH patients (Marks et al., 2000).

2. Methods

2.1. Preparation of methyl(4S)-4-(3,4-difluorophenyl)-6-[(methyloxy)methyl]-2-oxo-3-[({3-[4-(2-pyridinyl)-1-piperidinyl]propyl}amino)carbonyl]-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate (l-771,688)

The preparation of L-771,688 (7) was carried out as outlined in Fig. 1. Accordingly, equimolar amounts of 2,4′-dipyridyl (1) and 3-bromopropylamine hydrobromide (2) were heated in a solution of dimethylformamide. The resulting white solid (3) was dissolved in methanol at 0°C and this solution was treated with sodium borohydride over a 2-h period. The reaction mixture was then stirred at ambient temperature overnight. Extractive workup gave intermediate 4 which was further hydrogenated catalytically with palladium hydroxide in methanol to afford 5 as yellow oil. The latter compound was combined with the 2-oxo-tetrahydropyrimidine (6) (Dhar et al., 1999) in tetrahydrofuran at ambient temperature to afford the title compound 7 as a foam. All intermediates were fully characterized chromatographically and spectroscopically, including 7, which had the following properties as the hydrochloride salt: ^{1}H nuclear magnetic resonance ($CDCl_3$) δ 2.05–2.20 (m, 4 H), 2.77–2.88 (m, 2 H), 3.00–3.20 (m, 4 H), 3.35–3.47 (m, 2 H), 3.47 (s, 3 H), 3.64–3.70 (m, 2 H), 3.706 (s, 3 H), 4.05 (br t, 1 H), 4.672 (s, 2 H), 6.59 (s, 1 H), 7.05–7.20 (m, 3 H), 7.78 (t, 1 H), 8.00 (d, 1 H), 8.43 (dt, 1 H), 8.66 (d, 1H), 8.96 (br t, 1 H, NH), 12.422(br s, 1 H). Melting point 188–191°C; $[\alpha]_D = +141$ ($c = 0.265$, MeOH). Analysis ($C_{28}H_{34}N_5O_5F_2Cl \cdot 0.6H_2O$) C, H, N. C, 52.36; H, 5.84; N, 10.90. Found: C, 52.24; H, 5.96; N, 10.80.

2.2. Binding affinity at cloned human, dog and rat α_1-adrenoceptor subtypes

The cloning of the human and rat α_1-adrenoceptor subtypes has been described elsewhere (Forray et al., 1994; Laz et al., 1994). The full-length DNA encoding the

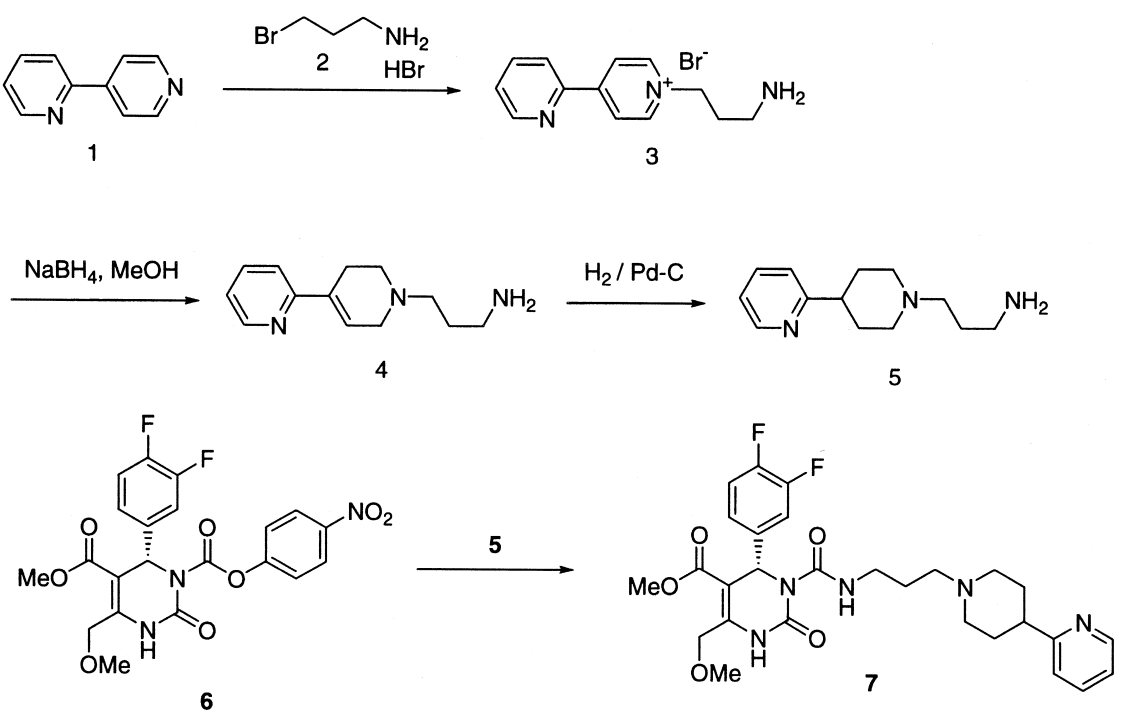

Fig. 1. Scheme outlining the synthesis of L-771,688.

dog α_{1A}-adrenoceptor was constructed by ligating *Xho*I/*Msc*I fragments of a genomic clone, representing the 5′ end of the receptor, with a *Msc*I/*Xba*I fragment of a cDNA clone, representing the 3′ end of the receptor, into the eukaryotic expressing vector pcEXV-3. Clones containing fragments were initially isolated from dog spleen genomic and dog liver cDNA libraries by reduced stringency homology screening using bovine and human α_{1A}-adrenoceptor probes, respectively. The full-length cDNA encoding the dog α_{1B}-adrenoceptor was isolated from dog brain plasmid cDNA library using exact probes based on the sequences of RDC5 (a code for the partial dog α_{1B}-adrenoceptor gene as described in GenBank accession number X14050) and a partial dog genomic clone isolated by homology with human α_{1B}-adrenoceptor probes. The full-length DNA encoding dog α_{1D}-adrenoceptor was constructed by ligating an *Xcm*I/*Sal*I fragment of a cDNA clone, representing the 5′ end of the receptor, with an *Xcm*I/*Sal*I fragment of a cDNA clone, representing the 3′ end of the receptor, into the eukaryotic expressing vector pcEXV-3. Clones containing the fragments were initially isolated from dog spleen genomic and dog brain cDNA libraries, respectively, by hybridization with human α_{1D}-adrenoceptor probes at reduced stringency.

The cDNA encoding each of the human, dog and rat α_1-adrenoceptor subtypes were transfected into either Chinese hamster ovary (CHO), human embryonic kidney (HEK-A293) or mouse fibroblast [$LM_{(tk-)}$, thymidine kinase negative mutant] cells and stable lines expressing each receptor were selected. Membranes were prepared from CHO cells expressing the human α_{1A}-, the rat α_{1A}-, α_{1B}- and α_{1D}-, the dog α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, $LM_{(tk-)}$ cells expressing the human α_{1B}- and HEK-A293 cells expressing the human α_{1D}-adrenoceptors and binding assay using [^{3}H]prazosin were performed as described previously (Forray et al., 1994). Binding data were analyzed using Graph-Pad Prism curve-fitting software. K_i values were calculated using the following formula, $K_i = IC_{50}/(1 + L/K_d)$, where L was the radioligand concentration used and K_d was the dissociation constant of the radioligand.

2.3. Binding affinities at α_1-adrenoceptors in tissue membranes

2.3.1. Preparation of tissue membranes

Frozen human tissues were obtained from National Disease Research Interchange (Philadelphia, PA). Animal tissues from rat and dog were obtained fresh and used immediately or stored frozen before use. Tissue membranes were prepared as described previously (O'Malley et al., 1998).

2.3.2. Binding assays

Tissue membrane pellets were resuspended in 50 mM Tris–hydrochloride (pH 7.4), 150 mM sodium chloride and 5 mM disodium-ethylenediamine tetraacetate (EDTA) buffer at the indicated tissue membrane concentrations (number in milliliter of binding assay buffer per gram of original tissue wet weight) as follows: human aorta (30), rat and human prostate (50), dog prostate (60), rat and human cerebral cortex, liver and dog aorta (100). [^{3}H]Prazosin or (±)-β-[^{125}I]-4-hydroxy-phenyl)-ethyl-aminomethyl-teralone ([^{125}I]HEAT) binding assays were performed as described (O'Malley et al., 1998).

2.4. [^{3}H]L-771,688 binding

[^{3}H]L-771,688 (13 Ci/mmol) was prepared by a catalytic reduction of the precursor, L-797,429, (3-[3-(3′,6′-dihydro-2′*H*-[2,4′]bipyridinyl-1′-yl)- propylcarbamoyl]-6-methoxymethyl-4-(3,4-difluorophenyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid methyl ester) in the presence of tritium gas followed by preparative high pressure liquid chromatography. Receptor membranes were prepared as described for [^{3}H]prazosin/[^{125}I]HEAT binding assays. To measure [^{3}H]L-771,688 binding, 980 μl of membranes (cloned human α_{1A} or rat tissues) were added to triplicate tubes containing 10 μl of dimethyl sulfoxide (DMSO) (for total binding) or phentolamine (10 μM final concentration, for nonspecific binding) or test compounds (at the desired final concentrations) and 10 μl of [^{3}H]L-771,688 (0.3–0.6 nM final concentration for routine studies and 10 pM–5 nM for saturation assays). [^{3}H]L-771,688 was diluted in DMSO/methanol/water (1:1:2) from stock solution to minimize its loss to the wall of test tubes. The binding reaction was conducted at 25°C for 1 h or various time intervals in the association rate studies. The reaction mixtures were filtered and washed as described previously (O'Malley et al., 1998).

2.5. α_1-Adrenoceptor antagonist activity at cloned receptors

CHO cells expressing the human α_{1A}-adrenoceptors were plated in 96-well plates and grown to confluence. Two days prior to the assay, 0.5 μCi [^{3}H]*myo*-inositol was added to the media. Immediately before the assay, the medium was removed and replaced by 180 μl of physiological buffered saline (PBS) containing 10 mM lithium chloride, and the cells were equilibrated with the new medium for 20 min at 37°C. In some experiments, L-771,688 or terazosin was also added during this period (pre-incubation), whereas in other experiments, the antagonists were added at the same time as the agonist, norepinephrine (co-incubation). All assays were performed in duplicate. The [^{3}H]inositol-phosphate accumulation was started by adding 20 μl of various concentrations of norepinephrine or PBS (basal accumulation). The plates were incubated in a CO_2 incubator for 60 min. The reaction was terminated by adding 40 μl of 50% trichloroacetic acid followed by a 10-min incubation at 4°C. After

neutralizing the acidified samples with 40 μl of 1 M Tris, the content of the wells was transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200–400 mesh, formate form). The filter plates were placed in a vacuum manifold. Each well was washed two times with 200 μl of water followed by 200 μl of 5 mM sodium tetraborate in 60 mM ammonium formate. The [^{3}H]inositol-phosphates were eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 M formic acid and the radioactivity was determined. Similar experiments were performed using cells expressing the human α_{1B}- and α_{1D}-adrenoceptors.

2.6. α_1-Adrenoceptor antagonism in isolated tissue preparations

Rat, dog and monkey prostate tissues were dissected free from adipose and connective tissues. For each rat prostate, two strips corresponding to two ventral lobes were obtained. Each dog or monkey prostate were cut into six to eight pieces longitudinally along the urethra opening. Human prostate chips from transurethral surgery of benign prostate hyperplasia (provided by Dr. J. Seidmon, Temple University and Dr. T. Malloy, Pennsylvania Hospital) and human bladder neck (Dr. T. Malloy) cut into $\sim 3 \times 3 \times 8$ mm were used the same day after operation or stored overnight in ice-cold Krebs solution (sodium chloride, 118 mM; potassium chloride, 4.7 mM; calcium chloride, 2.5 mM; potassium phosphate monobasic, 1.2 mM; magnesium sulfate, 1.2 mM; sodium bicarbonate, 25 mM; dextrose, 11 mM) or normal saline, if needed. Each rat thoracic aorta was cut into four piece of rings. Rat caudal artery from each animal was spirally cut and divided into two equal-size pieces.

Isolated tissues were mounted on glass tissue holders and placed in 5-ml tissue baths containing Krebs solution (supplemented with 1 μM normetanephrine, 1 μM (±)propranolol, 0.1 μM desipramine, 1 μM idazoxan and 7.6 mg/l disodium-EDTA when norepinephrine was used as an agonist). Contractile tension was recorded by means of Statham force transducers and 7700 series Hewlett-Packard recorders. One gram (rat prostate, aorta and caudal artery and monkey prostate or bladder neck), 1.5 g (dog prostate) or 0.75 g (human prostate and bladder neck) of tension was applied to the tissues. The tissue baths were kept at 37°C and aerated with 95% O_2–5% CO_2. The tissues were allowed to equilibrate for 1 h and were washed every 10 min. After a single priming dose (at concentrations which elicited approximately maximal responses from preliminary studies) of phenylephrine (3 μM for rat prostate and 10 μM for dog prostate), norepinephrine (1 μM for rat aorta) or a highly selective α_{1A}-adrenoceptor agonist A61603 (*N*-[5-4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-terahydro-naphthlen-1-yl] methanesulfonamide hydrobromide) [(Knepper et al., 1995), (10 nM for rat prostate, 100 nM for human bladder neck and rat caudal artery, 300 nM for human prostate/bladder neck)], tissues were washed every 10 min for 1 h. Agonists were then added cumulatively to the baths. The tissues were then washed every 10 min over 1-h period. After washout, the tissues were treated with vehicle (0.1% DMSO) or various concentrations of antagonists for 1 or 2 h. Cumulative concentration–response curves for agonists in the presence of antagonists and vehicle were again determined. In some experiments, the first cumulative concentration–response curves were omitted. The contractile responses were expressed as the percentages of the maximal responses of the first concentration–response curves or the priming responses if only one cumulative concentration–response was obtained. EC_{50} values were calculated for each groups using GraphPad Inplot software. K_b values were calculated as follows: $K_b = B/(x-1)$,

Table 1
Inhibition of [^{3}H]prazosin binding to cloned human, rat and dog α_1-adrenoceptors by L-771,688 and terazosin

Compound	K_i (nM)[a]		
	α_{1A}	α_{1B}[b]	α_{1D}[b]
L-771,688			
Human	0.36 ± 0.08	362 ± 38 (1006×)	618 ± 128 (1725×)
Rat	0.43 ± 0.02	566 ± 65 (1316×)	642 ± 2 (1493×)
Dog	1.04 ± 0.13	611 ± 240 (588×)	1102 ± 377 (1060×)
Terazosin			
Human	4.40 ± 1.58	2.61 ± 0.04 (0.59×)	4.04 ± 0.17 (0.92×)
Rat	5.28 ± 1.38	2.55 ± 0.62 (0.48×)	3.43 ± 0.74 (0.65×)
Dog	18.64 ± 6.69	3.37 ± 0.71 (0.18×)	5.58 ± 2.10 (0.30×)

[a]Values are group means ± S.E.M. ($n = 3$).
[b]Values in parentheses are selectivities versus the α_{1A}-adrenoceptor subtype.

Table 2
Binding affinities (K_i, nM) for L-771,688 and terazosin at α_1-adrenoceptors in various tissues

	L-771,688	Terazosin
Human		
Prostate[a]	0.13 ± 0.032	3.0 ± 0.46
Cerebral cortex[b]	0.066 ± 0.015 (0–27%) 81 ± 18 (73–100%)	1.9 ± 0.27
Aorta[b]	410 ± 100	2.3 ± 0.31
Rat		
Prostate[a]	0.11 ± 0.016 (75–100%) 220 (0–25%)	1.9 ± 0.57
Liver[a]	160	1.0 ± 0.17
Dog		
Prostate[b]	0.49 ± 0.087 (45–100%) 610 ± 150 (0–55%)	35 ± 11
Aorta[b]	340	2.0 ± 0.26

Values in parentheses are percentages of high and low affinity sites. K_i values are means ± S.E.M. from three or more determinations, values without S.E.M. are means of two experiments.
[a][^{3}H]prazosin.
[b][^{125}I]HEAT.

where B is the concentration of antagonist used and x is the ratio of EC_{50} values in the presence and absence (vehicle group) of antagonist.

2.7. Source of compounds

L-771,688 was synthesized in Synaptic Pharmaceutical, Paramus, NJ. [^{3}H]L-771,688, A61603, GG818 (5-(2-{4-[5-(4-fluoro-phenyl)-4-(2,2,2 -trifluoro-ethoxymethyl) methyl -oxazol-2-yl]piperidin-1-yl}-ethyl)-2-methoxy-benzenesulfonamide), BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione), L-765,314 (4- amino- 2- [4-1-benzyloxycarbonyl)-2(S)-[[1,1 -dimethyl-ethyl)amino]carbonyl] -piperazinyl-6,7- dimethoxyquinazoline) and terazosin were prepared in Merck Research Laboratories, West Point, PA. Other chemicals were from commercial sources.

3. Results

3.1. Binding affinity of l-771,688 at cloned α_1-adrenoceptor subtypes

L-771,688 inhibited the binding of [^{3}H]prazosin to membranes from cells expressing the human α_{1A}-adrenoceptors with a very high affinity (Table 1). It exhibited lower affinities for the human α_{1B}- and α_{1D}-adrenoceptors with resulting high α_{1A}-adrenoceptor selectivity ratios (Table 1). Similar high affinities and selectivities for the α_{1A}-adrenoceptors were seen with cloned receptors from the rat and dog (Table 1). In comparison, terazosin had lower affinity at the α_{1A}-adrenoceptors and was essentially nonselective versus the α_{1B}- and α_{1D}-adrenoceptor subtypes across species (Table 1).

3.2. Binding affinity at α_1-adrenoceptor in tissue membranes

L-771,688 inhibited specific [^{3}H]prazosin binding to α_1-adrenoceptors in human prostate tissue in a manner best fit by a one-site competition model with a K_i value of 0.13 nM (Table 2). In rat and dog prostate, however, high and low affinity sites were observed with L-771,688 (Table 2). The binding profiles, therefore, were consistent with a nearly exclusive α_{1A}-adrenoceptor population in human prostate, however, the existence of lower affinity α_{1B}- and/or α_{1D}-adrenoceptor is evident in rat and dog prostates. L-771,688 also inhibited specific [^{125}I]HEAT binding in human cerebral cortex in a way that was best fit by a two-site competition with K_i values for the high affinity sites being nearly identical for the α_{1A}-adrenoceptor (cf. Table 1). The fact that L-771,688 displayed mainly low affinity in human cerebral cortex suggested that it expressed a mixture of α_{1A}- and α_{1B}- and/or α_{1D}-adrenoceptors where the non-α_{1A}-adrenoceptor subtype(s) predominated. L-771,688 inhibited specific [^{3}H]prazosin or [^{125}I]HEAT binding in rat liver and human and dog aorta which was best fit by a one site model, but with higher K_i values, therefore consistent with a population of α_{1B} and/or α_{1D}-adrenoceptors (Table 2). In contrast to L-771,688, terazosin inhibited specific [^{3}H]prazosin or [^{125}I]HEAT binding in all the tissues best fit by a one-site model with K_i values of 1.0–35 nM (Table 2). These data, therefore, illustrated no subtype selectivity of terazosin. In addition, terazosin exhibited at least a 10-fold lower affinity for the presumed α_{1A}-adrenoceptors than L-771,688 in the various tissue types, in agreement with its activity at the cloned α_{1A}-adrenoceptors (Table 1).

3.3. [^{3}H]L-771,688 binding

In order to further define and characterize the interaction of L-771,688 with the α_{1A}-adrenoceptors, L-771,688 was radioactively labeled and used as a radioligand for binding assays in cloned α_{1A}-adrenoceptor membranes and rat tissue membranes. Specific [^{3}H]L-771,688 binding increased linearly with increasing membrane concentrations of human α_{1A}-adrenoceptor (25–200 μg protein/ml assay), submaxillary gland (5–40 mg tissue/ml assay) and rat prostate (10–80 mg/ml assay) (data not shown). Specific [^{3}H]L-771,688 binding was saturable and produced K_d values of 71, 43 and 90 pM in cloned human α_{1A} membranes, rat submaxillary gland and rat prostate membranes, respectively (data not shown). The B_{max} values were 1.6 pmol/mg protein in cloned human α_{1A}-adrenoceptor membranes and 8.7 and 1.6 pmol/g tissue in rat submaxillary gland and rat prostate, respectively.

The rate of association of [^{3}H]L-771,688 to its binding sites in cloned human α_{1A}-adrenoceptor membranes (Fig. 2A), rat submaxillary gland and rat prostate was rapid, reaching equilibrium within 30–60 min. The calculated association rate constants [$k_1 = (k_{obs} - k_{-1})/$[[^{3}H]L-771,688] were 0.24, 0.49 and 0.19 min^{-1} nM^{-1} in cloned human α_{1A}-adrenoceptor membranes (Fig. 2A), rat submaxillary gland and rat prostate (data not shown), respectively. The dissociation of [^{3}H]L-771,688 binding was relatively slow with dissociation rate constants (k_{-1}) of 0.0067, 0.015 and 0.014 min^{-1} in cloned human α_{1A}-adrenoceptor membranes (Fig. 2B), rat submaxillary gland and rat prostate, respectively(data not shown). The K_d values obtained from the ratio of k_{-1}/k_1 were 28–73 pM which were in good agreement with the K_d values from equilibrium studies.

Specific [^{3}H]L-771,688 binding to cloned human α_{1A}-adrenoceptors was inhibited with high potency by subtype selective compounds, GG818 (Adkison et al., 1998; $K_i = 0.026 \pm 0.002$ nM) and L-771,688 ($K_i = 0.052 \pm 0.008$ nM) and subtype non-selective α_1-adrenoceptor antagonists, prazosin ($K_i = 0.088 \pm 0.0.032$ nM) and terazosin ($K_i = 1.8 \pm 0.65$ nM). In contrast, L-765,314, an α_{1B}-adrenoceptor selective antagonist (Patane et al., 1998; K_i

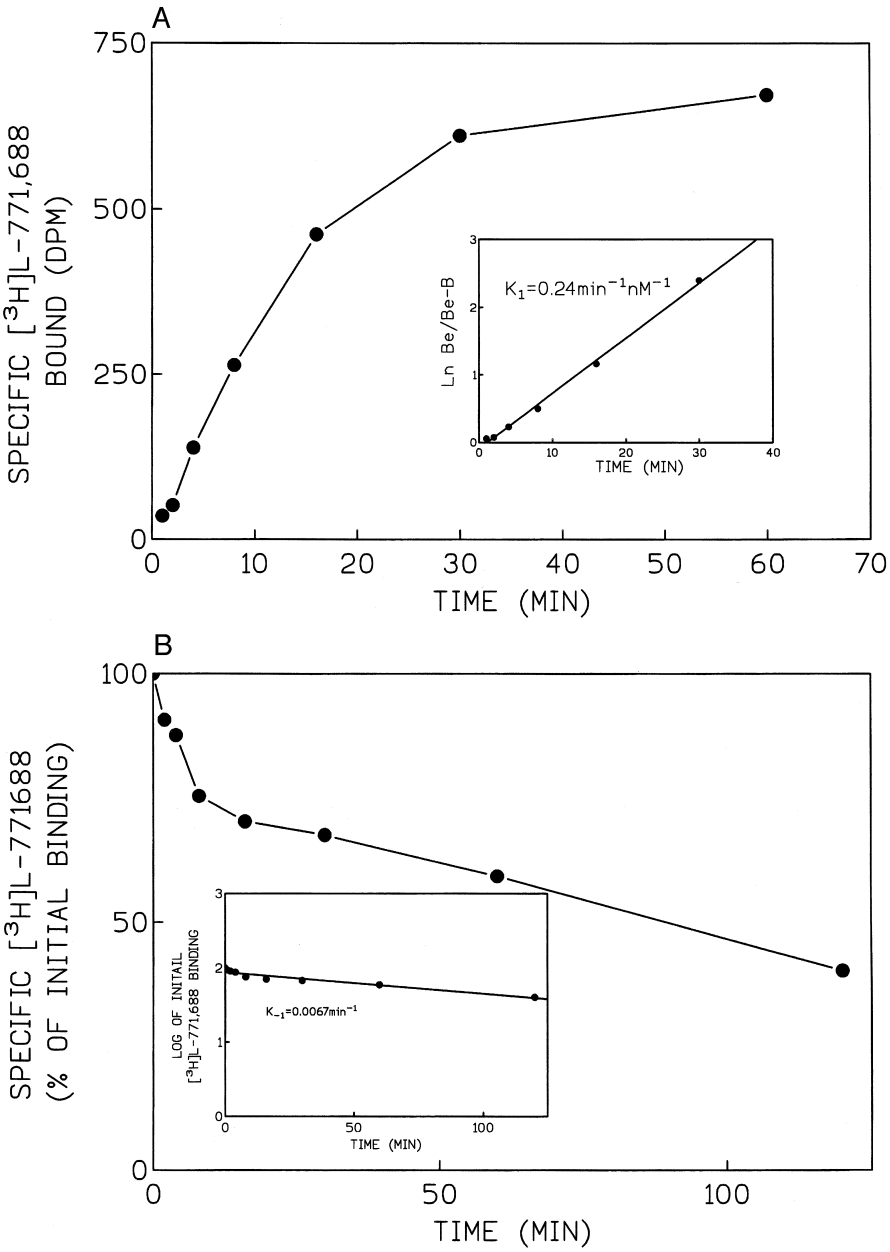

Fig. 2. (A) Time course of association of [^{3}H]L-771,688 binding to cloned human α_{1A}-adrenoceptor membranes. Data points shown were mean of triplicate determination from a single determination. The experiments were repeated twice with similar results. Inset: pseudo-first-order kinetic plot of specific [^{3}H]L-771,688 binding. On the ordinate, B was the amount of specific binding at time t and Be was the amount of specific binding at equilibrium. The slope of the plot was the observed rate constant (k_{ob}) for the pseudo-first-order reaction. The second-order association rate constant (k_1) was calculated from $k_1 = (k_{ob} - k_{-1})/[[^3\text{H}]\text{L-771,688}]$ where k_{-1} was the dissociation rate constant determined from Fig. 1B and [[^{3}H]L-771,688] was the concentration of radioligand used in the experiment. (B) Dissociation of specific [^{3}H]L-771,688 binding to cloned human α_{1A}-adrenoceptor membranes. Data points were mean of triplicate determination from a single experiment. The experiments were repeated twice with similar results. Inset: a semilogarithmic plot of percent initial binding remained at various times after initiation of dissociation vs. time. Dissociation was initiated by adding 1 μM of unlabeled L-771,688 to prevent rebinding of [^{3}H]L-771,688. k_{-1} is calculated from the formula, $k_{-1} = 2.3 \times$ slope.

$= 120 \pm 61$ nM) and BMY 7378, an α_{1D}-adrenoceptor selective antagonist (Goetz et al., 1995; $K_i = 180 \pm 27$ nM) had low potency in inhibiting specific [^{3}H]L-771,688 binding.

The relative amount of [^{3}H]L-771,688 (0.5 nM) binding in various rat tissue membranes was highest in submaxillary gland (9.5 pmol/g tissue), followed by brain (5.8 pmol/g tissue), vas deferens (4.3 pmol/g tissue), kidney (3.4 pmol/g tissue), heart (1.5 pmol/g tissue), urethra (1.1 pmol/g tissue) and prostate (0.88 pmol/g tissue). In contrast, low specific [^{3}H]L-771,688 binding was observed in rat urinary bladder(0.55 pmol/g tissue), liver (0.44 pmol/g tissue), aorta (0.11 pmol/g tissue) and spleen (0.11 pmol/g tissue).

3.4. α_1-Antagonist activity at cloned human α_1-adrenoceptors

When added simultaneously with norepinephrine (co-incubation), L-771,688 (10 nM) produced competitive antagonism of inositol-phosphate formation (parallel rightward shift of norepinephrine dose–responses to the right without reduction of maximal responses, EC_{50}: control, 65 ± 8 nM; treated 30 ± 4 μM) yielding a K_b value of 0.022 nM. However, with a 20-min preincubation, there was a 34% reduction in the maximal activation by norepinephrine in addition to a right-ward shift of norepinephrine dose–response (data not shown). L-771,688 was considerably weaker to inhibit norepinephrine-stimulated inositol-

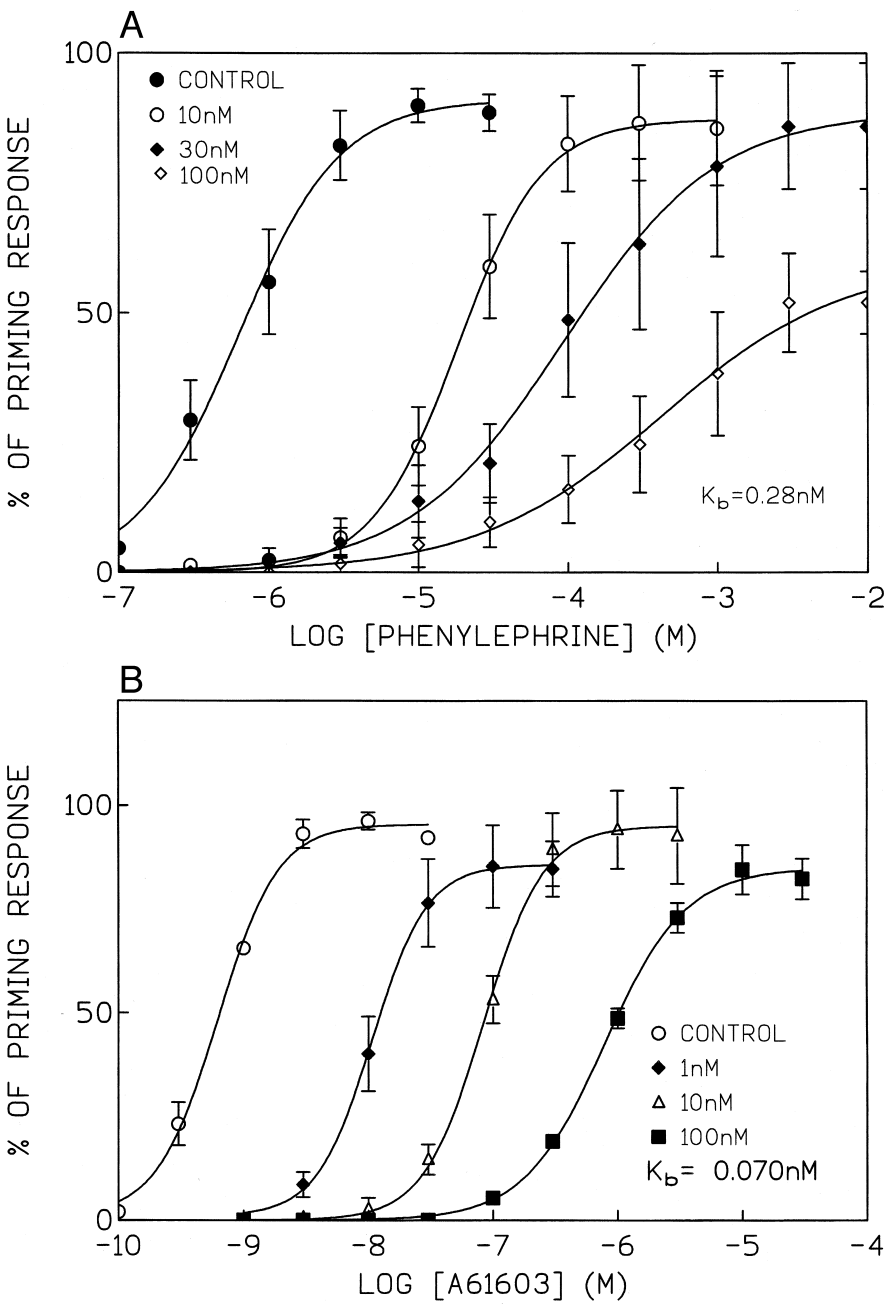

Fig. 3. Effect of L-771,688 on phenylephrine (Panel A) and A61603 (Panel B) induced contractile responses in isolated rat prostate (1-h pretreatment with L-771,688). Contractile responses were expressed as percent of the priming responses using 3 μM of phenylephrine (panel A) or 10 nM of A61603 (panel B). Data points shown were mean ± S.E.M. from three preparations. K_b value given in panel A was mean value from two lower concentrations of L-771,688 used. K_b values given in panel B were from all three concentrations of L-771,688. Schild plot of data from panel B yielded a slope not significantly different from unity and a pA_2 of 10.3.

phosphate formation in cell lines expressing human α_{1B}- and α_{1D}-adrenoceptors than α_{1A}-adrenoceptors with K_b values of 84 and 3200 nM, respectively. L-771,688 exhibited surmountable competitive-like inhibition at the α_{1B} and α_{1D} receptors, whether the antagonists were co-incubated or pre-incubated. Terazosin, by contrast, was essentially equipotent at the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes with K_b values of 0.4. 0.4 and 1.1 nM, respectively.

L-771,688, when tested in the absence of norepinephrine at concentrations as high as 100 μM, did not stimulate the accumulation of inositol-phosphates in cell lines expressing the α_{1A}, α_{1B} or α_{1D}-adrenoceptor subtypes (data not shown). Therefore, L-771,688 did not exhibit any detectable agonist-like activity.

3.5. α_1-Antagonism in isolated tissue preparations

3.5.1. Rat, dog, monkey and human isolated prostate and bladder neck

L-771,688 was tested as an α_1-adrenoceptor antagonist against phenylephrine- (nonselective) or A61603 (α_{1A}-adrenoceptor selective)-induced contractions of several isolated tissues. L-771,688 was a potent antagonist of phenylephrine-induced (Fig. 3A) and A61603-induced (Fig. 3B) contractions in rat prostate. L-771,688 up to 1 μM had no effect on KCl (100 mM) induced contraction indicating lack of nonspecific effect on contractile mechanism (data not shown). L-771,688 (10 and 30 nM) produced competitive antagonism of phenylephrine-induced contractions. However, at the higher concentration (100 nM), it produced an insurmountable inhibition of the phenylephrine response(Fig. 3A). K_b values calculated from two lower concentrations of L-771,668 gave a mean of 0.28 ± 0.10 nM. By contrast, the inhibition of A61603-induced contractions of rat prostate by L-771,688 at all tested concentrations (1, 10 and 100 nM) were surmountable by increasing concentrations of A61603 with K_b values of 0.070 ± 0.005 nM (Fig. 3B). Schild plot from data in Fig. 3B gave a slope of 0.95 ± 0.020, not significantly different from unity and a pA_2 value of 10.3 indicating competitive antagonism (data not shown). L-771,688 (1 and 10 nM) also potently and competitively inhibited phenylephrine-induced contractions of isolated dog prostate (K_b values of 0.22 ± 0.01 nM) (Fig. 4) and A61603-induced contractions in human bladder neck (K_b values of 0.021 ± 0.002 nM) (Fig. 5A). In contrast, L-771,668 antagonized A61603-induced contractions of human prostate (Fig. 5B) and monkey bladder neck [(K_b of 0.022 nM at 1 nM of L-771,688, some reduction in maximal responses at 10 nM of L-771,688) (Table 3)] with a reduction of maximal responses.

Terazosin was shown to be a competitive α_1-adrenoceptor antagonist on the rat and dog prostate against phenylephrine (Table 3) and in rat and human prostate against A61603-induced contractions (Table 3). Terazosin was considerably less potent than L-771,688 (see Table 3 for summary of data for terazosin and L-771,688).

3.5.2. Rat isolated aorta and caudal artery

L-771,688 (up to 1 μM) had little or no effect on norepinephrine-induced contractions in the isolated rat aorta

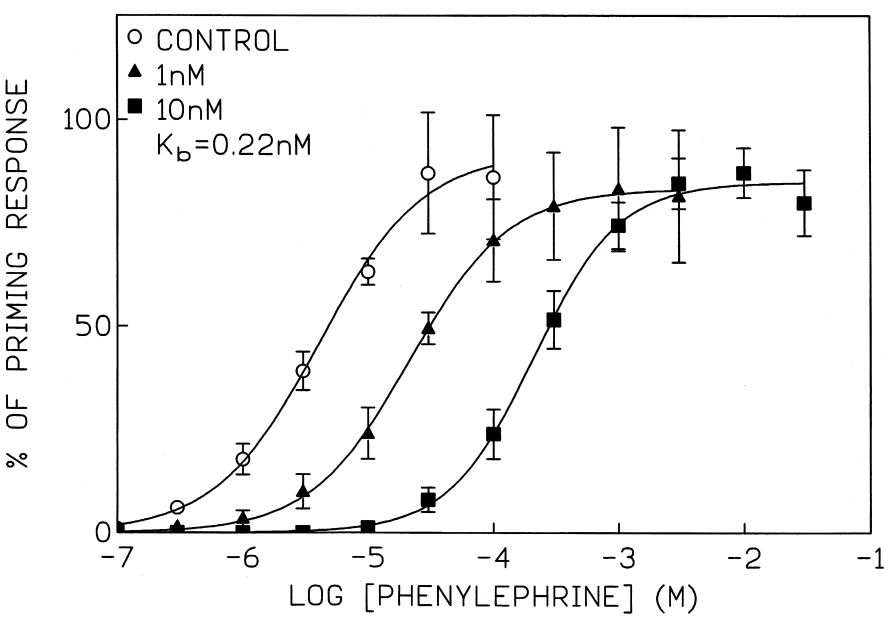

Fig. 4. Effect of L-771,688 on phenylephrine-induced contractile responses of isolated dog prostate (2-h pretreatment with L-771,688). Contractile responses were expressed as percent of the priming responses using 10 μM of phenylephrine. Data points shown were mean ± S.E.M. from four preparations.

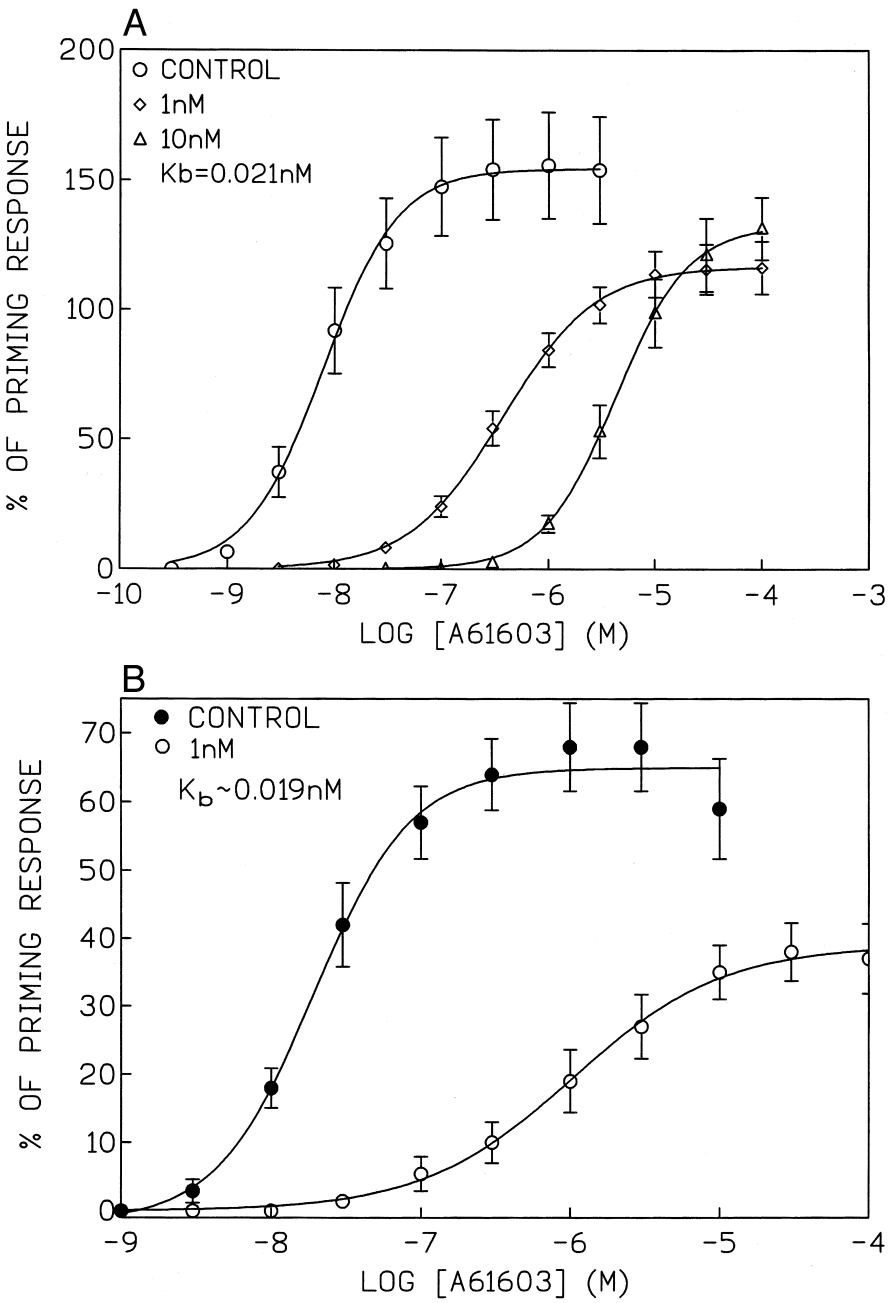

Fig. 5. Effect of L-771,688 on A61603-induced contraction of human bladder neck (panel A) and on A61603-induced contraction of human prostate (panel B). L-771,688 pretreatment was 2 h. Contractile responses were expressed as percent of the priming responses using 100 (panel A) or 300 nM of A61603 (panel B). Data points shown were mean ± S.E.M. from four preparations. Note in panel A that the control maximal responses to A61603 were higher than the priming responses. The reason for this observation is not known to us, but could be related to equilibrium conditions after the overnight storage of tissue.

(Table 3). Rat aorta, therefore, does not appear to contain contractile α_{1A}-adrenoceptors and, indeed, is a vascular tissue that is known to contain predominantly the α_{1D}-adrenoceptor mediated contraction (Kenny et al., 1995). On the other hand, L-771,688 was a potent antagonist of contractions elicited by the α_{1A}-adrenoceptor selective agonist, A61603, in rat caudal artery (Table 3) which is consistent with the literature suggesting that the rat caudal artery contains a functional α_{1A}-adrenoceptor subtype (Lachnit et al., 1997). In contrast to L-771,688, terazosin antagonized norepinephrine-induced contraction in rat aorta with K_b values similar to those obtained in rat, dog and human prostate tissues (Table 3). Terazosin, therefore, was equally potent as an α_1-adrenoceptor antagonist in tissues containing α_{1A}- and/or non-α_{1A}-adrenoceptor subtypes. L-771,688 did not affect resting contractile tension of any

Table 3
Summary of antagonist potency in isolated tissues

Tissues	Agonists	K_b (nM)	
		L-771,688	Terazosin
Rat prostate	Phenylephrine	0.28 ± 0.10	21 ± 4
	A61603	0.070 ± 0.005	25 ± 3
Dog prostate	Phenylephrine	0.22 ± 0.02	130 ± 33
Human prostate	A61603	~ 0.019[a]	25 ± 1
	A61603	~ 0.022[a]	–
Human bladder neck	A61603	0.021 ± 0.002	–
Monkey bladder neck	A61603	0.022	–
Rat aorta	Norepinephrine	>1000	19
Rat caudal artery	A61603	0.047 ± 0.02	–
	Norepinephrine	–	27

Values were mean ± S.E.M. Values without S.E.M. were from a single experiment.

[a] K_b values should be considered as estimates since some reduction in maximal responses were observed.

of these tissues at the tested concentrations and therefore showed no agonist-like activity.

4. Discussion

L-771,688 displayed high affinity (0.36 nM) and selectivity (≥ 500 over α_{1B} or α_{1D}) for the α_{1A}-adrenoceptor subtype in radioligand binding assays using cloned human, rat and dog α_1-adrenoceptors.The reason for the approximately three-fold difference in affinity between cloned α_{1A}-adrenoceptors expressed in cells and human and rat prostate tissues in competition binding experiments is not clear. It should be noted that the competition binding assays for the cloned α_{1A}-adrenoceptor and tissues were performed by two different laboratories. We considered that the difference may be due to inter-laboratory variability due to slight variations of experimental conditions. It is interesting to note that the direct [^{3}H]L-771,688 binding to the cloned α_{1A}-adrenoceptor expressed in CHO cells and α_1-adrenoceptor binding in tissues were done in the same laboratory yielding values in closer agreement. L-771,688 had no significant effect against many other G-protein-coupled-receptors and several ion channels including adrenoceptors ($\alpha_{2A,\ 2B,\ 2C,}$ $\beta_{1,\ 2,\ 3}$), 5-HT receptors ($5HT_{1A,\ 1D,\ 1E,\ 1F,\ 2,\ 7}$), dopamine receptors ($D_1$, D_2), histamine receptor (H_1, H_2), muscarine receptors (M_1–M_5), opioid receptors (μ, δ, κ,), Ca^{++} (L-channel) and Na^+ channels up 1 μM indicating at least 1000-fold selectivity. This α_{1A}-adrenoceptor subtype selectivity of L-771,688 was the highest as compared to compounds reported by others including Rec15/2793 (3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxylicacid{3-[4-(methoxy-phenyl)-piperazin-1-yl]-propyl}-amide(Testa et al., 1997; human $\alpha_{1B}/\alpha_{1A} = 27\times$; $\alpha_{1D}/\alpha_{1A} = 9\times$), KMD-3213 [(−)-(*R*)-1-(3-hydroxypropyl)-5-[2-[[2-(2,2,2-trifuoroethoxy) phenoxy] ethyl]-amino]propyl]indoline-carboxamide (Shibata et al., 1995; $\alpha_{1B}/\alpha_{1A} = 583\times$; $\alpha_{1D}/\alpha_{1A} = 55\times$), GG818 (Adkison et al., 1998; $\alpha_{1B}/\alpha_{1A} = 39\times$; $\alpha_{1D}/\alpha_{1A} = 75\times$), RS-100975 (3-(3{4-fluoro-2-(2,2,2-trifluooethoxy)-phenyl]-piperazin-1-yl}propyl)-5-methyl-1*H*-pyrimidine-2,4-dione) (Williams et al., 1999; $\alpha_{1B}/\alpha_{1A} = 60$ $\alpha_{1D}/\alpha_{1A} = 50\times$), tamsulosin (Shibata et al., 1995; $\alpha_{1B}/\alpha_{1A} = 15\times$; $\alpha_{1D}/\alpha_{1A} = 3.3\times$), and RWJ-69736 (*N*-(3-{4-[2-methyl-ethoxy)phenyl]piperazinyl}propyl)-2-(2oxopiperidyl) aceta-mide) (Pulito et al., 2000; $\alpha_{1B}/\alpha_{1A} = 340\times$; $\alpha_{1D}/\alpha_{1A} = 223\times$). The high affinity of L-771,688 for the α_{1A}-adrenoceptors was also demonstrated in human, rat and dog prostate tissues known to contain predominantly α_{1A}-adrenoceptor subtypes. L-771,688 competes with [^{3}H]prazosin binding to α_1-adrenergic receptors in human prostate with high affinity ($K_i = 0.13$ nM). In the rat and dog prostate, in addition to the majority of high affinity sites for L-771,688, some low affinity sites for L-771,688 were also observed in some preparations indicating the existence of some non-α_{1A}-adrenoceptors. L-771,688 competed with [^{125}I]HEAT binding in human cerebral cortex with the majority of sites having low affinity ($K_i = 81$ nM) and a small fraction of sites having a high affinity ($K_i = 0.066$ nM) similar to that for human prostate and cloned human α_{1A}-adrenoceptors. The results suggested that the human cortex had predominantly non-α_{1A}- and a small fraction of α_{1A}-adrenoceptors. In contrast, L-771,668 competed with [^{3}H]prazosin/[^{125}I]HEAT binding with relatively low affinity (160–410 nM) at an apparent single site in rat liver and human and dog aorta. The data is consistent with the report that rat liver expressed mainly the α_{1B}-adrenergic receptor subtype (Han et al., 1987). An earlier study by Yamada et al. (1994) showed that tamsulosin was 10-fold more potent in human prostate than aorta and suggested a subtype difference between these two tissues. The much larger difference in the K_i values for L-771,688 in human prostate and aorta (0.13 vs. 410 nM) gave further support that human aorta contains non-α_{1A}-adrenoceptors.

The high affinity (43–90 pM) and selectivity of L-771,688 to α_{1A}-adrenoceptors were further demonstrated by direct binding studies of [^{3}H]L-771,688 to cloned human α_{1A}-adrenoceptors and rat tissues. Specific [^{3}H]L-771,688 binding is competed by α_{1A}-adrenoceptor antagonists, L-771,688 and GG818 (Adkison et al., 1998), and α_1-adrenoceptor subtype nonselective antagonist, terazosin and prazosin at concentrations known to be effective on α_{1A}-adrenoceptors. In contrast, L-765,314 (Patane et al., 1998) and BMY 7378 (Goetz et al., 1995), selective α_{1B}- and α_{1D}-adrenoceptor antagonists, were much weaker in inhibiting specific [^{3}H]L-771,688 binding. Moreover, specific [^{3}H]L-771,688 binding was high in rat tissues known to contain high α_{1A}-adrenoceptors such as submaxillary glands, brain, vas deferens and kidney (Morrow and Creese, 1986; Han et al., 1987; Michel et al., 1989; Blue et al., 1995; O'Malley et al., 1998) but low in rat liver spleen and aorta, tissues known to contain mainly α_{1B}- and α_{1D}-adrenoceptors (Han et al., 1987; Kenny et al., 1995; Chang

et al., 1998). [^{3}H]L-771,688 therefore, represents a highly selective radioligand for labeling the α_{1A}-adrenoceptor.

L-771,688 antagonized norepinephrine-induced inositol-phosphate responses when L-771,668 and norepinephrine were co-incubated with the cells, yielding K_b values of 0.02, 84 and 3200 nM for cells expressing cloned human α_{1A}, α_{1B} and α_{1D}-adrenoceptors, respectively, without reduction of maximal responses indicative of competitive antagonism. L-771,688 also antagonized phenylephrine- (α_1-adrenoceptor subtype nonselective) or A-61603- (α_{1A}-adrenoceptor subtype selective) (Knepper et al., 1995) induced contraction in isolated rat, dog and human prostate and monkey and human bladder neck with K_b values of 0.02–0.28 nM. Further, L-771,688 potently antagonized A-61603-induced contraction in rat tail artery which was previously reported to contain mainly the α_{1A}-adrenoceptor (Lachnit et al., 1997). L-771,688 (1 μM) failed to affect norepinephrine-induced contraction in rat aorta which contains α_{1D}-adrenoceptors (Kenny et al., 1995).

In general, in the antagonist studies parallel shifts of concentration responses to the right without significant reduction of maximal contractile responses by L-771,688 were obtained indicative of competitive and reversible antagonism. However, in some preparations such as human prostate, rat prostate and monkey bladder neck, some reduction in maximal response was observed especially at the higher concentrations of L-771,688. Reduction in maximal inositol-phosphate responses to norepinephrine in cloned human α_{1A}-adrenoceptor cells was also observed if the cells were preincubated with L-771,688. The results were also consistent with other data (not shown) that preincubation of human cloned α_{1A}-adrenoceptor membranes with L-771,688 resulted in reduction of maximal number of specific [^{3}H]prazosin binding sites. However, co-incubation of cloned human α_{1A}-adrenoceptor cells with L-771,668 and norepinephrine resulted in no reduction of maximal inositol-phosphate responses to norepinephrine. The results were also consistent with no reduction in maximal number of specific [^{3}H]prazosin binding sites when L-771,688 was co-incubated with cloned human α_{1A}-adrenoceptor membranes and [^{3}H]prazosin (data not shown). Similar insurmountable antagonist behavior has been observed with other high affinity α_{1A}-adrenoceptor antagonists such as KMD-3213 (Moriyama et al., 1997), tamsulosin (Moriyama et al., 1997) and RWJ-69736 (Pulito et al., 2000) and some angiotensin AT_1 receptor antagonists such as E3174 (losartan metabolite), MK-966 (Chang et al., 1994) and candesartan (Ojima et al., 1997). The reduction in maximal responses and maximal number of binding sites when pretreated with L-771,688 may be due to the slow dissociation of L-771,688 ($T_{1/2}$ = 67–150 min from the rate of dissociation of [^{3}H]L-771,688 binding studies) as previously suggested for the angiotensin AT_1 receptor antagonists (Chang et al., 1994; Ojima et al., 1997; Fierens et al., 1999). It is clear however that L-771,688 is a freely reversible α_{1A}-adrenoceptor antagonist.

The high potency of L-771,688 on α_1-adrenoceptor mediated responses in prostate and bladder neck and on α_1-adrenoceptor binding assays in animal and human prostate tissues is consistent with previous evidence suggesting the preferential expression of α_{1A}-adrenoceptor subtype in these tissues. Prostate selective α_1-adrenoceptor antagonists may provide better efficacy in the treatment of benign prostate hyperplasia with fewer side effects especially concerning cardiovascular effects. Indeed, in in vivo animal models, L-771,688 was found to have a potent antagonist effect on urethral pressure with minimal effect on blood pressure (Broten T.B. et.al., personal communication). Similar conclusions were obtained with other α_{1A}-adrenoceptor selective antagonists such as SNAP 6991 (Broten et al., 1999), KMD-3213 (Akiyama et al., 1999) and RWJ-69736 (Pulito et al., 2000).

Recently, L-771,688 became the first truly α_{1A}-adrenoceptor selective antagonist to be tested in the clinic. In this study, L-771,688 improved peak urine flow rates in a dose-dependent manner after a single dose in benign prostate hyperplasia patients and was well-tolerated (Marks et al., 2000). Therefore, the types of studies reported here and elsewhere characterizing selective α_{1A}-adrenoceptor antagonists appear to be predictive of their efficacy in benign prostate hyperplasia patients. The true utility of compounds like L-771,688 for the treatment of benign prostate hyperplasia, however, does require further study.

References

Adkison, K.K., Halm, K.A., Shaffer, J.E., Drewry, D., Sinhababu, A.K., Berman, J., 1998. Discovery of a potent and selective alpha 1A antagonist: utilization of a rapid screening method to obtain pharmacokinetic parameters. Pharm. Biotechnol. 11, 423–443.

Akiyama, K., Hora, M., Tatemichi, S., Masuda, N., Nakamura, S., Yamagishi, R., Kitazawa, M., 1999. KDM-3213, a uroselective and long acting α_{1A}-adrenoceptor antagonist, tested in a novel rat model. J. Pharmacol. Exp. Ther. 291, 81–91.

Blue, D.R., Bonhaus, D.W., Ford, A.P.D.W., Pfister, J.R., Sharif, N.A., Shieh, I.A., Vimont, R.L., Williams, T.J., Clarke, D.E., 1995. Functional evidence equating the pharmacologically defined α_{1A} and cloned α_{1C}-adrenoceptor: studies in isolated perfused kidney of rat. Br. J. Pharmacol. 115, 283–294.

Broten, T., Ransom, R., Scott, A., Schorn, T., Kling, P., Pettibone, D., Siegl, P., Nagarathnam, D., Miao, S., Wong, W.C., Lagu, B., Marzabadi, M.R., Forray, C., Cluchowski, C., 1999. In vivo pharmacology of SNAP 6991(L-780,959), an α_{1A} selective adrenergic receptor antagonist. FASEB J. 13, A142, (Abstracts).

Bylund, D.B., Bond, R.A., Clarke, D.E., Eikenburg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K., Molinoff, P.B., Ruffolo, R.R., Strosberg, A.D., Trendelenburg, U.G., 1998. Adrenoceptors. IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media, London, UK, pp. 58–74.

Chang, R.S.L., Bendesky, R.J., Chen, T.B., Faust, K.A., Kling, P.A., O'Malley, S.S., Naylor, E.M., Chakravaty, P.K., Patchett, A.A., Greenlee, W.J., Clineschmidt, B.V., Lotti, V.J., 1994. In vitro pharmacology of MK-996, a new potent and selective angiotensin II (AT1) receptor antagonist. Drug Dev. Res. 32, 161–171.

Chang, R.S.L., O'Malley, S.S., Chen, T.B., Marzabadi, M.R., Hong, X., Gluchowski, C., 1996. Distribution of alpha-1 adrenergic receptor subtypes in human tissues. FASEB J. 10, A424, (Abstracts).

Chang, R.S.L., Chen, T.B., O'Malley, S.S., Lagu, B., Nagarathnam, D., Forray, C., Marzabadi, M., Wong, W., Dhar, T., Hong, X., Gluchowski, C., DiSalvo, J., Patane, M., Bock, M., 1998. Potency of α_{1A} (SNAP 6201 and SNAP 5399), α_{1B}(L-765,314) and α_{1D}(BMY 7378) subtype selective antagonists in isolated rat, dog monkey and human tissues: abstracts of XIIIth International Congress of Pharmacology. Nauyn-Schmiedeberg's Arch. Pharmacol. 358 (Suppl. 2) R593.

Chapple, C.R., 1995. alpha-Adrenergic blocking drugs in bladder outflow obstruction: what potential has alpha(1)-adrenoceptor selectivity? Br. J. Urol. 76 (Suppl. 1) 47–55.

Dhar, T.G.M., Nagarathnam, D., Marzabadi, M., Lagu, B., Wong, W.C., Chiu, G., Tyagarajan, S., Miao, S.W., Zhang, F., Sun, W., Tian, D., Shen, Q., Wetzel, J.M., Forray, C., Chang, R.S.L., Broten, T.P., Schorn, T.W., Chen, T.B., O'Malley, S., Ransom, R.W., Schneck, K., Bendesky, R., Harrell, C.M., Vyas, K.P., Zhang, K., Gilbert, J., Pettibone, D.J., Patane, M.A., Bock, M.G., Freidinger, R., Gluchowski, C., 1999. Design and synthesis of novel α_{1a} adrenoceptor selective antagonists: approaches to eliminate opioid active metabolites via modification of linker and 4-methoxycarbonyl-4-phenylpiperidine moiety. J. Med. Chem. 42, 4778–4793.

Fierens, F., Vanderheyden, P.M.L., De-Backer, J.P., Vauquelin, G., 1999. Binding of the antagonist [^{3}H]candesartan to angiotensin II AT1 receptor-transfected Chinese hamster ovary cells. Eur. J. Pharmacol. 367, 413–422.

Forray, C., Bard, J.A., Wetzel, J.M., Chiu, G., Shapiro, E., Tang, R., Lepor, H., Hartig, P.R., Weinshank, R.L., Branchek, T.A., Gluchowski, C., 1994. The alpha 1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human alpha 1c subtype. Mol. Pharmacol. 45, 703–708.

Goetz, A.S., King, H.K., Ward, S.D., True, T.A., Rimele, T.J., Saussy, D.L. Jr., 1995. BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. Eur. J. Pharmacol. 272, R5–R6.

Han, C., Abel, P.W., Minneman, K.P., 1987. Heterogenety of α_1-adrenoceptors revealed by chlorethylclonidine. Mol. Pharmacol. 32, 505–510.

Kenny, B.A., Chalmers, D.H., Philpott, P.C., Naylor, A.M., 1995. Characterization of a functional α_{1D}-adrenoceptor mediating the contractile response of rat aorta to norepinephrine. Br. J. Pharmacol. 115, 981–986.

Knepper, S.M., Buckner, S.A., Brune, M.E., DeBernardis, J.F., Meyer, M.D., Hancock, A.A., 1995. A-61603, a potent alpha 1-adrenergic receptor agonist, selective for the alpha 1A receptor subtype. J. Pharmacol. Exp. Ther. 274, 97–103.

Lachnit, W.G., Tran, A.M., Clarke, D.E., Ford, A.P.D.W., 1997. Pharmacological characterization of an alpha(1A)-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. Br. J. Pharmacol. 120, 819–826.

Laz, T.M., Forray, C., Smiith, K.E., Bard, J.A., Vaysse, P.J., Branchek, T.A., Weinshank, R.L., 1994. The rat homologue of the bovine α_{1C}-adrenergic receptor shows the pharmacological properties of the classical α_{1A} subtype. Mol. Pharmacol. 46, 414–422.

Marks, L.P., Curtis, S.P., Narayan, P., Gihooly, P., Sanchez, M., Castellanos, R., Bugianesi, K., Miller, J., Gertz, B., 2000. Effects of a highly selective alpha-1a antagonist on urinary flow rate in men with symptomatic BPH. American Urological Association Meeting (May 3, Atlanta, GA) Abstract 2770.

Michel, A.D., Loury, D.N., Whiting, R.L., 1989. Identification of a single α_1-adrenoceptor corresponding to α_{1A}-subtype in rat submaxillary gland. Br. J. Pharmacol. 98, 883–889.

Moriyama, N., Akiyama, K., Murata, S., Taniguchi, J., Ishida, N., Yamazaki, S., Kawabe, K., 1997. KMD-3213, a novel alpha(1A)-adrenoceptor antagonist, potently inhibits the functional alpha(1)-adrenoceptor in human prostate. Eur. J. Pharmacol. 331, 39–42.

Morrow, A.L., Creese, I., 1986. Characterization of α_1-adrenergic receptor subtype in rat brain: a reevaluation of [^{3}H]WB 4101 and [^{3}H]prazosin binding. Mol. Pharmacol. 29, 321–330.

Nagarathnam, D., Wetzel, J.M., Miao, S.W., Marzabadi, M.R., Chiu, G., Wong, W.C., Hong, X., Fang, J., Forray, C., Branchek, T.A., Heydorn, W.E., Chang, R.S.L., Broten, T., Schorn, T.W., Gluchowski, C., 1998. Design and synthesis of novel alpha 1a adrenoceptor-selective dihydropyridine antagonists for the treatment of benign prostatic hyperplasia. J. Med. Chem. 41, 5320–5333.

Ojima, M., Inada, Y., Shibouta, Y., Wada, T., Sanada, T., Kubo, K., Nishikawa, K., 1997. Candesartan (CV-11974) dissociates slowly from the angiotensin AT1 receptor. Eur. J. Pharmacol. 319, 137–146.

O'Malley, S.S., Chen, T.B., Francis, B.E., Gibson, R.E., Burns, H.D., DiSalvo, J., Bayne, M.L., Wetzel, J.M., Nagarathnam, D., Marzabadi, M., Gluchowski, C., Chang, R.S.L., 1998. Characterization of specific binding of [125I]L-762,459, a selective alpha 1A-adrenoceptor radioligand to rat and human tissues. Eur. J. Pharmacol. 348, 287–295.

Patane, M.A., Scott, A., Broten, T.P., Chang, R.S.L., Ransom, R.W., DiSalvo, J., Forray, C., Bock, M., 1998. 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino] carbonyl]-piperazinyl]-6, 7-dimethoxyquinazoline (L-765,314): a potent and selective alpha1b adrenergic receptor antagonist. J. Med. Chem. 41, 1205–1208.

Price, D.T., Schwinn, D.A., Lomasney, J.W., Allen, L.F., Caron, M.G., Lefkowitz, R.J., 1993. Identification, quantitation and localization of mRNA for three distinct alpha$_1$ adrenergic receptor subtypes in human prostate. J. Urol. 150, 546–551.

Pulito, V.L., Li, X., Varga, S.S., Mulcahy, L.S., Clark, K.S., Halbert, S.A., Reitz, A.B., Murray, W.V., Jolliffe, L.K., 2000. An investigation of the uroselective properties of four novel α_{1A}-adrenergic receptor subtype-selective antagonists. J. Pharmacol. Exp. Ther. 294, 224–229.

Richardson, C.D., Donatucci, C.F., Page, S.O., Wilson, K.H., Schwinn, D.A., 1997. Pharmacology of tamsulosin: saturation binding isotherms and competition analysis using cloned α_1-adrenergic receptor subtypes. Prostate 33, 55–59.

Shibata, K., Foglar, R., Horie, K., Obika, K., Sakamoto, A., Ogawa, S., Tsujimoto, G., 1995. KMD-3213, a novel, potent, alpha 1a-adrenoceptor-selective antagonist: characterization using recombinant human alpha 1-adrenoceptors and native tissues. Mol. Pharmacol. 48, 250–258.

Testa, R., Guareneri, L., Angelico, P., Poggesi, E., Taddei, C., Sironi, G., Colombo, D., Sulpizio, A.C., Naselsky, D.P., Hieble, J.P., Leonardi, A., 1997. Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739(SB 216469): role of alpha-1L adrenoceptor in tissue selectivity, Part II. J. Pharmacol. Exp. Ther. 281, 1284–1293.

Wetzel, J.M., Miao, S.W., Forray, C., Borden, L.A., Branchek, T.A., Gluchowski, C., 1995. Discovery of α_{1a}-adrenergic receptor antagonists based on the L-type Ca^{2+} channel antagonist niguldipine. J. Med. Chem. 38, 1579–1581.

Williams, T.J., Blue, D.R., Daniels, D.V., Davis, B., Elworthy, T., Gever, J.R., Kava, M.S., Morgans, D., Padilla, F., Tassa, S., Vimont, R.L., Chapple, C.R., Chess-Williams, R., Eglen, R.M., Clarke, D.E., Ford, A.P.D.W., 1999. In vitro α_1-adrenoceptor pharmacology of RO 70-0004 and RS-100329, novel α_{1A}-adrenoceptor selective antagonists. Br. J. Pharmacol. 127, 252–258.

Yamada, S., Suzuki, M., Tanaka, C., Mori, R., Kimura, R., Inagaki, O., Honda, K., Asano, M., Takenaka, T., Kawabe, K., 1994. Comparative-study on alpha(1)-adrenoceptor antagonist binding in human prostate and aorta. Clin. Exp. Pharmacol. Physiol. 21, 405–411.

Yazawa, H., Honda, K., 1993. Alpha 1-adrenoceptor subtype in the rat prostate is preferentially the alpha 1A type. Jpn. J. Pharmacol. 62, 297–304.