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Naftopidil, a Novel α_1-Adrenoceptor Antagonist, Displays Selective Inhibition of Canine Prostatic Pressure and High Affinity Binding to Cloned Human α_1-Adrenoceptors

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ABSTRACT—The pharmacological profiles of the α_1-adrenoceptor antagonists naftopidil, tamsulosin and prazosin were studied in an anesthetized dog model that allowed the simultaneous assessment of their antagonist potency against phenylephrine-mediated increases in prostatic pressure and mean blood pressure. The intravenous administration of each of these compounds dose-dependently inhibited phenylephrine-induced increases in prostatic pressure and mean blood pressure. To further assess the ability of the three compounds to inhibit phenylephrine-induced responses, the doses required to produce a 50% inhibition of the phenylephrine-induced increases in prostatic and mean blood pressure and the selectivity index obtained from the ratio of those two doses were determined for each test compound. Forty minutes after the intravenous administration of naftopidil, the selectivity index was 3.76, and those of tamsulosin and prazosin were 1.23 and 0.61, respectively. These findings demonstrated that naftopidil selectively inhibited the phenylephrine-induced increase in prostatic pressure compared with mean blood pressure in the anesthetized dog model. The selectivity of naftopidil for prostatic pressure was the most potent among the test compounds. In addition, using cloned human α_1-adrenoceptor subtypes, naftopidil was selective for the α_{1d}-adrenoceptor with approximately 3- and 17-fold higher affinity than for the α_{1a}- and α_{1b}-adrenoceptor subtypes, respectively. The selectivity of naftopidil for prostatic pressure may be attributable to its high binding affinity for α_{1a}- and α_{1d}-adrenoceptor subtypes.

Keywords: Naftopidil, α_1-Adrenoceptor antagonist, Prostate pressure, Benign prostatic hyperplasia, Anesthetized dog

Benign prostatic hyperplasia (BPH) is a condition characterized by a nodular enlargement of prostatic tissue resulting in obstruction of the urethra (1). Since the incidence of BPH is age-related, the clinical and economic impact of this disease will continue to progress as the average human lifespan increases (2). Symptoms such as bladder outlet obstruction, which is commonly encountered in BPH patients, may result from a constriction of the urethra by the enlarging prostate that surrounds it (1). In symptomatic BPH, there are two components that are associated with urethral obstruction: a passive component due to the enlarged prostate tissue and a dynamic component due to the sympathetic tone of prostatic and urethral smooth muscle. It has been estimated that the dynamic component contributes about 40% of the total tone that the hyperplastic prostate exerts on the urethra (3). The sympathetic innervation and the existence of α-adrenoceptors in the human prostate have been demonstrated by histochemical studies (4, 5), functional studies (6–9) and radioligand binding studies (10, 11). A number of in vitro studies have revealed that the contraction of the urethra and prostate in both humans and animals by sympathetic stimulation is predominantly mediated by an α_1-adrenoceptor subtype (8, 12, 13). These findings form the basis for the therapeutic use of α_1-adrenoceptor antagonists to treat the obstruction of the urethra associated primarily with BPH.

Clinical studies have confirmed that therapy with α_1-

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adrenoceptor antagonists such as prazosin significantly increases the urinary flow rate in men with BPH (14–17). Thus, α_1-adrenoceptor antagonists have received a great deal of attention in the treatment of bladder outlet obstruction in men with BPH. However, α_1-adrenoceptor antagonists occasionally induce orthostatic hypotension as a side effect. This is mainly due to a decrease in peripheral resistance mediated by the blockade of vascular α_1-adrenoceptors. Therefore, it would be expected that a more selective antagonist of prostatic α_1-adrenoceptors would be a better therapeutic drug for urinary obstruction in BPH because it would generate fewer cardiovascular side effects.

Naftopidil ((±)-1-[4-(2-methoxyphenyl)piperazinyl]-3-(1-naphthyloxy)propan-2-ol) is a novel α-adrenoceptor antagonist that has been found to be effective for the treatment of bladder obstruction in men with symptomatic BPH. The presumed mechanism underlying the efficacy of naftopidil is the blockade of α_1-adrenoceptors in the smooth muscle of the prostate (11). In the present study, the pharmacological selectivity effect of naftopidil on prostatic pressure and blood pressure were examined in an anesthetized dog model, which allowed the simultaneous assessment of antagonist potency against phenylephrine-mediated increases in prostatic pressure and blood pressure. The effects of naftopidil were also compared with those of two other α_1-adrenoceptor antagonists clinically used for the therapy of BPH, namely tamsulosin and prazosin.

Recently, it was found that α_1-adrenoceptors comprise a heterogeneous family (18). Two natively expressed subtypes (α_{1A} and α_{1B}) can be distinguished pharmacologically, while three subtypes (α_{1a}, α_{1b} and α_{1d}) have been cloned (19–22). By using *in situ* hybridization to determine the distribution of the three α_1-adrenoceptor subtypes in hypertrophied prostatic tissue, that α_{1a}- and α_{1d}-subtypes were found in the interstitium of the prostate. However, the α_{1b}-subtype was very faintly recognized (23). We compared the subtype selectivity of naftopidil, tamsulosin and prazosin by using CHO cells stably expressing the three cloned human α_1-aderenoceptor subtypes (α_{1a}-, α_{1b}- and α_{1d}-subtypes). In addition, we also evaluated the subtype selectivity of naphtyl-OH-naftopidil, phenyl-OH-naftopidil and desmethyl-naftopidil, which were the main metabolites of naftopidil in humans and dogs (24, 25).

MATERIALS AND METHODS

Anesthetized dog model for the measurement of prostatic pressure and blood pressure

Adult male mongrel dogs (7–17 kg) were intubated through the trachea during anesthesia with sodium pentobarbiton (30 mg/kg, i.v.). After tracheal intubation, anesthesia was maintained with isoflurane at an inhaled concentration of 1.15% delivered through an isoflurane vaporizer (FORAWICK; Shinano, Tokyo). The dogs were respirated with room air using a respirator (SN-480-3, Shinano) adjusted to maintain blood gases in the range PCO_2 35±5 mmHg. Body temperature was maintained at 37–38℃ with a heating pad. Blood pressure was measured with a pressure amplifier (AP-641G; Nihon Kohden, Tokyo) via a pressure transducer (P10EZ, Nihon Kohden) connected to a catheter inserted into the left femoral artery. The heart rate was measured with a cardiotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse wave. A laparotomy was performed to cannulate both ureters to prevent changes of fluid volume within the bladder. A 7F cardiac catheter (with a 1.5-ml capacity balloon tip) was inserted into the bladder via the urethra. The balloon was inflated with water and the catheter slowly withdrawn at 10 mm/min using an autonomic pulling unit (AU-601G, Nihon Kohden) until the balloon became lodged in the prostate, which was confirmed by the recording of the urethral pressure. The balloon pressure was recorded with a pressure amplifier (AP-641G, Nihon Kohden) via a pressure transducer (P10EZ, Nihon Kohden). Phenylephrine and α_1-adrenoceptor antagonists were administered i.v. through a catheter in the right cephalic vein. After a stabilizing period following the surgical procedure (30 min), in which the prostatic and blood pressures were continuously monitored, three intravenous administrations of phenylephrine (3 μg/kg) were made at 10-min intervals, and the prostatic and blood responses to the last challenge were considered the basal values. Intravenous injections of phenylephrine (3 μg/kg) were administered approximately 20, 30 and 40 min after the i.v. administration of the test compounds.

Expression of the cloned human α_1-adrenoceptor subtypes

The cloning and stable expression of the human α_1-adrenoceptor genes (α_{1a}, α_{1b} and α_{1d}) were achieved as described previously (26). Briefly, stable cell lines were obtained by transfection of the pSVK3neo containing the human α_{1a}-, α_{1b}- or α_{1d}-adrenoceptor gene into CHO-K1 cells, using the Lipofectin™ technique as described previously (27). Briefly, CHO-Kl cells were grown as monolayers in Ham's F-12 medium containing L-glutamine supplemented with 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). Stable clones were then selected for resistance to G418 (600 μg/ml).

[^{125}I]-HEAT binding

CHO cell membrane preparations were made as

described previously (26). Briefly, subconfluent 150-mm plates of CHO cells were washed twice with 10 ml of phosphate-buffered saline (PBS; 139 mM NaCl, 2.7 mM KCl, 8.8 mM Na_2HPO_4, 1.48 mM KH_2PO_4, pH 7.5) and then harvested by scraping. Cells were pelleted by centrifugation at 500×*g* for 5 min, washed, and the pellet was homogenized in 2 ml of ice-cold buffer A (250 mM sucrose, 5 mM Tris HCl, 1 mM $MgCl_2$, pH 7.4) and centrifuged at 1,000×*g* at 4℃ for 10 min to remove nuclei. The supernatant was then centrifuged at 35,000×*g* for 20 min at 4℃, and the pellet was homogenized and then frozen at −80℃ until assayed. The protein concentration was measured by a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA).

Radioligand binding studies with [^{125}I]-HEAT were performed as described previously (21). Briefly, the measurement of specific [^{125}I]-HEAT binding was performed by incubating 0.1 ml of membrane preparation (approximately 10 μg of protein) with [^{125}I]-HEAT in a final volume of 0.25 ml buffer B (50 mM Tris HCl, 10 mM $MgCl_2$, 10 mM EGTA, pH 7.4) for 60 min at 25℃ in the presence or absence of test compounds. The incubation was terminated by adding ice-cold buffer B and immediately filtering through Whatmann GF/C glass-fiber filters with a Brandel cell harvester (Model-30; Gaithersburg, MD, USA). Each filter was collected and the radioactivity was measured. Binding assays were performed in duplicate. For competition curve analysis, each assay contained about 70 pM [^{125}I]-HEAT. At this concentration, nonspecific binding, defined as binding displaced by 10 μM phentolamine, represented about less than 10% of the total binding.

Stastistical analyses

Values are expressed as the mean±S.E.M. The significance of differences was calculated by Dunnett's test. P values of 0.05 or less were considered significant. The ability of test compounds to inhibit phenylephrine-induced increases in prostatic pressure and blood pressure was estimated by the ED_{50} values, i.e., the dose (μg/kg) of drug necessary for the 50% inhibition of the phenylephrine-induced pressure responses. Computer analyses with LIGAND were used to evaluate the dissociation constant and receptor density.

Drugs used in the study

The following drugs were used: naftopidil ((±)-1-[4-(2-methoxyphenyl)piperazinyl]-3-1-naphthyloxy)propan-2-ol), naphthyl-OH-naftopidil ((±)-1-[4-(2-methoxyphenyl) piperazinyl]-3-[(4-hydroxy-1-naphthyl)oxy]propan-2-ol), phenyl-OH-naftopidil ((±)-1-[4-(4-hydroxy-2-methoxyphenyl)piperazinyl]-3-(1-naphthyloxy)propan-2-ol), desmethylnaftopidil ((±)-1-[4-(2-hydroxyphenyl)piperazinyl]-3-(1-naphthyloxy)propan-2-ol) (Boehringer Mannheim, Mannheim, Germany); prazosin hydrochloride (Orion Co., Ltd., Espoo, Finland); tamsulosin hydrochloride (synthesized at Asahi Chemical Industry Co., Tokyo); L-phenylephrine hydrochloride (Sigma Chemical Co.,

Table 1. Effects of naftopidil on prostatic pressure, mean blood pressure and heart rate in anesthetized dogs

Drugs	Dose (μg/kg)	Δ Change		
		Prostatic pressure (mmHg)	Mean blood pressure (mmHg)	Heart rate (beats/min)
Vehicle		−1.3±0.7	−3.0±2.6	2.5±1.8
Naftopidil	10	−1.7±0.2	−7.0±1.0	1.3±2.2
	30	−1.4±1.2	−10.5±2.0	0.3±2.2
	100	−2.8±0.8	−17.5±2.0**	4.2±1.1
	1000	−3.2±0.5	−31.5±5.6**	1.0±3.4
Vehicle		−2.2±1.0	−2.2±1.3	−0.7±1.3
Tamsulosin	1	−1.8±0.4	−9.0±0.9	7.3±2.1*
	3	−3.5±0.5	−20.0±3.4**	6.3±1.0*
	10	−4.0±0.7	−23.7±1.4**	3.8±2.3
Vehicle		−2.2±1.0	−2.2±1.3	−0.7±1.3
Prazosin	1	−2.7±0.5	−10.3±1.3*	2.0±1.2
	3	−5.3±0.9*	−15.2±2.3**	7.0±4.6
	10	−2.4±0.3	−22.8±3.0**	10.3±3.3*

Each value represents the mean±S.E.M. from 6 dogs. Asterisks denote significant difference vs vehicle by the Dunnett's test: *P<0.05, **P<0.01.

St. Louis, MO, USA); pentobarbital (Dinabot, Chicago, IL, USA); isoflurane (Dinabot); 2-[β-(4-hydroxy-3-[^{125}I]-iodophenyl)ethylamino-methyl] tetralone ([^{125}I]-HEAT) (specific activity 2,200 Ci/mmol) (New England Nuclear, Boston, MA, USA); Ham's F12 medium and G418 (Gibco BRL, Life Technologies, Gaithersburg, MD, USA). All other chemicals were of reagent grade. CHO-K1 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). The expression vector pSVK3 was from Pharmacia (Upsala, Sweden). Naftopidil was dissolved in 0.1 M phosphate buffer solution. Prazosin hydrochloride and tamsulosin hydrochloride were dissolved in distilled water.

RESULTS

Effects of α_1 antagonists in the anesthetized dog model

In the isoflurane-anesthetized dogs after 20 min of dosing, naftopidil, tamsulosin and prazosin seemed not to cause marked effects on prostatic pressure and heart rate. At higher doses, all drugs induced decreases in mean blood pressure (Table 1).

The i.v. administration of 3 μg/kg phenylephrine produced increases in both prostatic pressure (11.1±0.6 mmHg, n=72) and mean blood pressure (19.7±0.6 mmHg, n=72) and decreases in the heart rate (13.5±1.0 beatas/min, n=72) responses (Fig. 1, control). The PBS or distilled water used as the compound solvent did not affect the phenylephrine-induced pressure and heart rate responses. The inhibitory effects of the three compounds on the phenylephrine-mediated increases in prostatic pressure and mean blood pressure are summarized in Tables 2 and 3, respectively. The i.v. administration of naftopidil at the doses of 10−1000 μg/kg dose-dependently inhibited phenylephrine-induced increases in prostatic pressure and mean blood pressure (Fig. 1). This inhibitory effect of naftopidil was sustained for 40 min after intravenous administration. The other α_1-adreno-

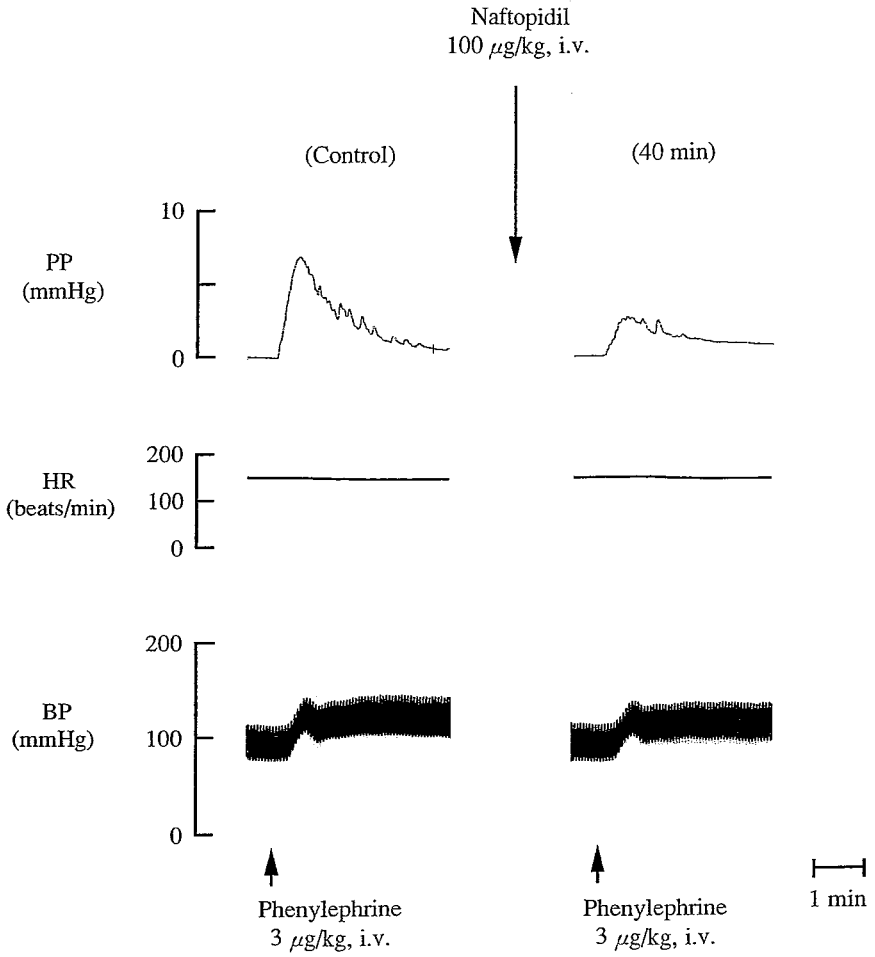

Fig. 1. Representative recording of the effect of naftopidil on phenylephrine-induced responses in prostatic pressure (PP), heart rate (HR) and blood pressure (BP) in an anesthetized dog. Phenylephrine (3 μg/kg) was injected before and 40 min after the administration of naftopidil (100 μg/kg, i.v.).

Table 2. Effects of naftopidil on phenylephrine-induced increases in prostatic pressure in anesthetized dogs

Drugs	Dose (μg/kg)	Inhibition (%)			
		Time after administration	20 min	30 min	40 min
Vehicle			−3.9±7.7	−0.2±6.3	−0.5±8.5
Naftopidil	10		12.1±10.6	10.6±7.3	3.3±7.8
	30		26.0±8.7	14.3±8.5	11.3±9.0
	100		49.0±9.6**	53.3±7.0**	47.4±11.4**
	1000		100.0±0.0**	100.0±0.0**	98.9±1.2**
Vehicle			−0.5±4.8	5.2±5.8	−1.3±5.6
Tamsulosin	1		7.6±9.3	14.5±11.4	1.5±8.6
	3		59.4±13.9**	67.1±8.6**	63.8±9.3**
	10		98.1±1.9**	100.0±0.0**	100.0±0.0**
Vehicle			−0.5±4.8	5.2±5.8	−1.3±5.6
Prazosin	1		−1.7±2.7	1.3±6.6	−9.1±3.1
	3		38.9±5.2**	31.3±4.8**	25.0±5.3**
	10		83.5±1.9**	74.7±4.4**	68.3±4.8**

Each value represents the mean±S.E.M. from 6 dogs. Asterisks denote significant difference vs vehicle by the Dunnett's test: *P<0.05, **P<0.01.

ceptor antagonists, tamsulosin (1−10 μg/kg) and prazosin (1−10 μg/kg), also dose-dependently inhibited phenylephrine-induced increases in prostatic and mean blood pressure. The three compounds dose-dependently inhibited phenylephrine-induced decreases in heart rate (data not shown).

The doses required to produce a 50% inhibition of the phenylephrine-induced increases in prostatic and mean blood pressure (ED_{50} value) and the selectivity index obtained from the ratio of those two doses were determined for each test compound. The ED_{50} values and selectivity indexes of naftopidil, tamsulosin and prazosin are shown in Table 4. In the inhibition of phenylephrine-induced in-

Table 3. Effects of naftopidil on phenylephrine-induced increases in mean blood pressure in anesthetized dogs

Drugs	Dose (μg/kg)	Inhibition (%)			
		Time after administration	20 min	30 min	40 min
Vehicle			2.7±4.9	0.1±4.2	−4.5±4.9
Naftopidil	10		3.3±5.4	−1.5±2.9	2.7±5.4
	30		13.8±1.9	7.1±3.2	5.8±1.9
	100		28.3±6.9**	30.6±5.5**	29.4±6.9**
	1000		66.8±5.9**	66.8±5.8**	62.4±5.9**
Vehicle			1.6±4.0	−2.3±3.2	−5.8±5.7
Tamsulosin	1		36.1±5.1**	35.9±5.3**	32.4±5.2**
	3		59.8±4.5**	58.8±5.5**	51.7±4.7**
	10		71.6±2.1**	73.6±5.2**	66.5±4.5**
Vehicle			1.6±4.0	−2.3±3.2	−5.8±5.7
Prazosin	1		29.8±5.7**	27.1±4.6**	25.0±2.8**
	3		51.5±5.7**	49.5±3.6**	48.8±4.9**
	10		59.3±3.1**	55.7±7.0**	56.2±5.6**

Each value represents the mean±S.E.M. from 6 dogs. Asterisks denote significant difference vs vehicle by the Dunnett's test: *P<0.05, **P<0.01.

creases in prostatic and mean blood pressure responses, the ED_{50} values of the naftopidil (i.v. administration after 20 min) were 105 and 366 μg/kg, respectively. The corresponding ED_{50} values of tamsulosin and prazosin were 2.46 and 1.90 μg/kg and 4.05 and 2.78 μg/kg, respectively. Thus, the inhibitory effects of naftopidil on increased prostatic and mean blood pressure were less potent than those of the other two compounds. However, at 20, 30 and 40 min after intravenous administration, the selectivity indices of naftopidil were 3.49, 3.80 and 3.76 for prostatic pressure and mean blood pressure, respectively, and the selectivity indices of tamsulosin and prazosin were 0.77, 0.94 and 1.23 and 0.69, 0.66 and 0.61, respectively. Naftopidil showed more than 3.5-fold selectivity for prostatic pressure relative to mean blood pressure in this anesthetized dog model. Tamsulosin inhibited mean blood pressure as potently as it did prostatic pressure. Prazosin, in contrast, inhibited mean blood pressure selectively compared with prostatic pressure.

[^{125}I]-HEAT binding studies with cloned human α_1-adrenoceptors

Membrane preparations from CHO cells stably expressing the cloned human α_1-adrenoceptor genes showed saturable binding of [^{125}I]-HEAT; the B_{max} and K_D values for the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors were 1.3±0.2, 5.5±0.1 and 1.1±0.1 pmol/mg protein (n=3 each) and 110±21, 60±1 and 300±26 pM (n=3 each), respectively. The potencies of the test compounds at the cloned human α_1-adrenoceptors are summarized in Table 5. Naftopidil and naphthyl-OH-naftopidil were found to be more potent for the α_{1d}-adrenoceptor than for the other α_1-adrenoceptor subtypes. Phenyl-OH-naftopidil and desmethylnaftopidil were more potent for the α_{1a}- and α_{1d}-adrenoceptors than for the α_{1b}-adrenoceptor subtype. Tamsulosin was more potent for the α_{1a}-adrenoceptor than for the other α_1-adrenoceptor subtypes. Prazosin showed small differences in the binding potencies for each α_1-adrenoceptor subtype. Naftopidil had approximately 3- and 17-fold higher potency for the α_{1d}-adrenoceptor than for the α_{1a}- and α_{1b}-adrenoceptor subtypes, respectively. With the exception of the metabolites of naftopidil, the selectivity of naftopidil for the α_{1d}-adrenoceptor subtype was greater than those of tamsulosin and prazosin.

Table 4. Comparative antagonist potencies for α_1-adrenoceptor antagonists as inhibitors of phenylephrine-induced increases in prostatic pressure and mean blood pressure in anesthetized dogs

Drugs	Time after administration	PP (ED_{50})	BP (ED_{50})	Ratio
Naftopidil	20 min	105	366	3.49
	30 min	90	343	3.80
	40 min	112	421	3.76
Tamsulosin	20 min	2.46	1.90	0.77
	30 min	2.10	1.97	0.94
	40 min	2.22	2.72	1.23
Prazosin	20 min	4.05	2.78	0.69
	30 min	5.04	3.31	0.66
	40 min	6.01	3.65	0.61

Data represent the doses (expressed in μg/kg) producing 50% inhibition of the prostatic pressure (PP) and mean blood pressure (BP) induced by phenylephrine and the ratio between the dose (BP/PP: selectivity index).

Table 5. Affinity of naftopidil at the cloned human α_1-adrenoceptors

Drugs	K_i (nM)		
	α_{1a}	α_{1b}	α_{1d}
Naftopidil	3.7±0.6	20±1	1.2±0.0
Naphtyl-OH-naftopidil	1.4±0.1	3.5±0.1	0.088±0.017
Phenyl-OH-naftopidil	9.7±3.1	261±26	15±4
Desmethylnaftopidil	4.8±0.2	38±3	6.0±0.4
Tamsulosin	0.019±0.002	0.29±0.02	0.063±0.011
Prazosin	0.17±0.02	0.25±0.03	0.066±0.000

CHO cell membranes stably expressing α_{1a}-, α_{1b}- α_{1d}-adrenoceptor were incubated with [^{125}I]-HEAT, in the absence or presence of increasing concentrations of test compounds. Each value represents the mean±S.E.M. from at least three tests, and the points were chosen to be the linear portion of the inhibition curve. K_i values were generated using the iterative curve-fitting program LIGAND. For all drugs examined, Hill slopes were not significantly different from unity.

DISCUSSION

In the present study, we investigated the effects of naftopidil, tamsulosin and prazosin on phenylephrine-induced increases in prostatic pressure and mean blood pressure in an anesthetized dog model and the profile of these compounds at cloned human α_1-adrenoceptor subtypes in vitro. First, the i.v. administration of naftopidil and other α_1-adrenoceptor antagonists dose-dependently inhibited the phenylephrine-induced increases in both prostatic pressure and mean blood pressure in the isoflurane-anesthetized dogs. The doses of naftopidil necessary for the 50% inhibition of the phenylephrine-induced increase in prostatic and mean blood pressure (ED_{50} value) were higher than those of tamsulosin and prazosin (Table 3). The binding properties of α_1-antagonists to prostatic α_1-adrenoceptors have been investigated, showing that phenylpiperazine derivatives such as naftopidil were weaker inhibitors of prostat-

ic α_1-adrenoceptors than sulfamoylethylamine (tamsulosin) and quinazoline (prazosin) derivatives (11). To further assess the ability of the three compounds to inhibit phenylephrine-induced prostatic pressure and mean blood pressure response, we calculated the selectivity index from the ratio of the two ED_{50} values. The selectivity indices showed that naftopidil was selective (by more than 3.5-fold) for prostatic pressure relative to mean blood pressure, whereas tamsulosin and prazosin showed no selectivity for prostatic pressure. The present findings on tamsulosin and prazosin are consistent with those of several previous studies (28–30). These results support that naftopidil may be a better therapeutic drug for urinary obstruction in patients with BPH. In fact, naftopidil has been shown to improve urinary outlet obstructions with a slight side effect (dizziness), which soon disappeared after the dose was decreased, and was regarded as promising for the treatment of bladder outlet obstruction due to BPH (31).

Second, naftopidil was found to have a higher affinity for the α_{1d}-adrenoceptor than for the α_{1a}- and α_{1b}-adrenoceptor subtypes in a radioligand binding study using cloned rat α_1-adrenoceptor subtypes (32). That study also demonstrated that the selectivity of naftopidil for the α_{1d}-adrenoceptor was higher than that of tamsulosin and prazosin. In the present study using cloned human α_1-adrenoceptor subtypes, naftopidil had a higher affinity for the α_{1d}-adrenoceptor subtype than for the other α_1-adrenoceptor subtypes (Table 4). Thus, our present findings support the idea that naftopidil can exert its α_{1d}-adrenoceptor-selective antagonism in humans. However, tamsulosin showed selectivity for α_{1a}-adrenoceptor relative to the other α_1-adrenoceptors, whereas prazosin displayed non-selectivity for the three cloned human α_1-adrenoceptors. These findings on tamsulosin and prazosin are consistent with those of a previous study (33). Although Forray et al. indicated that the α_1-adrenoceptor that mediates the contraction of human prostate smooth muscle had the pharmacological properties of the α_{1A} subtype (34), our present findings suggest that the selectivity of naftopidil for the α_{1a}-adrenoceptor subtype can not explain the uroselectivity of naftopidil. One possibility is that agents having selectivity for α_{1d}-adrenoceptor related to the α_{1b}-subtype may selectively inhibit phenylephrine-induced increase in prostatic pressure compared with mean blood pressure. In this regard, naftopidil, tamsulosin and prazosin had 17-, 4.6- and 3.8-fold higher potency for the α_{1d}-subtype than for the α_{1b}-subtype, respectively. In addition, the α_{1a}- and α_{1d}-adrenoceptor were present in the interstitum of the prostate, but the α_{1b}-adrenoceptor was hardly detectable (23). Futhermore, a recent study using α_{1b}-adrenoceptor knockout mice has suggested that the α_{1b}-adrenoceptor participates in the regulation of vascular contractile and blood pressure responses (35). Further studies are clearly required to determine functions of α_1-adrenoceptor subtypes in the prostate and blood vessels.

We also evaluated the human α_1-adrenoceptor subtype selectivity of three main metabolites of naftopidil. Naphtyl-OH-naftopidil was more potent than naftopidil in all cell lines. Naphtyl-OH-naftopidil decreased blood pressure in anesthetized dogs and inhibited noradrenaline-induced contraction of rabbit aorta. The effects of this metabolite were approximately similar to those of naftopidil (data not shown). After oral administration of naftopidil to humans or dogs, however, naphtyl-OH-naftopidil was not detected in serum or urine, and its glucuronate conjugate was detected (24, 25). Phenyl-OH-naftopidil, a metabolite in humans, and desmethyl-naftopidil, a metabolite in humans and dogs, were less potent than naftopidil in all cell lines. After oral administration of naftopidil to humans or dogs, phenyl-OH-naftopidil and desmethylnaftopidil in serum were of equal or less concentration than naftopidil (24, 25). These findings indicated that naftopidil played the major role in the improvement of bladder outlet obstruction for patients with BPH.

In conclusion, our study has demonstrated that α_1-adrenoceptor antagonists can reduce urethral pressure, in particular at the prostatic site, in anesthetized male dogs. The results are consistent with the efficacy of these compounds against BPH. Naftopidil had greater selectivity for reducing prostatic pressure related to mean blood pressure in this model than did the other two α_1-adrenoceptor antagonists examined. The selectivity of naftopidil for prostatic pressure may be attributable to its high binding affinity for α_{1a}- and α_{1d}-adrenoceptor subtypes.

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