Vascular $\alpha_1$-Adrenoceptor Subtype Selectivity and $\alpha_1$-Blocker-Induced Orthostatic Hypotension

Hitoshi Take$^1$, Katsushi Shibata$^1$, Takeo Awaji$^1$, Akira Hirase$^1$, Ichiro Ikegaki$^2$, Toshio Asano$^2$, Tatsuyuki Takada$^2$ and Gozoh Tsujimoto$^1$,*

$^1$Department of Molecular, Cell Pharmacology, National Children's Medical Research Center, 3–35–31 Taishido, Setagaya-ku, Tokyo 154–8509, Japan
$^2$First Laboratory for Pharmacological Research, Institute for Life Science Research, Asahi Chemical Industry, 632–1 Mifuku, Ohito-cho, Tagata-gun, Shizuoka 410–2321, Japan

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ABSTRACT—Newly developed $\alpha_1$-adrenoceptor antagonists including naftopidil are free from the "prazosin-like" side effect of orthostatic hypotension and associated symptoms. We investigated the mechanism for the differential effects of naftopidil and prazosin on the development of postural hypotension, with special attention on their selectivity for the $\alpha_1$-adrenoceptor subtype. We observed that head-up tilt caused a similar extent of drop in mean arterial pressure in control, naftopidil (1 mg/kg)- or prazosin (10 $\mu$g/kg)-treated rats; however, the tilt-induced postural hypotension was recovered within 2 min in the naftopidil-treated group, but not in the prazosin-treated group. Comparing an inhibitory effect on noradrenaline-induced contraction in the rat aorta and portal vein, we found that naftopidil was sixfold less potent in the portal vein, while prazosin showed similar potency in both tissues. Reverse transcription-polymerase chain reaction analysis showed that the expression of $\alpha_{1d}$-adrenoceptor mRNA predominated in the aorta, while that of $\alpha_{1b}$-adrenoceptor mRNA predominated in the portal vein. Using cloned rat $\alpha_1$-adrenoceptor subtypes, we found that naftopidil was selective for the $\alpha_{1d}$-subtype with approximately ninefold higher affinity than at the other subtypes. These results show that the pharmacological character of naftopidil, combined with the differential expression of the $\alpha_{1d}$-adrenoceptor subtype in the artery and the vein, may partly explain the differential effect of naftopidil and prazosin on head-up tilt-induced hemodynamic responses.

Keywords: $\alpha_1$-Adrenoceptor, Naftopidil, Prazosin, Postural hypotension, Reverse transcription-polymerase chain reaction

The sympathetic nervous system plays an important role in regulating the tone of the peripheral circulation. Catecholamines cause vascular smooth muscle contraction by activating $\alpha_1$-adrenoceptors. Recently, it was found that $\alpha_1$-adrenoceptors comprise a heterogeneous family (1). Two natively expressed subtypes ($\alpha_{1A}$ and $\alpha_{1B}$) can be distinguished pharmacologically, while three subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$) have been cloned (2–5). Each subtype has a characteristic pharmacological profile, and a marked species heterogeneity in the tissue distribution has been identified (6). Of the $\alpha_1$-adrenoceptor subtypes, tissue distribution of the $\alpha_{1A}$-adrenoceptor is markedly different in humans; thus, the $\alpha_{1x}$-adrenoceptor mRNA expression predominates in many human tissues including the liver, heart and prostate, as opposed to a restricted distribution in other species (6, 7). The $\alpha_{1B}$-adrenoceptor is widely distributed and mediates a variety of functions in the rat including smooth muscle contraction (8) and regulation of hepatic glycogen metabolism (9). Although the functional role is not well understood, the $\alpha_{1D}$-subtype is shown to localize in the rat arteries (10) and participate in the contractile response of the rat aorta to noradrena-
line (11, 12). However, there is still little information available about the physiological role of each $\alpha_1$-adrenoceptor subtype in the regulation of hemodynamics in particular.

Orthostatic hypotension and associated symptoms is a major clinical side effect for $\alpha_1$-adrenoceptor antagonists such as prazosin. These side effects have been attributed to a reduction in peripheral resistance and an increase in venous pooling mediated by a blockade of vascular $\alpha_1$-adrenoceptor (13, 14). Recently, it was suggested that some $\alpha_1$-adrenoceptor subtype-selective antagonists may have fewer orthostatic side effects (13, 15). Among those agents, the newly developed antihypertensive $\alpha_1$-adrenoceptor antagonist naftopidil (16) was reported not to cause "prazosin-like" alterations of the cardiovascular responses to orthostatic stress in clinical studies (17, 18).

In the present study, we have investigated the mechanism for the differential hemodynamic effect of naftopidil and prazosin by utilizing the rat as a model. We first confirmed that a tilt-induced postural hypotension recovered in naftopidil-treated rats, but not in prazosin-treated rats, and then we performed a series of physiological and molecular biological studies.

MATERIALS AND METHODS

Materials

Sources of drugs were as follows: 2-[3-[4-hydroxy-3-[125]iodophenyl]ethylamino-methyl] tetralone ([125]I-HEAT) (specific activity 2,200 Ci/mmol), (New England Nuclear, Boston, MA, USA); methoxamine HCl, phenylephrine HCl and (±)-noradrenaline bitartrate, desmethylimipramine HCl (Sigma, St. Louis, MO, USA); phen tolamine HCl (Ciba-Geigy, Summit, NJ, USA); prazosin HCl and doxazosin mesylate (Pfizer, Brooklyn, NY, USA); naftopidil ((±)-1-[4-(2-methoxyphenyl) piperazinyl]-3-(1-naphthyl) propan-2-ol) (Asahi Chemical Industry, Tokyo); (−)-YM617 (tamsulosin) ([−]-)(−)-5-[2-[2-(o-ethoxyphenox)ethyl]amino]propyl]-2-methoxy-benzesulfoxamide hydrochloride) (Yamanei Pharmaceutical Co., Tokyo); 5-methylurapidil (Research Biochemicals, Natick, MA, USA); deoxy cortisolone acetate, (±)-propranolol hydrochloride, alpha-chloralose (Nacalai Tesque, Kyoto); urethane (Tokyo Kasei Kogyo, Tokyo); sodium pentobarbital (Dinabot, Chicago, IL, 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 (ATCC; Rockville, MD, USA). The expression vector pSVK3 was from Pharmacia (Upsala, Sweden).

Postural change in arterial blood pressure

Male normotensive Sprague-Dawley rats weighing 270 to 310 g were briefly restrained in a 15 × 7 cm plastic cylinder to facilitate the combined administration of alpha-chloralose (40 mg/kg), urethane (40 mg/kg) and sodium pentobarbital (15 mg/kg), injected into the tail vein in a volume of 1 ml/kg of 5% glucose (19). Following anesthesia, the rats were placed in a supine position on a tilt board, and the four legs and upper front teeth were firmly affixed. The left common carotid artery was cannulated with PE-50 catheters for monitoring arterial pressure using a pressor transducer (NEC San-ei Instruments, Tokyo). The transducer was positioned at the level of the heart so that changes in posture by tilting would not influence arterial pressure measurements. The left femoral vein was cannulated for drug injection. In tilt experiments, the rats were subjected to a consistent, reproducible 45° head-up position from the horizontal without moving or touching the rats. Each tilt was timed to 2 min, after which the rats were returned to their original supine position.

About 30 min was allowed after anesthesia for preparation and equilibration prior to the start of the experiment. Basal mean arterial blood pressure (MAP) and blood pressure tilt response (BPTR) were determined as a control. Following the control tilt experiments, the rats were treated with either prazosin (10 μg/kg of body wt.), naftopidil (1 mg/kg of body wt.) or vehicle, and then after at least 10 min, tilt experiments were reexamined. Supine MAP immediately prior to tilt and MAP at 10, 30, 60 and 120 sec into each tilt maneuver were recorded. A mean basal value for each of these parameters was then averaged for each rat. BPTR and the drop in MAP obtained were compared between with and without drug treatment at each point described above (19, 20). The experimental animal care protocol was approved by the Animal use and Care Committee of the National Children's Medical Research Center.

Contractile response of rat aorta and portal vein

Rats were anesthetized with sodium pentobarbital and exsanguinated from the carotid artery. Thoracic aortic and portal veins were excised and ring preparations (3–4 mm in length) from each artery and vein were made in oxygenated Krebs-Henselte solution (118 mM NaCl, 4.7 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHPO₄ and 11 mM glucose). Each ring was suspended in a 20-ml organ bath containing Krebs-Henselte solution (pH 7.4) at 37°C and aerated continuously with a mixture of 95% O₂: 5% CO₂. Rings of aorta and portal vein were stretched to resting tensions of 1.0 and 0.5 g, respectively. Force generation was monitored with a TB-611T isometric transducer (Nihon
Kohden, Tokyo). Desmethylinipramine (100 nM), deoxytartorone acetate (5 μM) and propranolol (1 μM) were added to the bath solution to block neuronal and extraneuronal uptake of noradrenaline and to block β-adrenoceptors, respectively (21). Cumulative concentration-response curves for noradrenaline were constructed in the absence and in the presence of nanotriplid or prazosin. The antagonists were allowed to equilibrate with the tissues for at least 30 min. Antagonist pA2 values were obtained according to the procedure of Arunlakshana and Schild (22).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

RT-PCR assays were performed as described (23). Briefly, total cellular RNA from thoracic aortae and portal veins (approximately 0.5 g each) were extracted with 5.5 M guanidine isocyanate and purified from the lysate by centrifugation through a cesium chloride cushion at 350,000 × g for 4 hr. The RNA pellet was collected in 200 μl of 0.1 M Tris HCl pH 7.5, 12.5 mM EDTA, 0.15 M NaCl, 1% SDS solution; extracted with phenol/chloroform (1:1 vol./vol.), ethanol-precipitated; dried; resuspended in 50 μl of RNase-free water; and quantified by absorbance measurements at 260 nm. In preliminary experiments, the integrity of the purified RNA collected with this method was confirmed by visualization of the 28S and 18S ribosomal RNA bands after the electrophoresis of the RNA through a 1% agarose-formaldehyde gel. To eliminate contaminating genomic DNA, prepared total cellular RNA samples were further treated with RNase-free DNasel (Stratagene, La Jolla, CA, USA). RNA samples (10 μg each) were incubated at 37°C for 30 min with 20 U of DNasel in DNase buffer (40 mM Tris HCl pH 7.5, 6 mM MgCl2, 2 mM CaCl2, 2 U of placental RNase inhibitor) in a 30-μl volume. The reaction was stopped by an extraction with phenol/chloroform (1:1 vol./vol.), and RNA samples were ethanol-precipitated, vacuum-dried, and then resuspended in RNase-free water. RNA samples were then reverse transcribed as follows: each sample contained 10 μg of total cellular RNA, 50 mM Tris HCl pH 8.3, 75 mM KCl, 0.5 mM MgCl2, 10 mM dithiothreitol, 0.5 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 20 U of RNase inhibitor, 100 pmol random hexamer (Takara, Kyoto) and 200 U moloney murine leukemia virus reverse transcriptase (Gibco) in a final volume of 20 μl. After incubation at 37°C for 60 min, the samples were heated at 94°C for 5 min to terminate the reactions and were stored at −20°C until use.

Oligonucleotide primers were constructed from the published cDNA sequences of rat α1α-, α1β- and α1δ- adrenoceptor and rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) cDNA. GAPDH served as a control for the efficacy of RNA isolation and cDNA synthesis.

The sequences of the rat α1α-adrenoceptor primers were as follows: 5'-CATCGTGTTGGTTGCTCTTC TGCTG-3' (coding sense), corresponding to bases 921–950 of the cloned full-length sequence; 5'-CACAGAGA CTTGGTGGGCCTTCCCGGTGCG-3' (anticoding sense), which corresponds to bases 1236–1207 (24). The sequences of the rat α1δ-adrenoceptor primers were as follows: 5'-CTATGTTGACCACCCTTCAGCCGCTA C-3' (coding sense), corresponding to bases 645–672 of the cloned full-length sequence; 5'-ATGAAGAGGAGG AGGAAAACATAGAAGATGAA-3' (anticoding sense), which corresponds to bases 1175–1147 (25). The sequences of the rat GAPDH primers were as follows: 5'-TCCCTCAAGATTGTCAGCAAA-3' (coding sense) corresponding to bases 506–525 of the cloned full-length sequence; 5'-ATGATCACAACGGAT ACATT-3' (anticoding sense) which corresponds to bases 813–794 (26). The predicted sizes of the amplified rat α1α-, α1δ-, α1δ-adrenoceptor and GAPDH PCR products were 316, 531, 173 and 308 bp, respectively.

Each reverse transcription mixture was diluted 1:5 in RNase-free water and 2 μl were then transferred to fresh tubes for amplification. Each sample contained the upstream and downstream primers (0.2 μM of each primer) spanning the given sequence for amplification, 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 10 mM MgCl2, 0.01% (wt./vol.) gelatin, and 2.5 U of Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA) in a final volume of 25 μl. The reaction mixture was then overlaid with 3 drops (approximately 50 μl) of mineral oil and amplified in a Perkin Elmer Cetus thermal cycler. The amplification profiles consisted of denaturation at 94°C for 1 min, primer annealing at 55°C for 30 sec, and extension at 72°C for 1 min, and the PCR was conducted for 30 cycles. Negative control reactions without template were routinely included in PCR amplifications with both primer sets.

To confirm that the PCR products obtained were the same as the published cDNA clone, nucleotide sequences of the PCR products were determined as follows: fragments of PCR products were gel purified and inserted into pBluescript II KS(+) (Stratagene), and subcloned clones were analyzed in an ABI 373A DNA Sequencer (Applied Biosystems, Foster City, CA, USA).
Stable expression of the rat receptor genes

The cDNAs for the rat α1a- and α1d-adrenoceptors were kind gifts from Dr. Dianne M. Perez (Department of Molecular Cardiology, Cleveland Clinic Research Institute, Cleveland, OH, USA) and Dr. Robert M. Graham (The Victor Chang Cardiac Research Institute, St. Vincent’s Hospital, Sydney, Australia) [3, 24]. The rat α1b-adrenoceptor clone was isolated from the rat liver cDNA library in our laboratory. The 1.4-kb, 1.5-kb and 1.7-kb cDNAs, which contain the entire rat α1a-, α1b- and α1d-adrenoceptor coding region, respectively, were inserted into the eukaryotic expression vector pSVK3 containing the neomycin-resistant gene (pSVK3neo).

Stable cell lines were obtained by transfection of the pSVK3neo containing the rat α1a-, α1b- or α1d-adrenoceptor cDNA construct into CHO-K1 cells, using the Lipofectin™ technique as described previously [27]. Briefly, CHO-K1 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).

[^21]I-HEAT binding

CHO cell membrane preparations were made as described previously [27]. 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 Na₂HPO₄, 1.48 mM KH₂PO₄, 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 MgCl₂, pH 7.4) and centrifuged at 1,000 × g at 4°C for 10 min to remove nuclei. The supernatant was then centrifuged at 35,000 × g for 20 min at 4°C, and the pellet was homogenized and then frozen at −80°C until assay. The protein concentration was measured by a bichinchonic acid protein assay kit (Pierce, Rockford, IL, USA).

Radioligand binding studies with [^21]I-HEAT were performed as described previously [4, 27]. Briefly, measurement of specific [^21]I-HEAT binding was performed by incubating 0.1 ml of membrane preparation (approximately 10 μg of protein) with [^21]I-HEAT in a final volume of 0.25 ml buffer B (50 mM Tris HCl, 10 mM MgCl₂, 10 mM EGTA, pH 7.4) for 60 min at 25°C in the presence or absence of competing drugs. The incubation was terminated by adding ice-cold buffer B and immediately filtering through Whatman 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 always performed in duplicate. For competition curve analysis, each assay contained about 70 pM [^21]I-HEAT. At this concentration nonspecific binding, defined as binding displaced by 10 pM phentolamine, represented about less than 10% of the total binding.

Statistical analyses

In the tilt and organ bath experiments, analysis of variance (ANOVA) with 99% confidence limits was performed, followed by the paired Student’s t-test on individual sets of data. Computer analyses with LIGAND (28) were used to evaluate dissociation constant and receptor density. Values are expressed as the mean ± S.E.M., and P values less than 0.05 were considered significant.

RESULTS

Head-up tilt experiments

In a series of preliminary experiments, we found that 1 mg/kg of naftopidil had an equipotent hypotensive effect to 10 μg/kg of prazosin in the rat; thus, a decrease in basal MAP of anesthetized rats after drug administration was 12.7 ± 1.7 mmHg for naftopidil and 12.0 ± 1.2 mmHg for prazosin (n = 5 each), respectively (the basal MAP without drug treatment was 140 ± 3.3 mmHg, n = 10). Using these doses, we compared the effect of naftopidil and prazosin on BPTR (Fig. 1). Graphed are the 2-min tilt-induced changes in MAP (Fig. 1, lower graphs). In the tilt experiments, both groups of rats developed a similar brisk drop in MAP following the tilt, and the tilt-induced drops in MAP were not significantly different compared to controls (17.5 ± 2.5 mmHg, n = 5 for naftopidil-treated rats, 18.0 ± 3.2 mmHg, n = 5 for prazosin-treated rats and 12.8 ± 3.6 mmHg for the control, n = 10, respectively) (Fig. 1, lower graphs). Although the drop in MAP on tilt was to the same extent for both prazosin- and naftopidil-treated rats, the time-course of changes in MAP following the tilt was markedly different; thus, the drop in MAP was sustained in prazosin-treated rats during the 2-min head-up tilt, while it recovered toward the pre-tilt level in naftopidil-treated rats, similar to the control rats (Fig. 1). In both groups vehicle administration did not significantly alter tilt-induced changes in MAP observed in controls (data not shown).

Organ bath experiments

To further investigate the mechanism for the differential effect of naftopidil and prazosin on the blood pressure compensation to tilt, we next compared effects of naftopidil and prazosin on noradrenaline-induced contractions in the rat thoracic aorta and portal vein. As shown in Fig. 2, both antagonists caused a concentration-dependent rightward shift in noradrenaline-induced contractile response of the aorta and portal vein.
a1-Adrenoceptor and Postural Hypotension

**Naftopidil**

<table>
<thead>
<tr>
<th>Control</th>
<th>1 mg/kg, i.v.</th>
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<tbody>
<tr>
<td>BP (mmHg)</td>
<td>control</td>
</tr>
<tr>
<td>2 min</td>
<td>100</td>
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</table>

**Prazosin**

<table>
<thead>
<tr>
<th>Control</th>
<th>10 μg/kg, i.v.</th>
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<tbody>
<tr>
<td>BP (mmHg)</td>
<td>control</td>
</tr>
<tr>
<td>2 min</td>
<td>100</td>
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**Fig. 1.** Effects of naftopidil and prazosin on head-up tilt-induced postural hypotension. Upper: Representative tracing of tilt-induced change in arterial blood pressure (BP). Lower: Time course for the change in mean arterial pressure (ΔMAP) before (○) and after (●) drug treatment. Head-up tilt-induced postural hypotension was tested before and 10 min after administration of naftopidil (1 mg/kg, i.v.) and prazosin (10 μg/kg, i.v.), and 2-min tilting was performed as shown by the horizontal bar. Each point represents the mean ± S.E.M. in ΔMAP in the control pretreatment and 10-min post-treatment tilt tests (n = 5 each). Asterisks: a significant difference between pre- and posttreatment value (P < 0.05).

Prazosin shifted the noradrenaline concentration-response curves of both tissues in parallel, which yielded pA_2_ values of 9.73 ± 0.09 for the aorta and 9.49 ± 0.10 for the portal vein (n = 4 each). On the other hand, naftopidil was found to be about sixfold more potent in the aorta than in the portal vein (pA_2_ values were 8.32 ± 0.33 and 7.55 ± 0.05 for the aorta and portal vein, respectively, n = 5 each, P < 0.05). In all experiments, Schild slopes were not significantly different from unity (Table 1), being consistent with competitive antagonism. As naftopidil was previously reported to have effects other than α_1a_-adrenoceptor blocking effect such as Ca^{2+}-channel-blocking activity (16), we further examined the effect of naftopidil on the K^+ (60 mM)-depolarized contraction in both tissues and found that naftopidil (up to 3 μM) had no effect (data not shown), confirming that the inhibitory effect of naftopidil on noradrenaline-induced contraction in the rat aorta is not mediated through its Ca^{2+}-channel-blocking activity.

**Expression of α_1_-adrenoceptor subtype mRNA**

We next performed a RT-PCR study to determine the α_1_-adrenoceptor subtype expressed in the rat aorta and portal vein. As shown in Fig. 3, in the rat portal vein, PCR product of the expected size was visible only for the α_1a_-adrenoceptor but not for the α_1d_- or α_1β_-adrenoceptor (lane 3), whereas in the aorta, that was visible only for the α_1d_-adrenoceptor (lane 4). The sequences of the PCR products obtained in the rat portal vein and aorta were confirmed to be identical to nucleotide positions 645–1175 of rat α_1a_-adrenoceptor cDNA and 1286–1458 of rat α_1d_-adrenoceptor cDNA, respectively (data not shown). No products were detected with the negative controls performed in the absence of cDNA (lane 6) or without the RT reaction (lane 1 for the portal vein; lane 2 for the aorta), assuring that the amplified products all originated from mRNA rather than from contaminating genomic DNA. In both tissues, similar amounts of GAPDH PCR products were obtained in each of the RT-PCR samples (Fig. 3).

[^25]-HEAT binding studies with cloned rat α_1_-adrenoceptors

Membrane preparations from CHO cells stably expressing the cloned rat α_1_-adrenoceptor genes showed saturable binding of [^25]-HEAT; B_max and K_D values for the α_1a_, α_1d_, and α_1β_-adrenoceptors were 790 ± 20, 1,600 ± 230 and 450 ± 30 fmol/mg protein (n = 3 each)
and 86±2.3, 72±10 and 54±9.1 pM (n=3 each), respectively. The potencies of α1-adrenoceptor agonists and antagonists at the cloned rat α1-adrenoceptors are summarized in Table 2. Noradrenaline and phenylephrine were found to be more potent at the α1a-adrenoceptor than the other α1-adrenoceptor subtypes. Prazosin and doxazosin showed very small differences in their binding potencies at each α1-adrenoceptor subtype, while naftopidil was found to be relatively selective for the α1d-adrenoceptor; thus, naftopidil had approximately ninefold higher potency at the α1d-adrenoceptor than at the α1a- and α1b-adrenoceptor subtypes (Table 2).

Table 1. Potency of naftopidil and prazosin to inhibit noradrenaline-induced contraction of rat aorta and portal vein

<table>
<thead>
<tr>
<th></th>
<th>Naftopidil</th>
<th>Prazosin</th>
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<tbody>
<tr>
<td></td>
<td>pA₂</td>
<td>Slope</td>
</tr>
<tr>
<td>Aorta</td>
<td>8.32±0.33</td>
<td>0.98±0.12</td>
</tr>
<tr>
<td>Portal vein</td>
<td>7.55±0.05*</td>
<td>1.03±0.03</td>
</tr>
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</table>

pA₂ values and slope factors were evaluated by linear regression analysis. Each value represents the mean±S.E.M. from 4–5 individual experiments. *P<0.05 vs aorta.
**Fig. 3.** The $\alpha_{1a}$, $\alpha_{1b}$, $\alpha_{1d}$-adrenoceptor and GAPDH mRNA expression in the rat aorta and portal vein. RT-PCR was performed with 100 ng of total RNA from aorta and portal vein using the primer set as described under the Materials and Methods. PCRs using as templates cDNAs from portal vein (lane 1, without reverse transcription; lane 3, with reverse transcription) and aorta (lane 2, without reverse transcription; lane 4, with reverse transcription) are shown. Also shown are PCRs with cDNA encoding the rat $\alpha_{1a}$, $\alpha_{1b}$, $\alpha_{1d}$-adrenoceptor or GAPDH as a positive control (lane 5) and without template as a negative control (lane 6). PCR products were electrophoresed on 5% polyacrylamide gel and stained with ethidium bromide. PCR products of 531 base pairs and 173 base pairs specific for the rat $\alpha_{1a}$ and $\alpha_{1d}$-adrenoceptor, respectively, were detected. PCR was performed for 30 cycles.

### Table 2. Pharmacological profile of the cloned rat $\alpha_1$-adrenoceptor subtypes

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$\alpha_{1a}$</th>
<th>$\alpha_{1b}$</th>
<th>$\alpha_{1d}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>1,000±100</td>
<td>1,000±270</td>
<td>46±4.2</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>16,000±2,100</td>
<td>170,000±16,000</td>
<td>11,000±2,500</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>3,200±400</td>
<td>23,000±19,000</td>
<td>370±110</td>
</tr>
<tr>
<td>Naftopidil</td>
<td>27±1</td>
<td>27±3</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.13±0.06</td>
<td>0.19±0.07</td>
<td>0.11±0.020</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>2.4±0.4</td>
<td>3.5±0.5</td>
<td>4.6±0.4</td>
</tr>
<tr>
<td>5-Methylurapidil</td>
<td>2.6±0.6</td>
<td>140±44</td>
<td>30±8</td>
</tr>
<tr>
<td>(−)-YM617</td>
<td>0.044±0.009</td>
<td>0.7±0.1</td>
<td>0.18±0.03</td>
</tr>
</tbody>
</table>

CHO cell membranes stably expressing the cloned rat $\alpha_{1a}$, $\alpha_{1b}$ or $\alpha_{1d}$-adrenoceptors were incubated with [125I]-HEAT in the absence or presence of increasing concentrations of various agonists and antagonists. Each value represents the mean±S.E.M. from 3–5 individual experiments performed in duplicate. At least ten concentrations of each ligand were tested, and the points were chosen to be the linear portion of the displacement curve. $K_i$ values were generated by 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 mechanism for the differential effects of naftopidil and prazosin on the development of postural hypotension. First, we observed that prazosin, but not naftopidil, caused postural hypotension in anesthetized rats. In organ bath experiments using rat aorta and portal vein as a model of arterial and venous tissue, we observed that naftopidil more potentlly inhibited the noradrenaline-induced contraction in the aorta than in the portal vein. RT-PCR analysis showed that the two tissues were markedly different in their expression of \( \alpha_1 \)-adrenoceptor-subtype mRNA: \( \alpha_{1d} \) and \( \alpha_{1b} \)-adrenoceptor mRNA predominated in the aorta and portal vein, respectively. Furthermore, naftopidil was found to be relatively selective for the \( \alpha_{1d} \)-subtype in radioligand binding study with the cloned rat \( \alpha_1 \)-adrenoceptors. Taken together, the results indicate that the relatively \( \alpha_{1d} \)-adrenoceptor-selective antagonist naftopidil may have more inhibitory effect on the arterial \( \alpha_1 \)-adrenoceptor-mediated contractile response than the venous one, thereby the drug may not cause the non-selective \( \alpha_1 \)-adrenoceptor antagonist prazosin-like alterations of the cardiovascular responses to orthostatic stress.

As indicated in the Introduction, the use of classical \( \alpha_1 \)-adrenoceptor antagonists such as prazosin is frequently limited by side effects such as orthostatic hypotension, fatigue and dizziness that occur in up to 20% of patients (13). Most adverse events can be attributed to reversible competitive antagonism of postsynaptic \( \alpha_1 \)-adrenoceptor in resistance arteries and capacitance veins that sustain high-levels of \( \alpha \)-adrenergic sympathetic tone. In particular, the venous dilating effect of these agents, which would impair cardiac output by reducing preload, is considered to be one of the major contributing factors for the postural hypotension. From our results of RT-PCR and the contractile studies, the \( \alpha_1 \)-adrenoceptor subtype that mediates noradrenaline-induced contraction in the rat aorta and portal vein appears to be different; hence a relatively \( \alpha_{1d} \)-adrenoceptor-selective antagonist naftopidil may be less potent in inhibiting the \( \alpha_{1d} \)-adrenoceptor-mediated contraction of the portal vein. These results, while obtained in the rat aorta and portal vein, may indicate that \( \alpha_1 \)-adrenoceptor subtype regulating arteries and veins can be different, and that drugs selective for the "arterial \( \alpha_1 \)-adrenoceptor subtype" such as naftopidil may have less inhibitory effect on the contraction of veins, which may partly explain the differential effect of naftopidil and prazosin on the head-up tilt-induced hemodynamic changes in the rat.

As \( \alpha_1 \)-adrenoceptor subtypes have species-related differences in their pharmacological properties and tissue distribution, it is difficult to directly extrapolate our results obtained in the rat to humans and to explain why naftopidil does not cause clinical "prazosin-like" postural hypotension (17). In fact, however, the \( \alpha_{1d} \)-adrenoceptor predominates in the human aorta (7) as is similar for the rat, although very little information is available regarding arterial and venous \( \alpha_1 \)-adrenoceptor subtypes in humans. Furthermore, our radioligand binding studies with human cloned \( \alpha_1 \)-adrenoceptors showed that naftopidil and its major metabolite in human (naphthyl)hydroxynaftopidil ((±)-1-[4-(2-methoxyphenyl)piperazinyl]-3-[(4-hydroxy-1-naphthyl)oxy]propan-2-ol) have higher affinity for the \( \alpha_{1d} \)-subtype than the others (unpublished observation). Thus, our present data may support the idea that naftopidil can exert its \( \alpha_{1d} \)-adrenoceptor-selective antagonism even in humans.

Interestingly, more recently developed \( \alpha_1 \)-adrenoceptor blockers such as tamsulosin and naftopidil have been reported to be well-tolerated in clinical studies (13, 15). Although clinical results should be carefully evaluated as pharmacokinetic factors and the dose used may influence the incidence of side effects, one hypothesis to explain the good tolerability of the newer \( \alpha_1 \)-adrenoceptor antagonists would be selectivity for an \( \alpha_1 \)-adrenoceptor subtype that regulates arterial blood pressure but is not involved in side effects such as orthostatic hypotension. In fact, tamsulosin, recently developed for the treatment of benign prostatic hypertrophy, is shown to have better tolerability than prazosin (29) and have lower affinity at the \( \alpha_{1b} \)-adrenoceptor (30). Also, as described above, naftopidil is found to have higher affinity at the \( \alpha_{1d} \)-adrenoceptor in humans as well as in the rat. Hence, these data may suggest that improved tolerability of the newly developed \( \alpha_1 \)-adrenoceptor antagonists is attributable to \( \alpha_1 \)-adrenoceptor subtype selectivity for the \( \alpha_{1d} \) and/or \( \alpha_{1b} \)-adrenoceptor, but not for the \( \alpha_{1a} \)-adrenoceptor. Further information regarding the tissue distribution of each \( \alpha_1 \)-adrenoceptor subtype and functional studies with more selective agents in humans are clearly required to determine the \( \alpha_1 \)-adrenoceptor subtype involved in the development of clinical side effects of this class of drugs.

In summary, the present study for the first time analyzed the mechanism for the \( \alpha_1 \)-adrenoceptor antagonist-related side effect of postural hypotension with special focus on the \( \alpha_1 \)-adrenoceptor subtype. As observed in the present study, targeting specific \( \alpha_1 \)-adrenoceptor subtypes in vascular smooth muscle may have particular utility in the drug therapy of hypertension. More information on the distribution of \( \alpha_1 \)-adrenoceptor subtypes in various vascular tissues and the use of very selective antagonists for specific vascular beds would be of value for the study of the human vascular \( \alpha_1 \)-adrenoceptor pharmacology, pathophysiology and more effective drug therapy.


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