ELSEVIER

European Journal of Pharmacology 329 (1997) 55–61

ejp

Characterization of subtype of α_1-adrenoceptor mediating vasoconstriction in perfused rat hind limb

Weizhong Zhu, Youyi Zhang, Chide Han *

Institute of Vascular Medicine, The Third Hospital, Beijing Medical University, Beijing 100083, People's Republic of China

Received 5 December 1996; revised 8 April 1997; accepted 11 April 1997

Abstract

The subtype of α_1-adrenoceptor mediating the exogenous noradrenaline-induced vasopressor response in perfused rat hind limb was determined by functional measurements and radioligand binding assays. The potencies (pA_2 values) of α_{1A}-adrenoceptor-selective antagonists, RS-17053 (*N*-[2-(2-cyclopropylmethoxy-phenoxy) ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), WB 4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4 benzodioxane), 5-methyl-urapidil, and the α_{1D}-adrenoceptor-selective antagonist, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4.5]decane-7,9-dione), to inhibit the noradrenaline-induced vasopressor response determined by Schild plot were 9.47 ± 0.21, 9.48 ± 0.19, 8.10 ± 0.27 and 6.66 ± 0.14, respectively, with no slope significantly different from unity. The affinities (K_i values) of these antagonists were determined by displacement of ^{125}I-BE 2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) binding from the cloned α_{1a}-, α_{1b}-, α_{1d}-adrenoceptor, stably expressed in human embryonic kidney (HEK) 293 cells. The pA_2 values of the above antagonists correlated well with the binding K_i values only for α_{1A}-adrenoceptors ($r = 0.93$), but not for α_{1B}-adrenoceptors ($r = 0.51$) and α_{1D}-adrenoceptors ($r = 0.13$). The concentration-vasopressor response curve for noradrenaline was not significantly affected by pretreatment with 50 μM chloroethylclonidine for 30 min. The results suggest that only α_{1A}-adrenoceptors mediate the noradrenaline-induced vasopressor response in perfused rat hind limb. © 1997 Elsevier Science B.V.

Keywords: α_{1A}-Adrenoceptor; Hind limb, isolated perfused, rat

1. Introduction

α_1-Adrenoceptors can be found at numerous end organs of the autonomic nervous system, especially vascular smooth muscle. The clinical potencies of prazosin and other α_1-adrenoceptor antagonists as antihypertensive drugs are evidence of the important physiological role of peripheral α_1-adrenoceptors in maintaining arterial pressure in animals and humans.

α_1-Adrenoceptors have been classified on the basis of pharmacological evidence into three subtypes termed α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor subtypes mainly because of differential affinities to subtype-selective antagonists. Selective α_{1A}-adrenoceptor antagonists include WB 4101 (Morrow et al., 1985; Morrow and Creese, 1986), 5-methyl-urapidil (Gross et al., 1988; Hanft and Gross, 1989), (+)-niguldipine (Boer et al., 1989), and RS-17053 (Ford et al., 1996). BMY 7378 appears to preferentially antagonize α_{1D}-adrenoceptors (Goetz et al., 1995). Chloroethylclonidine is an alkylating antagonist that irreversibly inactivates the α_{1B}- and α_{1D}-adrenoceptor, but not the α_{1A}-adrenoceptor subtype (Perez et al., 1991; Forray et al., 1994; Han et al., 1987; Minneman et al., 1988). The heterogeneity of α_1-adrenoceptors is further supported by results of receptor gene cloning studies which show that three molecular subtypes (α_{1a}-, α_{1b}-, α_{1d}-adrenoceptor) exist and correspond directly to the receptors expressed in intact tissues (Ford et al., 1994). One of the most useful approaches for characterizing subtype distribution in a tissue is to determine correlations between potencies of antagonists obtained in functional experiments with the tissue and affinities obtained from radioligand binding assays with cloned α_1-adrenoceptor subtypes.

* Corresponding author. Tel.: (86-10) 6209-2306; Fax: (86-10) 6201-5681; e-mail: hanqd@mail.bjmu.edu.cn

PII S0014-2999(97)00127-1

Investigations employing functional, radioligand binding and molecular methods have demonstrated the existence of multiple α_1-adrenoceptor subtypes in vascular smooth muscle of isolated rat, rabbit, dog, and human blood vessels, such as aorta (Buckner et al., 1996), renal artery (Han et al., 1990; Piascik et al., 1994), and mesenteric artery (Han et al., 1990; Piascik et al., 1994), etc. These arteries essentially function as conduit vessels which direct blood flow into organs; the contractile properties of these vessels have a minor influence on the regulation of vascular resistance. It is more important to determine the distribution and function of α_1-adrenoceptor subtypes in a resistance vascular bed. In the rat perfused mesenteric arterial bed, noradrenaline-induced increases of perfusion pressure are mainly mediated by the activation of α_{1A}-adrenoceptors based on the high affinities for 5-methylurapidil (pA_2 9.0–9.2) and WB 4101 (pA_2 9.6, Kong et al., 1994; Cunningham et al., 1994; Williams and Clarke, 1995) and the lack of affinity for chloroethylclonidine (Williams and Clarke, 1995). The α_1-adrenoceptor in the rat kidney vascular bed has also been characterized as an α_{1A}-adrenoceptor subtype (Eltze et al., 1991; Blue et al., 1991, 1992, 1995). A consistent observation in isolated perfused kidney studies is the weak inhibitory effect of chloroethylclonidine on the vasopressor response to α_1-adrenoceptor stimulation. In addition, the potencies of several subtype-selective antagonists on the noradrenaline-induced vasocontractile response in the isolated rat perfused kidney correlated significantly with their binding affinity in the cloned α_{1a}-adrenoceptor ($r = 0.85$). In contrast, correlation with binding affinity for the cloned α_{1b}-adrenoceptor ($r = 0.02$) or α_{1d}-adrenoceptor ($r = 0.12$) is poor (Blue et al., 1995). Those results confirm that the α_{1A}-adrenoceptor is the major subtype in renal resistance arterioles. Other reports show that, in isolated perfused rat mesentery or kidney, sympathetic nerve stimulation provokes vasoconstriction which is extremely sensitive to low concentrations of α_{1A}-adrenoceptor antagonists and nonsensitive to chloroethylclonidine (Kong et al., 1994; Blue et al., 1991, 1992). Therefore, α_{1A}-adrenoceptors may be located at the neurovascular synapses where they receive direct sympathetic nerve innervation (Kong et al., 1994; Williams and Clarke, 1995; Eltze et al., 1991; Blue et al., 1992).

The distribution of α_1-adrenoceptor subtypes in other resistance vasculature has not been reported yet. Skeletal muscle is the largest organ in the body, comprising an estimated 35–45% of body weight in non-obese individuals. The muscle resistance vessels have a key role in regulating the total systemic vascular resistance and hence arterial blood pressure. In the present study, we characterized subtypes of the α_1-adrenoceptor distributed in the resistance vasculature of skeletal muscle by determining functional potencies of α_1-adrenoceptor subtype-selective antagonists and comparing them with binding affinities at cloned α_1-adrenoceptor subtypes.

2. Materials and methods

2.1. Isolated perfused rat hind limb

2.1.1. Preparation of isolated perfused rat hind limb

The animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). Surgery was performed as described by Colquhoun et al. (1990). Flow was restricted to two hind limbs by cannulation with PE-50 polyethylene to two lateral common iliac arteries. Perfusion was performed in a temperature-controlled cabinet, and the arterial perfusion medium was temperature-equilibrated by passage through a heat exchanger. The perfusion Krebs solution contained the following (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 1.27; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 8.3, and 2% hydroxyethyl starch, pH 7.4 at 28°C. To ensure that the perfusion buffer was particle-free, the buffer was filtered under pressure through a 0.45-μm pore filter before use. The perfusion solution was saturated with a mixture of 95% O_2-5% CO_2. The perfusion pressure was monitored through a junction located as close as practicable to the iliac canal, using a pressure transducer connected to a polygraph. Desmethylimipramine 1 μM, normetanephrine 1 μM (to block neuronal and extraneuronal uptake of noradrenaline, respectively), indomethacin 10 μM (to inhibit prostanoid production), propranolol 10 μM (to block β-adrenoceptors) and yohimbine 0.1 μM (to block α_2-adrenoceptors) were also included in the perfusion solution except where described otherwise.

2.1.2. Determination of noradrenaline-induced vasopressor responses and potencies of α_1-adrenoceptor antagonists

Cumulative concentration-response curves were obtained by infusion of each concentration of agonist for 5–6 min. After the highest concentration, the preparation was allowed to recover to show reversibility. With the exception of those with chloroethylclonidine, all experiments with antagonists were performed as follows. After control noradrenaline concentration-response curves were made, the hind limbs were perfused for 45 min with Krebs solution containing antagonist (three different concentrations with 0.5 log M increments) or vehicle. A second curve for noradrenaline was then made in the presence of antagonist or vehicle (time control). Perfusion pressure was determined from a calibrated recorder trace, and concentration-response relationships were determined from these readings, so that a graphical representation could be made. EC_{50} was calculated by computer analysis using non-linear regression. The pA_2 and slope for the antagonist were determined by Schild plot. In time control experiments, the repeated response curves did not differ as to EC_{50}, maximum response, and basal pressure ($n = 5$, data not shown). Experiments with chloroethylclonidine were performed in the following way. After a control concentration-response curve for noradrenaline, the hind limbs were

perfused with chloroethylclonidine 50 μM for 30 min and then with Krebs solution free of chloroethylclonidine for 30 min before the second concentration-response curves for noradrenaline were made.

2.2. Radioligand binding assays

2.2.1. Cell culture

Subclones of HEK 293 (human embryonic kidney 293) cells transfected with hamster α_{1b}, bovine α_{1a} or rat α_{1d} cDNA were kindly provided by Dr. Kenneth P. Minneman. Subclones were propagated in Dulbecco's modified Eagle's medium containing 10% calf serum, 4.5 g/l glucose, and selective antibiotics (0.6 mg/ml histidinol for pREP8/α_{1a}, 0.05 mg/ml hygromycin for pREP4/α_{1b}, 0.15 mg/ml geneticin for pREP9/α_{1d}).

2.2.2. Membrane preparation

Cells were harvested by scraping confluent 75 cm^2 flasks and pelleted by centrifugation at 500 × *g* for 5 min, washed with 10 ml phosphate buffer solution (20 mM Na_2HPO_4 containing 154 mM NaCl, pH 7.6) and centrifuged again. The cells were homogenized with a Polytron (speed 6, 10 s) in 10 ml phosphate buffer solution. The membranes were collected by centrifugation at 20 000 × *g* for 10 min, resuspended in phosphate buffer solution and centrifuged again. The membranes were then resuspended in phosphate buffer solution (one flask HEK 293 cell transfected with α_{1b}/15 ml final resuspension, one flask HEK 293 cell transfected with α_{1a} or α_{1d}/10 ml final resuspension).

2.2.3. Radioligand binding assays

BE 2254 was radioiodinated to a theoretical radioactivity of 2200 Ci/mmol as described by Engel and Hoyer (1981) and stored at −20°C in methanol. Specific ^{125}I-BE 2254 binding was measured by incubating the tissue preparation with ^{125}I-BE 2254 in PBS in a final volume of 250 μl for 20 min at 37°C in the presence or absence of competing drugs. After 20 min, the incubation was terminated by adding 10 ml of 10 mM Tris-HCl (pH 7.4) and the mixture was filtered under vacuum using a glass-fiber filter. Each filter was washed with 10 ml of 10 mM Tris-HCl, dried and its radioactivity (cpm) was measured. Non-specific binding was less than 15%. To determine the affinity of 5-methyl-urapidil, BMY 7378, RS-17053, prazosin, and WB 4101 to α_1-adrenoceptors, the potencies of these antagonists for competing for the specific ^{125}I-BE 2254 binding sites were determined by incubation of a single concentration of ^{125}I-BE 2254 (40–50 pM) in the presence or absence of 16 concentrations of the antagonist. The IC_{50} values were determined as the *x* intercept on a Hill plot, and K_i values were calculated by the method of Cheng and Prusoff (1973).

2.3. Drugs and chemicals

Drugs were obtained from the following sources: noradrenaline, yohimbine, propranolol, desmethylimipramine, normetanephrine, prazosin, indomethacin (Sigma, St. Louis, MO, USA); WB 4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4 benzodioxane), 5-methyl-urapidil, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4.5]decane-7,9-dione) (Research Biochemicals International, Natick, MA, USA); RS-17053 (*N*-[2-(2-cyclopropylmethoxy-phenoxy) ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride (Roche Bioscience, USA); BE 2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) (Beiersdorf, Hamburg, Germany); [^{125}I]Na^+ (Beijing Institute of Atomic Energy, Chinese Academy of Science).

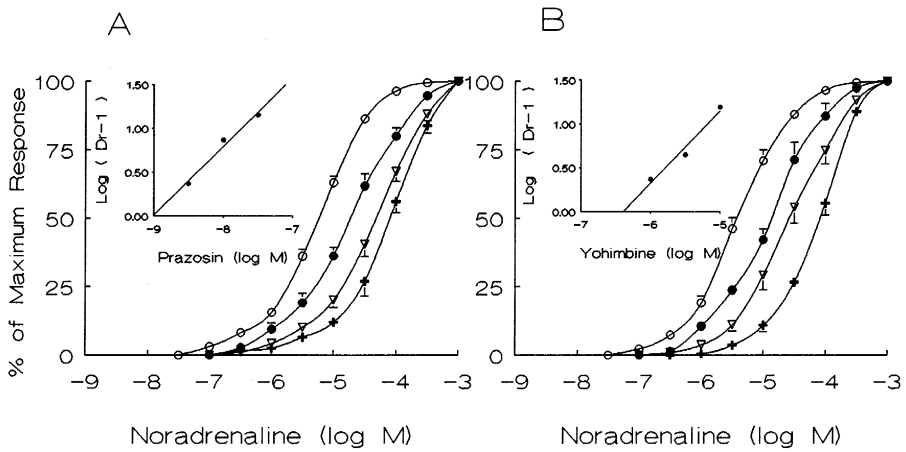

Fig. 1. The antagonist effect of prazosin and yohimbine against noradrenaline-induced contractile response in rat perfused hind limb. (A) In the absence (open circles, $n = 11$) and presence of prazosin: 3 nM (filled circles, $n = 5$), 10 nM (open triangle, $n = 5$), 30 nM (cross, $n = 5$). (B) In the absence (open circles, $n = 9$) and presence of yohimbine: 1 μM (filled circles, $n = 5$), 3 μM (open triangle, $n = 5$), 10 μM (cross, $n = 5$). Each point represents the mean and S.E.M. The inset shows results of the Schild plot.

Table 1
Comparison of the functional potencies (pA_2) of α_1-adrenoceptor subtype-selective antagonists to inhibit noradrenaline-induced vasopressor response in perfused rat hind limb to the radioligand binding affinities (pK_i) at cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors

	n	pA_2	Slope	Cloned α_{1a}			Cloned α_{1b}			Cloned α_{1d}		
				n	pK_i	n_H	n	pK_i	n_H	n	pK_i	n_H
Prazosin	5	9.04 ± 0.12	0.85 ± 0.13	4	9.34 ± 0.10	0.67 ± 0.04	4	9.16 ± 0.08	0.92 ± 0.02	4	9.69 ± 0.16	0.78 ± 0.01
WB 4101	5	9.48 ± 0.19	0.82 ± 0.10	4	9.28 ± 0.13	0.75 ± 0.05	5	7.29 ± 0.17	0.85 ± 0.10	4	8.42 ± 0.08	0.55 ± 0.10
RS-17053	6	9.47 ± 0.21	0.96 ± 0.14	4	8.59 ± 0.08	0.93 ± 0.08	4	7.06 ± 0.09	0.86 ± 0.05	4	7.40 ± 0.11	0.93 ± 0.10
5-MU [a]	5	8.10 ± 0.27	1.08 ± 0.23	5	8.24 ± 0.11	0.85 ± 0.10	5	6.40 ± 0.13	0.75 ± 0.12	4	6.76 ± 0.14	0.85 ± 0.15
BMY 7378	6	6.66 ± 0.14	0.81 ± 0.08	4	6.11 ± 0.10	1.10 ± 0.10	4	6.40 ± 0.16	1.10 ± 0.10	4	8.29 ± 0.16	0.85 ± 0.10

[a] 5-Methyl-urapidil.

2.4. Animal care

Experiments were performed with male, 180–200 g hooded Wistar rats. The rats were housed in groups, at room temperature (22–25°C) and provided with a conventional diet and water ad libitum. Experiments were approved by the Committee on the Ethical Aspects of Research Involving Animals of the Beijing Medical University.

2.5. Statistics

The results were expressed as means ± S.E.M. Comparisons were made using an analysis of variance or paired *t*-test, P values < 0.05 being considered as significant.

3. Results

3.1. pA_2 values of α_1-adrenoceptor subtype-selective antagonists

Prazosin (3–30 nM in the absence of yohimbine) or yohimbine (1–10 μM in the absence of prazosin) competitively antagonized noradrenaline-induced vasoconstriction (Fig. 1), with pA_2 values of 9.04 ± 0.12 and 6.35 ± 0.14, and slope in the Schild plot of 0.85 ± 0.13 and 0.86 ± 0.06, respectively. In the presence of 0.1 μM yohimbine to block α_2-adrenoceptors, prazosin (1–10 nM), RS-17053 (1–10 nM), WB 4101 (1–10 nM), 5-methyl-urapidil (10–100 nM), and BMY 7378 (0.3–3 μM) produced parallel shifts to the right of concentration-response curves for noradrenaline and did not significantly reduce the maximum response. Schild regression analyses yielded lines with slopes not significantly different from unity, and showed high potencies for RS-17053, WB 4101 and 5-methyl-urapidil, but a low potency for BMY 7378 (Table 1).

3.2. pK_i values of α_1-adrenoceptor subtype-selective antagonists

The α_1-adrenoceptor subtype-selective antagonists, WB 4101, RS-17053, 5-methyl-urapidil and BMY 7378, inhibited binding of ^{125}I-BE 2254 to α_{1A}-, α_{1B}-, α_{1D}-adrenoceptors stably expressed in the HEK 293 cell line in a concentration-dependent manner. Their K_i values are shown in Table 1.

3.3. Comparison between the functional potencies (pA_2) for α_1-adrenoceptor subtype antagonists and the binding affinities (pK_i) at the cloned α_1-adrenoceptor subtypes

The potencies (pA_2) for the α_1-adrenoceptor subtype-selective antagonists on the contractile response to nor-

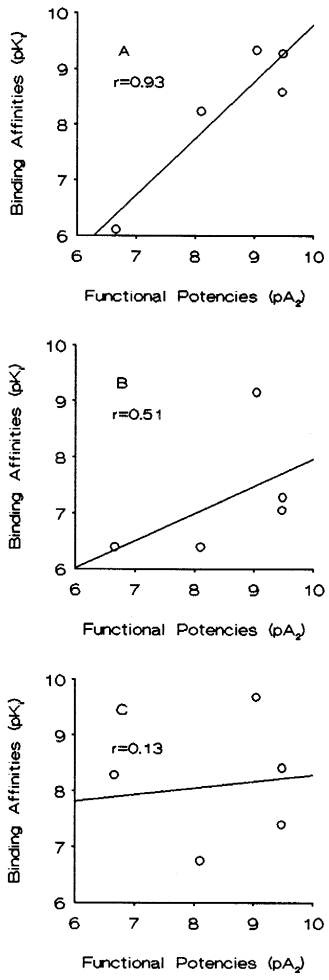

Fig. 2. Correlations between the potencies (pA_2) of the α_1-adrenoceptor antagonists for inhibition of noradrenaline-induced vasopressor response in rat perfused hind limb and binding affinities (pK_i) at the cloned α_{1a}-(A), α_{1b}- (B) and α_{1d}-adrenoceptor (C).

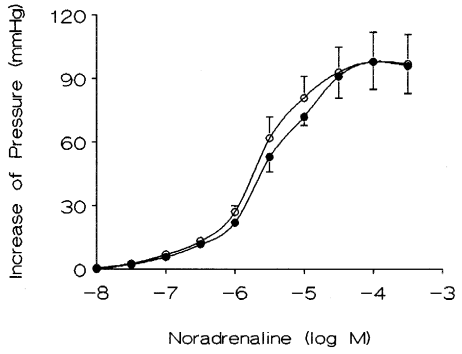

Fig. 3. The effect of chloroethylclonidine (50 μM) pretreatment on noradrenaline-induced pressure response in perfused rat hind limb. Vehicle, open circles; chloroethylclonidine (50 μM), filled circles. Each point represents the mean, and the S.E.M. is shown by a vertical line.

adrenaline in rat perfused hind limb correlated well with the binding affinities (pK_i) at the cloned α_{1a}-adrenoceptor ($r = 0.93$, Fig. 2A). In contrast, the functional pA_2 values correlated poorly with binding pK_i values at cloned α_{1b}- ($r = 0.51$, Fig. 2B) and α_{1d}-adrenoceptors ($r = 0.13$, Fig. 2C).

3.4. Effect of chloroethylclonidine on concentration-response curves for noradrenaline in rat perfused hind limb

Chloroethylclonidine 50 μM pretreatment for 30 min did not change the concentration-response curves for noradrenaline significantly. The EC_{50} (2.10 ± 0.19 vs. 3.19 ± 0.65 μM, $n = 5$) and maximum increase of perfusion pressure (96 ± 13 vs. 98 ± 13 mmHg, $n = 5$) were not significantly different in the two groups (Fig. 3).

4. Discussion

The vasopressor responses induced by exogenous noradrenaline or by sympathetic activity were mediated by both α_1-adrenoceptor and α_2-adrenoceptor in cat and dog hind limb vasculature (Gardiner and Peters, 1982; Karasawa and Koss, 1993). Medgett and Ruffolo (1988) reported that α_1- and α_2-adrenoceptors exist in the femoral vascular bed of rat hind limb and that both mediate exogenous noradrenaline-induced vasoconstriction. However, only the α_1-adrenoceptor was involved in neurogenic vasoconstriction. The present study showed that, in perfused rat hind limb, the vasopressor response to exogenous noradrenaline was antagonized by prazosin approximately 1000-fold more potently than by yohimbine. The pA_2 values for prazosin (9.20 ± 0.12 vs. 9.04 ± 0.12) were not significantly different in the absence and in the presence of yohimbine at concentrations sufficiently high (0.1 μM) to block α_2-adrenoceptors possibly participating in noradrenaline-induced vascular constriction. These results indicate that the exogenous noradrenaline-induced vasopressor response is predominantly mediated by α_1-adrenoceptors in the perfused rat hind limb vasculature.

The functional experiments of the present study showed that the α_{1A}-adrenoceptor-selective antagonists, RS-17053, WB 4101 and 5-methyl-urapidil, inhibit the noradrenaline-induced vasopressor response with high potencies, which are consistent with the K_i values reported from binding assays (Michel et al., 1995; Ford et al., 1996). The α_{1D}-adrenoceptor also has relatively high affinities for these antagonists compared with those of the α_{1B}-adrenoceptor. In contrast, BMY 7378 has an approximately 100-fold higher affinity at α_{1D}- than at α_{1A}- and α_{1B}-adrenoceptors (Goetz et al., 1995). In accordance with this, the pA_2 value for BMY 7378 obtained in the present study is consistent with its low affinity at α_{1A}-adrenoceptor. The fact that slopes of Schild plot for all antagonists mentioned were not significantly different from unity also supports the assumption that there is a single subtype of α_1-adrenoceptor existing in the rat hind limb vascular bed. Further, we performed radioligand binding assays in subcloned HEK 293 cells stably transfected with α_{1A}-, α_{1B}- or α_{1D}-adrenoceptor to measure K_i values of these compounds, and compared these K_i values with the pA_2 values obtained from the functional experiments. As expected, the results showed that the correlation was much higher at cloned α_{1a}- than at the cloned α_{1b}- or α_{1d}-adrenoceptor. Another strategy to determine α_1-adrenoceptor subtypes in a tissue is to assess the ability of chloroethylclonidine to irreversibly inactivate α_1-adrenoceptor-specific binding or to block α_1-adrenoceptor agonist-induced responses. Both α_{1B}- and α_{1D}-adrenoceptors are sensitive to chloroethylclonidine, while the α_{1A}-adrenoceptor is not. In the present study, preparations were pre-perfused with 50 μM chloroethylclonidine for 30 min, which should block most responses induced by either α_{1B}- or α_{1D}-adrenoceptors according to other reports and our own experience (Perez et al., 1991; Forray et al., 1994; Han et al., 1987; Minneman et al., 1988). Under these conditions, the noradrenaline-induced vasopressor response was not changed significantly, indicating that the response was mediated only by α_{1A}-adrenoceptors.

In conclusion, only the α_{1A}-adrenoceptor contributes to the exogenous noradrenaline-induced vasopressor response in rat hind limb, since the potencies of four subtype-selective antagonists correlated well with their binding affinities only for the α_{1A}-adrenoceptors but not for α_{1B}- and α_{1D}-adrenoceptors, and the response was not influenced by chloroethylclonidine pretreatment. Several questions need to be answered by further experiments. Firstly, whether or not neurogenic vasoconstriction in the rat hind limb is also mediated only by α_{1A}-adrenoceptors. Secondly, whether or not only α_{1A}-adrenoceptors distribute in the hind limb vasculature, or all subtypes of α_1-adrenoceptor are present but only α_{1A}-adrenoceptors play a functional role in vasoconstriction. The latter is possible, as we have demonstrated by means of RNase protection assays and solution

hybridization that mRNAs for all three subtypes of α_1-adrenoceptor are expressed in rat aorta (Xu and Han, 1996), although it is commonly accepted that only the α_{1D}-adrenoceptor mediates the vasoconstriction response. Finally, as in all three resistance vascular beds investigated thus far, that is the rat renal (Eltze et al., 1991; Blue et al., 1991, 1992, 1995), mesenteric (Kong et al., 1994; Cunningham et al., 1994; Williams and Clarke, 1995) and hind limb vasculature, only α_{1A}-adrenoceptors mediate the vasoconstriction response, it is speculated that this may be the case in all resistance vasculature. If so, the α_{1A}-adrenoceptor will be the major subtype involved in the regulation of systemic peripheral blood pressure. On the other hand, based on the fact that mainly α_{1A}-adrenoceptors mediate noradrenaline-induced smooth muscle contraction in the human prostate (Forray et al., 1994; Marshall et al., 1995; Hatano et al., 1994), much effort has been put into the search for α_{1A}-adrenoceptor-selective antagonists to develop drugs for the treatment of benign prostate hypertrophy. One of the α_{1A}-adrenoceptor-selective antagonists, tamsulosin, it still marketed. Orthostatic hypotension as a side-effect seems less with tamsulosin in clinical trials (Na et al., 1996), although Kenny et al. (1996) have demonstrated that in the anaesthetized dog, tamsulosin inhibits phenylephrine-induced increases in prostatic and blood pressure with similar affinity. Therefore further study in vivo is essential to define if it is true that mainly α_{1A}-adrenoceptors are involved in the regulation of peripheral blood pressure.

Acknowledgements

This study is supported by grants from the National Natural Science Foundation of China, and a grant from the China Medical Board of New York Inc., No. 93-951.

References

Blue, D.R. Jr., Vimont, R.L., Clarke, D.E., 1991. Antagonism of α_1-adrenoceptor subtypes by niguldipine and 5-methyl-urapidil in rat kidney. Br. J. Pharmacol. 102 ((Suppl.)), 196.

Blue, D.R. Jr., Vimont, R.L., Clarke, D.E., 1992. Evidence for a noradrenergic innervation to α_{1A}-adrenoceptors in rat kidney. Br. J. Pharmacol. 107, 414–417.

Blue, D.R. Jr., 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 α_{1C}-adrenoceptor: studies in the isolated perfused kidney of rat. Br. J. Pharmacol. 115, 283–294.

Boer, R., Grassegger, A., Schudt, C., Glossmann, H., 1989. (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors. Eur. J. Pharmacol. 172, 131–145.

Buckner, S.A., Oheim, K.W., Morse, P.A., Knepper, S.M., Hancock, A.A., 1996. α_1-Adrenoceptor-induced contractility in rat aorta is mediated by the α_{1D} subtype. Eur. J. Pharmacol. 297, 241–248.

Cheng, Y.C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Colquhoun, E.Q., Hettiarachchi, M., Ye, J.M., Rattigen, S., Clark, M.G., 1990. Inhibition by vasodilators of norepinephrine and vasoconstrictor-mediated, but not skeletal muscle contraction-induced oxygen uptake in the perfused rat hindlimb: implications for non-shivering thermogenesis in muscle tissue. Gen. Pharmacol. 21, 141–148.

Cunningham, D., Kim, Y., Vargas, H.M., 1994. Role of alpha$_1$ adrenoceptor (AR) subtypes in the isolated rat mesenteric vascular bed. FASEB J. 8, A874.

Eltze, M., Boer, R., Sanders, K.H., Kolassa, N., 1991. Vasodilatation elicited by 5-HT$_{1A}$ receptor agonists in constant-pressure-perfused rat kidney is mediated by blockade of α_{1A}-adrenoceptors. Eur. J. Pharmacol. 202, 33–44.

Engel, G., Hoyer, D., 1981. ^{125}IBE 2254, a new high affinity radioligand for α_1-adrenoceptors. Eur. J. Pharmacol. 73, 221–224.

Ford, A.P.W.D., Williams, T.J., Blue, D.R. Jr., Clarke, D.E., 1994. α_1-Adrenoceptor classification: sharpening Occam's razor. Trends Pharmacol. Sci. 15, 167–170.

Ford, A.P.W.D., Arredondo, N.F., Blue, D.R. Jr., Bonhaus, D.W., Jasper, J., Kava, M.S., Lesnick, J., Pfister, J.R., Shieh, I.A., Vimont, R.L., Williams, T.J., Mcnal, J.E., Stamey, T.A., Clarke, D.E., 1996. RS-17053 (*N*-[2-(2-cyclopropylmethoxy-phenoxy) ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functional α_1-adrenoceptors in human prostate: implications for adrenoceptor classification. Mol. Pharmacol. 49, 209–215.

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 α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1C}-subtype. Mol. Pharmacol. 45, 703–708.

Gardiner, J.C., Peters, C.J., 1982. Postsynaptic α_1- and α_2-adrenoceptor involvement in the vascular response to neuronally released and exogenous noradrenaline in the hindlimb of the dog and cat. Eur. J. Pharmacol. 84, 189–198.

Goetz, A.S., King, H.K., Ward, S.D.C., True, T.A., Rimele, T.J., Saussy, D.L., 1995. BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptor. Eur. J. Pharmacol. 272, R5–R6.

Gross, G., Hanft, G., Rugevics, C., 1988. 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor. Eur. J. Pharmacol. 151, 333–335.

Han, C., Abel, P.W., Minneman, K.P., 1987. α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329, 333–335.

Han, C., Li, J., Minneman, K.P., 1990. Subtypes of α_1-adrenoceptors in rat blood vessels. Eur. J. Pharmacol. 190, 97–104.

Hanft, G., Gross, G., 1989. Subclassification of α_1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. Br. J. Pharmacol. 97, 691–700.

Hatano, A., Takahashi, H., Tamaki, M., Komeyama, T., Koizumi, T., Takeda, M., 1994. Pharmacological evidence of distinct α_1-adrenoceptor subtypes mediating the contraction of human prostatic urethra and peripheral artery. Br. J. Pharmacol. 113, 723–728.

Karasawa, Y., Koss, M.C., 1993. Distribution of neuronally activated postjunctional adrenoceptors in cat forelimb vasculature. J. Cardiovasc. Pharmacol. 22, 594–599.

Kenny, B.A., Miller, A.M., Williams, I.J., O'Connell, J., Chalmers, D.H., Naylor, A.M., 1996. Evaluation of the pharmacological selective profile of alpha 1 adrenoceptor antagonists at prostatic alpha 1 adrenoceptors: binding, functional and in vivo studies. Br. J. Pharmacol. 118, 871–978.

Kong, J.Q., Taylor, D.A., Fleming, W.W., 1994. Functional distribution and role of alpha-1 adrenoceptor subtypes in the mesenteric vasculature of the rat. J. Pharmacol. Exp. Ther. 268, 1153–1159.

Marshall, I., Burt, R.P., Chapple, C.R., 1995. Noradrenaline contractions

of human prostate by α_{1A}- (α_{1C}-) adrenoceptor subtype. Br. J. Pharmacol. 115, 781–786.

Medgett, I.C., Ruffolo, R.R. Jr., 1988. Alpha-1 adrenoceptor-mediated vasoconstriction in rat hindlimb: innervated alpha-2 adrenoceptors in the saphenous arterial bed. J. Pharmacol. Exp. Ther. 246, 249–254.

Michel, M.C., Kenny, B., Schwinn, D.A., 1995. Classification of α_1-adrenoceptor subtypes. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 1–10.

Minneman, K.P., Han, C., Abel, P.W., 1988. Comparison of α_1-adrenergic receptor subtypes distinguished by chloroethylclonidine and WB 4101. Mol. Pharmacol. 33, 509–514.

Morrow, A.L., Creese, I., 1986. Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4101 and [^{3}H]prazosin binding. Mol. Pharmacol. 29, 321–330.

Morrow, A.L., Battaglia, G., Norman, A.B., Creese, I., 1985. Identification of subtypes of [^{3}H]prazosin-labelled α_1-receptor binding sites in rat brain. Eur. J. Pharmacol. 109, 285–287.

Na, Y., Wang, D., Jin, J., Gu, Y.L., Xu, Z.Y., Gu, F.L., 1996. Selective α_{1A} adrenoceptor antagonists for the treatment of BPH. Chin. J. Urol. 17, 327–329.

Perez, D.M., Piascik, M.T., Graham, R.M., 1991. Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. Mol. Pharmacol. 40, 876–883.

Piascik, M.T., Smith, M.S., Soltis, E.E., Perez, D.M., 1994. Identification of the mRNA for the novel α_{1D}-adrenoceptors and two other α_1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. 46, 30–40.

Williams, T.J., Clarke, D.E., 1995. Characterization of α_1-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in the isolated perfused mesentery of rat. Br. J. Pharmacol. 114, 531–536.

Xu, K., Han, C., 1996. Quantification of mRNAs for three α_1-adrenoceptor subtypes in rat aorta by solution hybridization. Life Sci. 59, 343–347.