Heterogeneity of α_1-adrenoceptor subtypes involved in adrenergic contractions of dog blood vessels

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1 We determined the α_1-adrenoceptor subtypes involved in adrenergic contractions of eight different blood vessels isolated from the dog.

2 Noradrenaline produced concentration-dependent contractions in all the blood vessels tested, which were competitively inhibited by prazosin, WB4101, HV723 and 5-methylurapidil. However, there was considerable difference between the vessels with regard to the pK_B values for all the antagonists. The α_1-adrenoceptors of dog vertebral and carotid arteries had high affinity for prazosin ($pK_B > 9.0$) but low affinity for WB4101 (< 8.5), 5-methylurapidil (< 7.5) and HV723 ($\leqslant 8.5$). By contrast, HV723 had higher affinity (> 9.0) than prazosin (< 8.3), WB4101 (< 8.7) and 5-methylurapidil (< 8.2) in the portal vein, mesenteric artery and vein, and renal artery. In the femoral artery and vein, however, the four antagonists showed pK_B values in the range 8.0–8.7.

3 Chloroethylclonidine ($10\,\mu M$) produced a remarkable reduction of the contractile responses to noradrenaline in the vertebral and carotid arteries as compared with those in the other vessels. Nifedipine inhibited the responses to noradrenaline in all the tissues tested, and had marked effects in the portal vein.

4 Sympathetic adrenergic contractions induced by transmural electrical stimulation were also inhibited by prazosin and HV723 at different potencies among tissues. The relative potencies of both the antagonists paralleled the relationship in inhibiting the responses to exogenous noradrenaline in each vessel.

5 According to recent α_1-adrenoceptor subclassification, the present results suggest that the contractions of blood vessels induced by endogenous and exogenous noradrenaline are mediated through different α_1-adrenoceptor subtypes heterogeneously distributed in each vessel; presumably, the α_{1B} subtype in the carotid and vertebral arteries, the α_{1N} subtype in the visceral region and the α_{1L} subtype in the femoral region. Regionally different expression of α_1-adrenoceptor subtypes may be in part associated with the regional heterogeneity of sympathetic responses in the blood vessels.

Keywords: α-Adrenoceptors; noradrenaline-induced contraction; dog blood vessels; α_1-adrenoceptor subclassification

Introduction

Heterogeneity of postjunctional α_1-adrenoceptors has been demonstrated in mammalian blood vessels, where different sensitivities to agonists and antagonists have been observed (McGrath, 1982; Drew, 1985; Bevan *et al.*, 1986; Takayanagi *et al.*, 1988; Docherty, 1989). The heterogeneity may be associated with different α_1-adrenoceptor subtypes (Flavahan & Vanhoutte, 1986; Minneman, 1988; Muramatsu *et al.*, 1990). Binding and/or molecular biological studies have revealed the existence of at least 4 α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1C} and α_{1D}) with high affinity for prazosin (1993 Receptor Nomenclature supplement, *Trends in Pharmacological Sciences*). The α_{1A} subtype shows high affinity for WB4101 (2-(2,6-dimethoxy-phenoxyethyl)aminomethyl-1,4-benzodioxane) and 5-methylurapidil, while the α_{1B} subtype is not sensitive to the drugs. The α_{1C} and α_{1D} subtypes are also highly sensitive to WB4101 but the α_{1D} subtype may be distinguished by its lesser sensitivity to 5-methylurapidil than the α_{1C} and α_{1A} subtypes (Morrow & Creese, 1986; Han *et al.*, 1987; Lomasney *et al.*, 1991; Perez *et al.*, 1991; Schwinn & Lomasney, 1992). On the other hand, another subclassification has been proposed from functional and binding studies, whereby the α_1-adrenoceptors can be separated into three subtypes (α_{1H}, α_{1L} and α_{1N}) (Muramatsu *et al.*, 1990; Oshita *et al.*, 1991; 1993; Ohmura *et al.*, 1992). Prazosin has a higher affinity for the α_{1H} subtype than for the α_{1L} and α_{1N} subtypes. The third α_{1N}-adrenoceptor subtype is distinguished by its higher affinity for HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxyl)ethyl)-amino)-prop-

yl)benzeneacetonitrile fumarate). The α_{1A}, α_{1B}, α_{1C} and α_{1D} subtypes mentioned above may be included in the α_{1H} group of the latter subclassification because of their high affinity for prazosin.

The objectives of this study were to identify the α_1-adrenoceptor subtypes involved in adrenergic contractions in various blood vessels of dogs according to the α_1-adrenoceptor subclassifications and to characterize the regional heterogeneity of sympathetic adrenergic responses with special reference to α_1-adrenoceptor subtypes.

Methods

Mongrel dogs of either sex (7–15 kg) were killed under pentobarbitone anaesthesia. Thereafter, the blood vessels listed in Table 1 were isolated and helically cut under a dissecting microscope (Muramatsu *et al.*, 1990), except for longitudinal preparations of the portal vein. To avoid the involvement of endothelium-derived relaxing factor in the mechanical response (Furchgott, 1981), the endothelial cells in the blood vessels were removed by rubbing them with filter paper. The functional loss of endothelial cells was confirmed by the loss of the relaxing response to acetylcholine ($1\,\mu M$) or substance P ($0.1\,\mu M$) in noradrenaline-precontracted arteries or veins (Muramatsu *et al.*, 1990). Each strip was mounted vertically in an organ bath containing 20 ml of modified Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, $NaHPO_4$ 1.2 and glucose 11.5. The medium was maintained at 37°C, pH 7.4, and equilibrated with a gas mixture consist-

Table 1 EC_{50} values and relative contractions caused by noradrenaline before and after 10 μM or 50 μM chloroethylclonidine (CEC)-treatment

		EC_{50}^a			Relative contraction[b]		
Blood vessel	Before CEC	Without CEC[c]	After 10 μM CEC	After 50 μM CEC	Without CEC	After 10 μM CEC	After 50 μM CEC
Vertebral artery	6.95 ± 0.06	6.95 ± 0.08	4.68 ± 0.48[d]	4.19 ± 0.08[d]	1.10 ± 0.07	0.58 ± 0.06[d]	0.55 ± 0.03[d]
Carotid artery	6.53 ± 0.12	6.40 ± 0.18	5.06 ± 0.37[d]	3.93 ± 0.19[d]	1.08 ± 0.03	0.72 ± 0.08[d]	0.46 ± 0.08[d]
Portal vein	7.35 ± 0.15	7.28 ± 0.45	7.64 ± 0.28	7.11 ± 0.22	1.00 ± 0.06	0.95 ± 0.03	0.90 ± 0.03
Mesenteric artery	6.45 ± 0.10	6.47 ± 0.25	6.25 ± 0.15	5.66 ± 0.30	1.03 ± 0.08	0.91 ± 0.07	0.79 ± 0.05[d]
Mesenteric vein	6.70 ± 0.13	6.51 ± 0.21	6.34 ± 0.08	6.14 ± 0.07	0.99 ± 0.07	0.93 ± 0.08	0.77 ± 0.03[d]
Renal artery	6.20 ± 0.08	6.19 ± 0.07	5.89 ± 0.15	5.87 ± 0.28	1.03 ± 0.10	0.93 ± 0.02	0.92 ± 0.06
Femoral artery	6.26 ± 0.06	6.26 ± 0.22	5.91 ± 0.09	5.40 ± 0.43	0.98 ± 0.04	0.94 ± 0.02	0.75 ± 0.08[d]
Femoral vein	6.50 ± 0.12	6.33 ± 0.34	6.32 ± 0.08	5.96 ± 0.28	1.02 ± 0.10	0.92 ± 0.07	0.82 ± 0.05[d]

[a]The concentrations of noradrenaline which produced a contraction to the level of a half maximal response before CEC treatment are represented as negative logarithm concentrations.
[b]The relative value of the maximal contraction induced by 100 μM noradrenaline after treatment with vehicle or CEC against the maximal contraction before the treatment in the same blood vessel.
[c]The vessels were treated by the same procedure as for CEC-treatment but the vehicle was used instead of CEC.
[d]Significantly different from the value without CEC ($P < 0.05$).
Means ± s.e. of 4–16 experiments.

ing of 95% O_2 and 5% CO_2. The tension was recorded isometrically through a force-displacement transducer. The preparations were equilibrated for 90 min before starting the experiments.

Noradrenaline was used as the agonist in all tissues. Desmethylimipramine (0.1 μM), deoxycorticosterone (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. Cumulative concentration-response curves for noradrenaline were obtained 5 or 6 times from the same strip and the third concentration-response curve was used as a control. In preliminary experiments, the reproducibility of the concentration-response curves obtained from the third to the sixth trial in the absence of the α-antagonist was confirmed. Three increasing concentrations of α-antagonist were added for 30 min before the fourth to sixth concentration-response curves. In each preparation, the pA_2 value was estimated from the ratio of median effective concentrations of noradrenaline in the presence or absence of blocking agents. When the straight line yielded a slope with unity, the pA_2 value estimated was represented as pK_B (Arunlakshana & Schild, 1959). In chloroethylclonidine (CEC) experiments, 10 or 50 μM CEC was added for 20 min after recording the third concentration-response curve for noradrenaline; then concentration-response curves were repeated after a 30 min washout period. Similar treatment with vehicle of CEC had no effect on the concentration-response curve for noradrenaline (i.e. less than 2 fold shift in the curve).

Transmural electrical stimulation was applied through a pair of platinum-wire electrodes at 10–15 min intervals (Muramatsu et al., 1989). The preparation was placed in parallel between the electrodes, the distance between them being about 2 mm. The stimulus parameters were 0.3 ms in duration and the supramaximum voltage (10 V) for 10 s. The stimulus frequency was 20 Hz in all preparations except the portal vein, in which 5 Hz was used. In this series of experiments, DG-5128 (10 μM) and propranolol (1 μM) were added to the bath medium to block prejunctional $α_2$-adrenoceptors and postjunctional β-adrenoceptors, respectively (Muramatsu et al., 1983; 1989). When studying the dog mesenteric artery and portal vein, α,β-methylene ATP (10 μM) was present throughout in order to block the sympathetic purinergic component (Machaly et al., 1988; Muramatsu et al., 1989). Atropine (1 μM) blocked muscarinic responses in the portal vein (Ichikawa et al., 1979).

Experimental values are given as means ± standard error of mean (s.e.mean). Results were analysed by Student's t test

(unpaired or paired comparison) and a probability of less than 0.05 was considered significant.

Drugs used were: (−)-noradrenaline bitartrate; desmethylimipramine hydrochloride; α,β-methylene ATP (Sigma, St. Louis, U.S.A.), deoxycorticosterone acetate, (±)-propranolol hydrochloride, atropine sulphate (Nacalai Tesque, Kyoto, Japan), prazosin (Taito-Pfizer, Tokyo, Japan), chloroethylclonidine dihydrochloride (CEC), WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane), 5-methylurapidil (Funakoshi, Tokyo, Japan), HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxyl)ethyl)amino)-propyl)-benzeneacetonitrile fumarate), (Hokuriku Seiyaku, Katsuyama, Fukui, Japan), tetrodotoxin (Sankyo, Tokyo, Japan), guanethidine sulphate (Tokyo-Kasei, Tokyo, Japan) and DG-5128 (2-(2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl) pyridine dihydrochloride sesquihydrate) (Daiichi Seiyaku, Tokyo, Japan).

Results

Effects of prazosin, WB4101, HV723 and 5-methylurapidil on the contractile responses to noradrenaline

Noradrenaline produced concentration-dependent contractions in the arteries and veins isolated from dogs. The pD_2 values for noradrenaline were within the range from 6.0 to 7.5 (Table 1). The concentration-response curves were attenuated by prazosin, WB4101, HV723 and 5-methylurapidil, resulting in a parallel shift of the curves to the right. The slopes of the Schild plot for all antagonists were not significantly different from unity, indicating that the antagonists competitively inhibited the contractile responses induced by noradrenaline in all tissues (Table 2). Figure 1 shows the representative Schild plots for prazosin, WB4101 and HV723 in the vertebral artery, femoral artery and portal vein. Prazosin inhibited the responses to noradrenaline at lower concentrations than WB4101 or HV723 in the vertebral and carotid arteries, thus higher pK_B values than those for WB4101 and HV723 were calculated. In the femoral artery and vein, the antagonist affinities did not differ among these four antagonists. On the other hand, the pK_B values for HV723 were higher than those for prazosin, WB4101 or 5-methylurapidil in the portal vein, mesenteric artery and vein, and renal artery. The pK_B values and slopes of the Schild plot for the $α_1$-adrenoceptor antagonists in these blood vessels are summarized in Table 2.

Effects of chloroethylclonidine (CEC) on the contractile responses to noradrenaline

Since CEC is thought to inactivate the α_{1B}-adrenoceptor subtype (Han et al., 1987), we examined its effects on the contractile responses to noradrenaline in various vascular tissues. As shown in Figure 2, pretreatment with CEC (10 and 50 μM) inhibited the concentration-response curves for noradrenaline in the dog vertebral artery. Similar results were obtained with the carotid artery. However, the attenuation by CEC was much less in the other blood vessels tested (Table 1). In the portal vein, CEC itself produced an irreversible contraction which lasted after CEC removal.

Effects of nifedipine on the contractile responses to noradrenaline

Nifedipine (1 μM) slightly but significantly attenuated the contractile responses to noradrenaline in all the tissues tested (Figure 3, Table 3). The maximum inhibition (47% reduction at 100 μM noradrenaline) was seen in the portal vein in which the spontaneous rhythmic contraction disappeared after exposure to nifedipine. The pD_2 values for noradrenaline were slightly (less than 4 times in affinity) reduced by nifedipine in the portal vein, mesenteric, renal and femoral arteries.

Effects of prazosin and HV723 on the contractile responses to transmural electrical stimulation

Transmural electrical stimulation produced transient contractions in the presence of propranolol, DG-5128, α,β-methylene ATP and/or atropine (see Methods for experimental details). These responses were abolished by guanethidine (3 μM) or tetrodotoxin (0.5 μM) (n = 3, for each drug and each vessel), suggesting sympathetic neurogenic responses. The contractions were also inhibited by prazosin and HV723 in concentration-dependent manner. However, the inhibitory potencies of both drugs varied among the tissues. Prazosin was more potent in inhibiting the neurogenic response than HV723 in the vertebral and carotid arteries, whereas the converse relationship was observed in the portal vein, mesenteric artery and vein, and renal artery (Table 4). In the femoral artery the inhibition by both prazosin and HV723 was equipotent, but the EC_{50} values were relatively low. These high potencies of both drugs in the femoral artery may be related to low concentrations of noradrenaline which diffuse from the sympathetic nerve terminals to the postjunc-
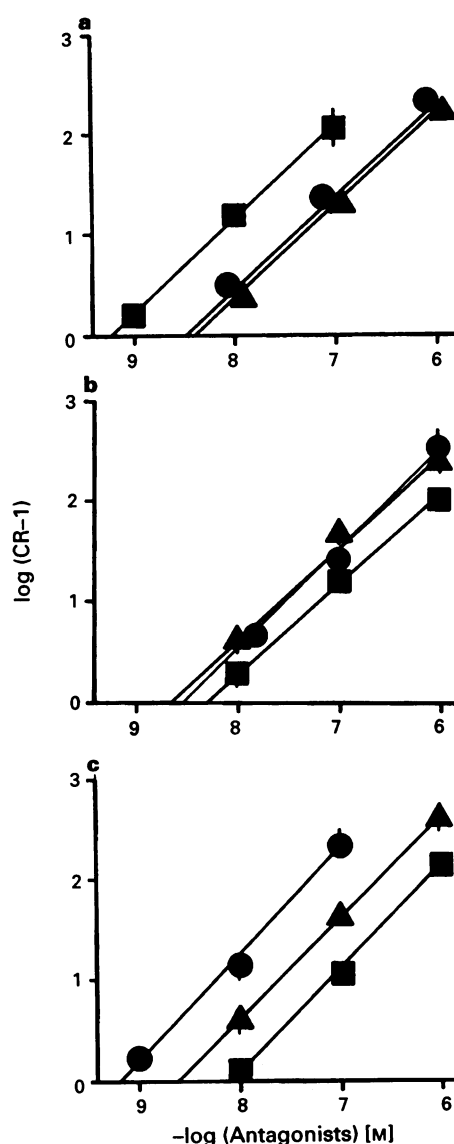

Figure 1 Representative Schild plots for competitive inhibition of noradrenaline-induced contraction by HV723 (●), WB4101 (▲) and prazosin (■) in the dog vertebral artery (a), femoral artery (b) and portal vein (c).

Table 2 α₁-Adrenoceptor affinities for prazosin, WB4101, HV723 and 5-methylurapidil in dog blood vessels

| | | pK_B/Slope (95% CL) | | |
Blood vessel	Prazosin	WB4101	5-Methylurapidil	HV723
Vertebral artery	9.24 ± 0.13	8.41 ± 0.11	7.29 ± 0.10	8.49 ± 0.15
	0.93 (0.75–1.12)	0.93 (0.79–1.07)	0.97 (0.81–1.13)	0.92 (0.75–1.10)
Carotid artery	9.55 ± 0.14	8.15 ± 0.15	7.21 ± 0.11	8.52 ± 0.10
	0.98 (0.73–1.13)	0.92 (0.81–1.03)	0.87 (0.73–1.00)	0.90 (0.77–1.04)
Portal vein	8.05 ± 0.07	8.63 ± 0.10	8.16 ± 0.06	9.11 ± 0.09
	1.03 (0.91–1.15)	0.97 (0.86–1.11)	1.08 (0.98–1.18)	1.09 (0.96–1.23)
Mesenteric artery	8.12 ± 0.10	8.76 ± 0.10	8.05 ± 0.10	9.15 ± 0.05
	0.94 (0.84–1.04)	0.95 (0.88–1.02)	1.14 (0.96–1.33)	0.96 (0.87–1.02)
Mesenteric vein	7.95 ± 0.10	8.51 ± 0.07	7.73 ± 0.16	9.04 ± 0.07
	0.95 (0.79–1.11)	0.97 (0.88–1.07)	1.04 (0.82–1.26)	0.96 (0.84–1.08)
Renal artery	8.05 ± 0.09	8.67 ± 0.10	8.21 ± 0.13	9.14 ± 0.12
	1.07 (0.91–1.23)	0.98 (0.86–1.11)	0.96 (0.78–1.15)	1.04 (0.85–1.23)
Femoral artery	8.29 ± 0.11	8.70 ± 0.16	8.43 ± 0.16	8.62 ± 0.13
	0.90 (0.75–1.04)	0.88 (0.73–1.03)	0.85 (0.66–1.05)	0.95 (0.70–1.20)
Femoral vein	8.33 ± 0.15	8.43 ± 0.11	8.00 ± 0.14	8.21 ± 0.18
	0.86 (0.68–1.04)	1.00 (0.84–1.16)	1.01 (0.74–1.27)	1.09 (0.75–1.42)

Data shown are means ± s.e. of 4–7 experiments.
All data were obtained from Schild plot analysis.

tional α_1-adrenoceptors and produce the contraction (Bevan & Su, 1973). The sympathetic contraction in the femoral artery developed slowly (time to peak: 115 ± 10 s in the

femoral artery vs 18 ± 2 s in the carotid artery, $n = 5$). The femoral vein was not examined because the sympathetic adrenergic contraction was small and unstable.
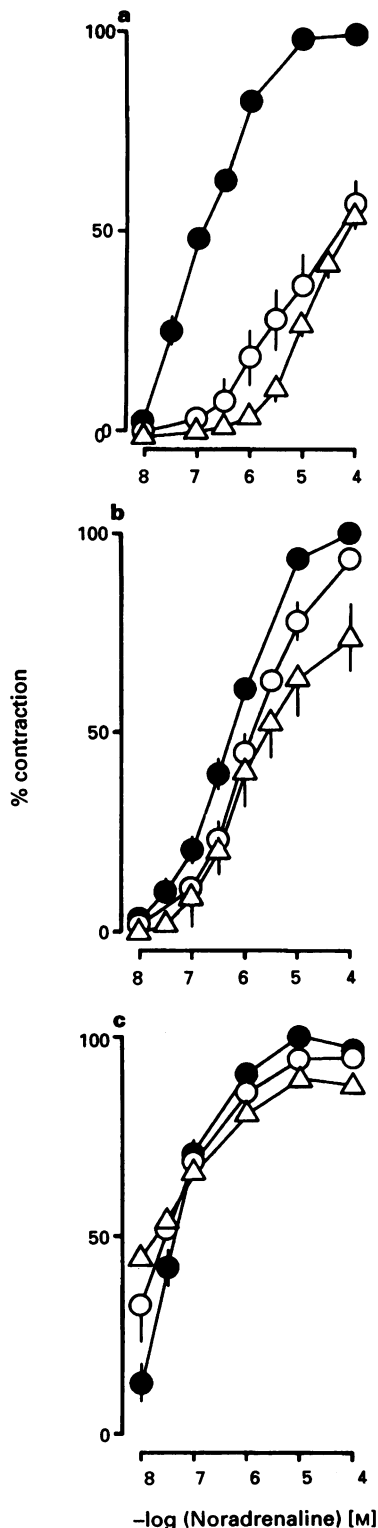

Figure 2 Effect of pretreatment with chloroethylclonidine (CEC) on noradrenaline-induced contractions of the dog vertebral artery (a), femoral artery (b) and portal vein (c). CEC (10 or 50 μM) was added for 20 min, then removed from the bath. The test response was recorded 30 min after washing out the CEC. The maximum contraction induced by noradrenaline before CEC treatment was taken as 100%. (●) Control; (○) 10 μM CEC-pretreated; (△) 50 μM CEC-pretreated. Each value is the mean with the s.e.mean of 6 experiments shown by vertical lines.
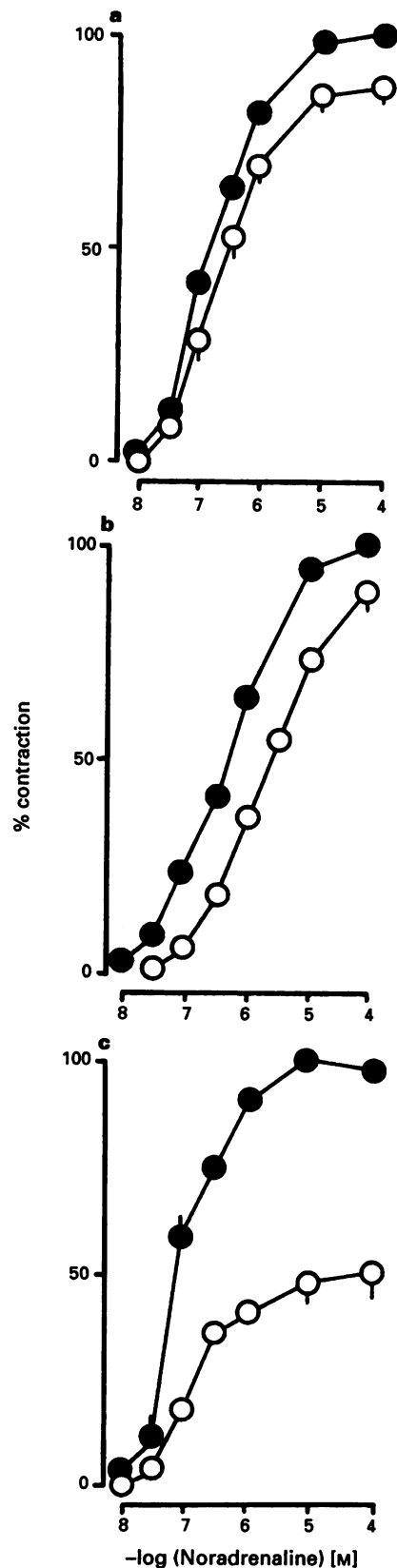

Figure 3 Effects of nifedipine (1 μM) on the concentration-response curves for noradrenaline in the dog vertebral artery (a), femoral artery (b) and portal vein (c). (●) Control; (○) in the presence of nifedipine. Means $\pm$ s.e.mean of 6 experiments.

Table 3 pD$_2$ values and relative contraction to noradrenaline before and after nifedipine-treatment

Blood vessel	Before nifedipine	pD$_2$ Without nifedipine[b]	After nifedipine	Relative contraction[a] Without nifedipine	After nifedipine
Vertebral artery	6.82 ± 0.05	6.75 ± 0.12	6.70 ± 0.09	1.12 ± 0.07	0.87 ± 0.04[c]
Carotid artery	6.95 ± 0.03	6.86 ± 0.04	6.82 ± 0.02	1.12 ± 0.08	0.83 ± 0.06[c]
Portal vein	7.10 ± 0.03	7.03 ± 0.06	6.73 ± 0.05[c]	1.05 ± 0.05	0.53 ± 0.06[c]
Mesenteric artery	6.61 ± 0.06	6.53 ± 0.10	6.06 ± 0.09[c]	0.98 ± 0.03	0.78 ± 0.03[c]
Mesenteric vein	6.33 ± 0.08	6.25 ± 0.10	6.07 ± 0.07	0.98 ± 0.06	0.81 ± 0.02[c]
Renal artery	6.23 ± 0.06	6.22 ± 0.05	5.66 ± 0.06[c]	1.10 ± 0.10	0.70 ± 0.03[c]
Femoral artery	6.33 ± 0.04	6.29 ± 0.07	5.77 ± 0.06[c]	1.07 ± 0.04	0.87 ± 0.04[c]
Femoral vein	6.72 ± 0.08	6.67 ± 0.10	6.51 ± 0.08	1.03 ± 0.04	0.90 ± 0.04[c]

[a]Relative value of maximal contraction against the maximal contraction induced by 100 μM noradrenaline before nifedipine (1 μM) or vehicle.
[b]The vessels were treated with vehicle instead of nifedipine.
[c]Significantly different from the value without nifedipine ($P < 0.05$).
Means ± s.e. of 4-12 experiments.

Table 4 EC$_{50}$ values for prazosin and HV723 in inhibiting the sympathetic adrenergic contraction induced by transmural electrical stimulation

Blood vessel	EC$_{50}$ (nM) Prazosin	HV723
Vertebral artery	1.31 ± .015	5.25 ± 0.56[a]
Carotid artery	2.14 ± 0.47	10.4 ± 3.88[a]
Portal vein	50.0 ± 8.29	8.09 ± 2.67[a]
Mesenteric artery	9.81 ± 4.38	0.97 ± 0.33[a]
Mesenteric vein	6.24 ± 1.28	1.15 ± 0.33[a]
Renal artery	26.1 ± 18.0	1.82 ± 0.23[a]
Femoral artery	1.06 ± 0.79	2.09 ± 0.15

[a]Significantly different from the value for prazosin ($P < 0.05$).
Means ± s.e. of 4-6 experiments.

Discussion

Noradrenaline produced concentration-dependent contractions in 8 different blood vessels isolated from the dog, and the responses were inhibited by prazosin, WB4101, HV723 and 5-methylurapidil. The slopes of Schild plots were close to unity, indicating that the contractile response to noradrenaline in each blood vessel is mediated through a single population of α$_1$-adrenoceptors. However, the pK_B values for the antagonists (especially prazosin, 5-methylurapidil and HV723) varied among the tissues. This suggests regional heterogeneity of the α$_1$-adrenoceptors involved in noradrenaline-induced contractions.

Recently, α$_1$-adrenoceptors were found not to be homogeneous. Binding and molecular biological studies have demonstrated that the α$_1$-adrenoceptors having high affinity for prazosin (p$K_B > 9$) are subdivided into at least 4 subtypes (α$_{1A}$, α$_{1B}$, α$_{1C}$ and α$_{1D}$), where the α$_{1A}$, α$_{1C}$ and α$_{1D}$ subtypes are more sensitive to WB4101 (p$K_B > 9$) as compared with the α$_{1B}$ subtype. 5-Methylurapidil shows higher affinity toward the α$_{1A}$ (p$K_B > 9$) and α$_{1C}$ (pK_B close to 8.5) subtypes than α$_{1B}$ and α$_{1D}$ subtypes (p$K_D < 8.0$) (Morrow & Creese, 1986; Han et al., 1987; Cotecchia et al., 1988; Voigt et al., 1990; Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1991). On the other hand, another subclassification (α$_{1H}$, α$_{1L}$, α$_{1N}$ subclassification) was proposed from functional and binding studies, where the α$_1$-adrenoceptors were first separated into prazosin-high and low affinity sites (Drew, 1985; Flavahan & Vanhoutte, 1986). The prazosin-high sites (α$_{1H}$) may include the α$_{1A}$, α$_{1B}$, α$_{1C}$ and α$_{1D}$ subtypes because of their high affinity for prazosin, while the low sites were further subdivided into α$_{1L}$ and α$_{1N}$ subtypes by HV723 (α$_{1N}$-high affinity drug) (Muramatsu et al., 1990; Muramatsu,

1992; Ohmura et al., 1992). WB4101 and 5-methylurapidil cannot discriminate between α$_{1L}$ and α$_{1N}$ subtypes. CEC produces a remarkable inactivation of the α$_{1B}$ and α$_{1C}$ subtypes (more than 70% inactivation at 10 μM CEC), but the other subtypes were less sensitive to CEC alkylation (Lomasney et al., 1991; Perez et al., 1991; Ohmura et al., 1992).

The present study shows that the α$_1$-adrenoceptors of dog vertebral and carotid arteries have high affinity for prazosin but low affinity for WB4101, 5-methylurapidil and HV723, and that CEC more potently inhibits the contractile responses to noradrenaline in both the arteries as compared with the other vessels tested. These results are consistent with the characteristics of α$_{1B}$ subtype mentioned above, indicating that the contractile response to noradrenaline is mediated through the α$_{1B}$ subtype in the vertebral and carotid arteries (Muramatsu et al., 1991).

By contrast, the results obtained in the other six blood vessels were complex. Unlike carotid and vertebral arteries, the α$_1$-adrenoceptors of the visceral and femoral vessels were less sensitive to CEC and showed lower affinity for prazosin (pK_B: approximately 8.0). The affinities for WB4101 were almost the same among the vessels tested and were lower than the affinities (pK_B close to 9.5) of α$_{1A}$, α$_{1C}$ and α$_{1D}$ subtypes. 5-Methylurapidil also showed higher affinities in the visceral and femoral vessels than the carotid and vertebral arteries (presumably α$_{1B}$ subtype). These results suggest that the α$_1$-adrenoceptors in the visceral and femoral vessels cannot be fully determined by the criteria presently proposed for α$_{1A}$, α$_{1B}$, α$_{1C}$ and α$_{1D}$ subtypes. Rather, the results seem to fit the α$_{1H}$, α$_{1L}$ and α$_{1N}$ subclassification, suggesting the involvement of α$_{1N}$ subtype in the portal vein, mesenteric artery and vein, and renal artery, and of α$_{1L}$ subtype in the femoral artery and vein.

In the original α$_{1A}$, α$_{1B}$ subclassification, nifedipine was proposed to produce a selective inhibition of α$_{1A}$-mediated responses because the α$_{1A}$ subtype was considered to be coupled to calcium channels (Han et al., 1987; Minneman, 1988). In this study, the α$_{1A}$ subtype with high affinity for prazosin, WB4101 and 5-methylurapidil and insensitive to CEC was not detected. However, nifedipine slightly but significantly inhibited the responses to noradrenaline in all tissues tested. Previously, we suggested that contractile responses mediated through α$_1$-adrenoceptor subtypes cannot be classified strictly by a difference in signal transduction mechanisms (Muramatsu et al., 1991; Ohmura et al., 1992). Therefore, it is more likely that α$_1$-adrenoceptors involved in noradrenaline-induced contraction in the dog blood vessels cannot be distinguished by nifedipine, as suggested in other species (Muramatsu et al., 1990; 1991; Oriowo & Ruffolo, 1992; Oriowo et al., 1992; Oshita et al., 1993; Sayet et al., 1993).

Sympathetic adrenergic contractions induced by trans-

mural electrical stimulation were also inhibited by prazosin and HV723 with variable potency among tissues. The variation in the inhibitory potencies between both the antagonists was similar to that in inhibiting the response to noradrenaline. Thus, it is likely that endogenous noradrenaline released from sympathetic nerve terminals acts upon the same α_1-adrenoceptor subtypes as does exogenous noradrenaline, producing adrenergic neurogenic contractions (Muramatsu, 1991a).

Regional heterogeneity of sympathetic transmission in blood vessels has been demonstrated (Lundberg *et al.*, 1984; Burnstock, 1988; Muramatsu, 1991b). In the dog, sympathetic contractions of the carotid and femoral arteries are adrenergic in nature, while both adrenergic and purinergic components are involved in the sympathetic contractions in the portal vein, mesenteric artery and vein, and saphenous

vein (Muramatsu *et al.*, 1984; 1989; Machaly *et al.*, 1988). This evidence, together with the results of this study, suggests that the regional heterogeneity of sympathetic responses may be caused by differences not only among the transmitters released but also in the α_1-adrenoceptor subtypes distribution.

In conclusion, there is evidence of regional differences in the distribution of α_1-adrenoceptor subtypes, which suggests regionally variable expression of α_1-adrenoceptor subtypes.

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