THE ROLE OF $\alpha_1$- AND $\alpha_2$-ADRENOCEPTORS IN CANINE SYSTEMIC CAPACITANCE VESSELS

—A Study with Measurement of Mean Circulatory Pressure—

ISAO HIROSE, M.D., HIROYASU ITO, M.D., KIJUN NAGATA, M.D.
TERUCHIKA SAHASHI, M.D., HISAYASU WADA, M.D., KUNIYUKI TAKAI, M.D.
AND SENRI HIRAKAWA, M.D.

We investigated the presence of $\alpha$-adrenoceptor subtypes in systemic capacitance vessels by examining the effects of $\alpha_1$- and $\alpha_2$-agonists or antagonists on the mean circulatory pressure (MCP). Dogs were anesthetized with pentobarbital, and after total spinal anesthesia, epinephrine was given intravenously to maintain mean blood pressure at about 80 mmHg.

1. With intravenous injection of phenylephrine ($\alpha_1$-agonist, 10 $\mu$g/kg, n = 7), and of BHT 920 ($\alpha_2$-agonist, 5 $\mu$g/kg, n = 7), MCP increased significantly from 9.8 ± 0.4 (mean ± SE) to 10.9 ± 0.3 mmHg (+11.2%, p < 0.01), and from 9.3 ± 0.4 to 10.3 ± 0.4 mmHg (+10.8%, p < 0.05), respectively.

2. Intravenous injection of prazosin ($\alpha_1$-antagonist, 150 $\mu$g/kg, n = 7) and of yohimbine ($\alpha_2$-antagonist, 30 $\mu$g/kg, n = 7) decreased MCP significantly from 9.9 ± 0.4 to 8.2 ± 0.5 mmHg (-17.2%, p < 0.01), and from 9.8 ± 0.2 to 7.6 ± 0.3 mmHg (-22.4%, p < 0.01), respectively.

3. Intravenous injection of phenylephrine (10 $\mu$g/kg, n = 7) after pretreatment with prazosin (150 $\mu$g/kg) decreased MCP significantly from 9.5 ± 0.3 to 7.8 ± 0.3 mmHg (-17.9%, p < 0.01). MCP decreased significantly from 9.9 ± 0.3 to 8.2 ± 0.3 mmHg (-17.2%, p < 0.01) after intravenous injection of BHT 920 (5 $\mu$g/kg, n = 7) following pretreatment with yohimbine (30 $\mu$g/kg).

These results suggest that the $\alpha_1$- and $\alpha_2$-adrenoceptor subtypes exist in systemic capacitance vessels, and that both play a mediating role in systemic venoconstriction induced by their agonists in areflex dogs.

Since Langer proposed that $\alpha$-adrenoceptors could be classified into two subtypes, $\alpha_1$- and $\alpha_2$-adrenoceptors, many investigators have examined whether these $\alpha$-adrenoceptor subtypes exist in the different tissues of a variety of species and it is now established that these $\alpha$-adrenoceptor subtypes are present in arteries and veins. Stimulation of these receptors in isolated veins and arteries causes their constriction. However, the role of the $\alpha$-receptor subtypes in vivo systemic capacitance vessels has not yet been established. We previously investigated the role of adrenoceptors in the control of vascular tone in capacitance and resistance vessels in anesthetized open-chest dogs by measuring changes in mean circulatory pressure (MCP). This study demonstrated that $\alpha$-receptors responsible for venoconstriction were present in

Key words:
Mean circulatory pressure (MCP)
Capacitance vessel
$\alpha_1$-adrenoceptor
$\alpha_4$-adrenoceptor
Canine

(Received March 4, 1989; accepted August 22, 1989)
The 2nd Department of Internal Medicine, Gifu University School of Medicine, Gifu, Japan
Mailing address: Senri Hirakawa, M.D., The 2nd Department of Internal Medicine, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan

Japanese Circulation Journal Vol. 54, February 1990
canine systemic capacitance vessels. We also found that venodilation, mediated through $\beta$-receptors, was much less prominent than venoconstriction mediated by $\alpha$-receptors$^{13}$.

In the present study, we examined the presence of the $\alpha$-adrenoceptor subtypes in canine systemic capacitance vessels, and their mediator role in whole-body venoconstriction by measuring changes in MCP in response to subtype-specific agonists and antagonists.

**METHODS**

Detailed procedures for measuring MCP were described elsewhere$^{13,14}$.

1) Experimental procedures

Dogs were anesthetized with pentobarbital (25–30 mg/kg iv) and were heparinized (300–400 U/kg iv). Total spinal anesthesia (TSA) was performed by injecting bupivacaine (7.5 mg/kg) into the subarachnoid space through a tube advanced from the occipital region. Immediately after this procedure, we infused epinephrine (E) using a microinfusion pump (STS-502, TERUMO Co., Ltd.) to maintain mean blood pressure at approximately 80 mmHg. The main reason for the necessity of the TSA was to eliminate the baroreceptor reflex induced by the drugs, and to eliminate modulation (facilitation or inhibition) of norepinephrine release from sympathetic nerve endings, induced by drugs acting on the presynaptic $\alpha_2$-adrenoceptor.

As shown in Fig. 1, left thoracotomy was performed and artificial respiration was provided by a respirator pump (ACOMA Co., Ltd.). A Courand catheter was advanced through the left carotid artery to the aorta to measure mean blood pressure, and another Courand catheter was advanced through the right jugular vein to the right atrium to measure right atrial pressure. An electromagnetic flow probe (MF-27, NIHON KODEN KOGYO Co., Ltd.) was placed around the root of the aorta to measure cardiac output. The bilateral femoral arteries were connected to the contralateral femoral veins with two vinyl tubes to make arterio-venous shunts (A-V shunts), and a constant flow pump (C-16, TOKYO RIKAKIKAI Co., Ltd.) was set midway in these circuits so that the arterial blood could be translocated into the veins, when necessary. A fibrillator was used to induce ventricular fibrillation.

2) Experimental procedures for measuring MCP

The MCP was measured as follows:

Ventricular fibrillation was induced with a fibrillator. At the same instant, A-V shunts were opened and a constant flow pump was activated. This procedure caused a rapid fall in the arterial pressure and a rapid rise in right atrial pressure, and these two pressures reached an equilibrium pressure equal to the MCP. After measuring the MCP, the constant flow pump was stopped and the A-V shunts were closed, and the heart was

*Japanese Circulation Journal Vol. 54, February 1990*
Fig. 2. Effect of phenylephrine (PHE, 10 μg/kg) on MCP and other hemodynamic parameters (n = 7, mean ± SE).
C = control, * = 0.01 < p < 0.05, ** = p < 0.01.
For abbreviations, see the glossary of terms.

returned to its beating condition with a defibrillator.

The interval from induction of ventricular fibrillation to measurement of MCP was within 5 seconds.

All data, including ECG, were recorded simultaneously on a multipurpose polygraph (PC-10A TOSHIBA Co., Ltd.) using a recorder (Mingograph 800), and heart rate was calculated from the ECG. In addition, circulatory parameters, other than MCP, were measured just before inducing ventricular fibrillation.

3) Drugs

α₁-agonist: phenylephrine (10 μg/kg)
α₂-agonist: prazosin (150 μg/kg)
α₂-agonist: BHT 920 (5 μg/kg)
α₂-agonist: yohimbine (30 μg/kg)

The drugs were diluted to the required doses in 5 ml of saline solution, and were injected into the canine forelimb vein over one minute.

In our preliminary study, performed under TSA and continuous intravenous infusion of E, we first determined the doses of the α₁- and α₂-agonists required to elevate the mean blood pressure by about 30 mmHg (phenylephrine; 10 μg/kg, BHT 920; 5 μg/kg). Next, we determined the doses of the antagonists (prazosin; 150 μg/kg, yohimbine; 30 μg/kg) necessary to inhibit the elevation of mean blood pressure induced by the agonists.

4) Glossary of terms

CO (ml/min/kg) = cardiac output (per kg body weight)
E = epinephrine
HR (beats/min) = heart rate
MCP (mmHg) = mean circulatory pressure
%ΔMCP (%) = percentage change in MCP
MBP (mmHg) = mean blood pressure
NE = norepinephrine
RAP (mmHg) = mean right atrial pressure
RVR (dyne·sec·cm⁻²) = resistance to venous return
TPR (dyne·sec·cm⁻²) = total peripheral resistance
%ΔTPR (%) = percentage change in TPR
TSA = total spinal anesthesia
TSA + E = condition of TSA where mean blood pressure was maintained at about 80 mmHg by constant intravenous infusion of E

TPR (dyne·sec·cm⁻²) = [(MBP/(CO × BW)) × 8 × 10⁴
RVR (dyne·sec·cm⁻²) = [(MCP−RAP)/(CO × BW)] × 8 × 10⁴

5) Estimation of dilation in the systemic capacitance and resistance vessels, and reproducibility of the measurements of TPR and MCP
Fig. 3. Effect of prazosin (PRA, 150 μg/kg) on MCP and other hemodynamic parameters (n = 7, mean ± SE).
For abbreviations, see the glossary of terms.

Fig. 4. Changes in MCP and other hemodynamic parameters in response to intravenous phenylephrine (PHE, 10 μg/kg) after pretreatment with prazosin (PRA, 150 μg/kg) (n = 7, mean ± SE).
PRA + PHE = intravenous injection of phenylephrine after pretreatment with prazosin.
For abbreviations, see the glossary of terms.

As reported previously, we estimated constriction or dilation of the systemic capacitance vessels from a rise or fall in the MCP, respectively. We also estimated constriction or dilation of the systemic resistance vessels from a rise or a fall in the TPR, respectively.

We made 2 consecutive measurements of MCP and TPR at 15 min intervals in 6 dogs denervated with TSA. The first and second MCP measurements were 10.0 ± 0.5 mmHg (mean ± SE) and 10.0 ± 0.5 mmHg, respectively, and the first and second TPR measurements were 5969 ± 426
dyne·sec·cm⁻² and 5929 ± 191 dyne·sec·cm⁻².

6) Statistical analysis

All data are expressed as mean ± SE. Statistical analysis was made using Student’s t-test. P values less than 0.05 were considered to be significant.

RESULTS

1) Effect of phenylephrine (α₁-agonist) on MCP and TPR

Figure 2 shows the changes, from the control values [C], in hemodynamic parameters in response to phenylephrine [PHE]. Parameters were measured following phenylephrine [PHE] administration when the MBP reached a plateau after the end of the drug’s intravenous injection.

Phenylephrine was injected into 7 dogs (11.0 ± 0.8 kg). Both MCP and TPR increased significantly from control levels of 9.8 ± 0.4 mmHg and 6119 ± 242 dyne·sec·cm⁻² to 10.9 ± 0.3 mmHg and 8223 ± 304 dyne·sec·cm⁻², respectively. %ΔMCP increased by 11.2% and %ΔTPR increased by 36.0%.

2) Effect of prazosin (α₁-antagonist) on MCP and TPR

Figure 3 shows the changes in the hemodynamic parameters induced by intravenous injection of prazosin [PRA] in another group of 7 dogs (12.1 ± 0.7 kg). MCP and TPR decreased significantly from 9.9 ± 0.4 to 8.2 ± 0.5 mmHg and from 5608 ± 288 to 4154 ± 396 dyne·sec·cm⁻², respectively. %ΔMCP decreased by 17.2% and %ΔTPR decreased by 17.0%.

3) Effect of phenylephrine (α₁-agonist) on MCP and TPR after treatment with prazosin (α₁-antagonist)

Figure 4 shows the changes in the hemodynamic parameters induced by intravenous injection of phenylephrine after treatment with prazosin in another 7 dogs (11.9 ± 1.0 kg).

The parameters were first measured during the control period [C]. Fifteen minutes later, prazosin was injected intravenously and after the MBP had fallen and reached a plateau, which was usually about 1 min after completion of the prazosin injection, the parameters (PRA) were again measured. MBP decreased from its control value of 83 ± 1 to 51 ± 1 mmHg and TPR decreased significantly from its control level of 5346 ± 382 to 3241 ± 233 dyne·sec·cm⁻² (%ΔTPR = -39.4%) following prazosin injection. Phenylephrine injected after prazosin had hardly any effect on MBP and TPR (TPR changed from 3241 ± 233 to 3453 ± 302 dyne·sec·cm⁻²; %ΔTPR = +6.5%), meaning that these values remained depressed. MCP measured at this time [PRA + PHE] was significantly lower than the control value (9.5 ± 0.3 mmHg vs 7.8 ± 0.3 mmHg; %ΔMCP = -17.9%).

*Japanese Circulation Journal* Vol. 54, February 1990
Fig. 6. Effect of yohimbine (YOH, 30 μg/kg) on MCP and other hemodynamic parameters (n = 7, mean ± SE).
For abbreviations, see the glossary of terms.

Fig. 7. Changes in MCP and other hemodynamic parameters in response to intravenous BHT 920 (BHT, 5 μg/kg) after pretreatment with yohimbine (YOH, 30 μg/kg) (n = 7, mean ± SE).
YOH + BHT = intravenous injection of yohimbine after pretreatment with BHT 920.
For abbreviations, see the glossary of terms.

4) Effect of BHT 920 (α₂-agonist) on MCP and TPR
Figure 5 shows the changes in the hemodynamic parameters caused by intravenous injection of BHT 920 in another 7 dogs (12.3 ± 0.4 kg).
MCP increased significantly from 9.3 ± 0.4 to 10.3 ± 0.4 mmHg, and TPR increased signifi-
Fig. 8. Percentage change in MCP (%ΔMCP) in response to intravenous prazosin (PRA) and to intravenous phenylephrine (PHE) after pretreatment with prazosin (left), percentage change in MCP (%ΔMCP) in response to intravenous yohimbine (YOH) and to intravenous BHT 920 (BHT) after pretreatment with yohimbine (right). For abbreviations, see the glossary of terms.

significantly from 5780 ± 306 to 7146 ± 600 dyne-sec-cm⁻² following intravenous injection of BHT 920. %ΔMCP was +10.8% and %ΔTPR was +23.6%.

5) Effect of yohimbine (α₂-antagonist) on MCP and TPR

Figure 6 shows the changes in the hemodynamic parameters due to intravenous injection of yohimbine in another 7 dogs (11.9 ± 1.1 kg).

MCP decreased significantly from 9.8 ± 0.2 to 7.6 ± 0.3 mmHg, and TPR decreased significantly from 6028 ± 557 to 3559 ± 276 dyne-sec-cm⁻² following intravenous injection of yohimbine. %ΔMCP was −22.4% and %ΔTPR was −41.0%.

6) Effect of BHT 920 (α₂-agonist) on MCP and TPR after treatment with yohimbine (α₂-antagonist)

Figure 7 shows the changes in the hemodynamic parameters caused by intravenous injection of BHT 920 after pretreatment with yohimbine in another 7 dogs (10.6 ± 0.4 kg). After MBP had reached a plateau, about 1 min following the end of intravenous injection of yohimbine, BHT 920 was injected. Yohimbine significantly decreased MBP from 84 ± 1 to 56 ± 1 mmHg, and significantly decreased TPR from 6560 ± 237 to 3866 ± 256 dyne-sec-cm⁻², with %ΔTPR being −41.1%. After intravenous injection of BHT 920, both MBP and TPR that had been lowered previously by yohimbine were elevated slightly. TPR increasing from 3866 ± 256 to 4374 ± 325 dyne-sec-cm⁻². %ΔTPR was +13.1%. These changes were not significant. However, when comparisons were made with the control values [C], the post-BHT 920 treatment TPR value was significantly lower (6560 ± 237 vs. 4374 ± 325 dyne-sec-cm⁻²), %ΔTPR was −33.6%. The MCP obtained following yohimbine and BHT 920 treatments was significantly lower than the control value [C] (9.9 ± 0.3 vs. 8.2 ± 0.3 mmHg), with %ΔMCP being −17.2%.

7) Comparison of %ΔMCP values in dogs injected with prazosin or prazosin plus phenylephrine, and in dogs injected with yohimbine or yohimbine plus BHT 920

Figure 8 shows comparison of %ΔMCP values obtained following injection of prazosin or prazosin + phenylephrine, and following injection of yohimbine or yohimbine + BHT 920. %ΔMCP decreased by 17.2% with prazosin, by 17.9% with prazosin + phenylephrine, by 22.4% with yohimbine and by 17.2% with yohimbine + BHT 920.

There were no significant differences in %ΔMCP between prazosin [PRA] and prazosin + phenylephrine [PRA + PHE] or between yohimbine [YOH] and yohimbine + BHT 920 [YOH + BHT].

DISCUSSION

According to a study on the selectivity of various drugs for α-adrenoceptor subtypes \(^{18}\) phenylephrine, BHT 920 and prazosin are the most selective α₁-agonist, α₂-agonist, α₄-antagonist, respectively. Yohimbine, and α₂-antagonist, is less selective than RX 781094 and rauwolscine. However, the selectivity of these drugs is now known to disappear at higher doses \(^{19}\). This fact raises the possibility that the changes in TPR and MCP seen in our study would not always reflect the responses to the drugs of the α-adrenoceptor subtypes in systemic resistance and capacitance vessels, if the drug doses were inappropriate.

Species differences in distribution of the α-adrenoceptor subtypes have been demonstrated in isolated arteries \(^{20}\). In one study carried out on dogs, the following two findings were obtained: \(^{11}\) 1) the vasoconstrictor response (TPR) to phenylephrine (0.5 µg/kg/min, 5 min) was almost eliminated after a high dose of prazosin.
(1 mg/kg), and was reduced by about 30% by pre-treatment with a high dose of rauwolscine (100 μg/kg), a diastereoisomer of yohimbine, with α₂-antagonism more potent than that of yohimbine; the vasconstrictor response to BHT 920 (1.0 μg/kg/min, 5 min) was almost eliminated after a high dose of rauwolscine, and was reduced by about 35% after pre-treatment with a high dose of prazosin. From the above data, one may speculate as follows: 1) the selectivity of prazosin for α₁-adrenoceptors is high, and 2) the selectivity of yohimbine for α₂-adrenoceptors is also high. In our study, the doses of prazosin (150 μg/kg) and yohimbine (30 μg/kg) were less than those used in the aforementioned study. We can speculate that the selectivity of each drug would be greater with the doses used in the present study. Our study shows that prazosin (150 μg/kg) and yohimbine (30 μg/kg) inhibited almost completely the increases in TPR caused by phenylephrine (10 μg/kg) and BHT 920 (5 μg/kg), respectively (Figs. 4 and 7).

Our results suggest that each of the drugs at the doses used was highly selective for the α₁- or α₂-adrenoceptors in canine systemic resistance vessels.

Epinephrine is a balanced α₁- and α₂-adrenoceptor agonist. The drop in TPR and MCP seen after a single injection of prazosin (Fig. 3) or a single injection of yohimbine (Fig. 6) is thought to result primarily from their blocking action on the stimulatory effects of epinephrine on the α₁- and α₂-receptors. The fact that the %ΔMCP values after prazosin + phenylephrine or yohimbine + BHT 920 treatments were almost identical to the %ΔMCP values obtained after injections of prazosin or yohimbine alone, indicates that stimulation of the α₁- or α₂-adrenoceptors with phenylephrine or BHT 920 had been effectively blocked (Fig. 8). It would have been more satisfactory than in the present study, were it possible to administer prazosin (and observe a decrease in MCP) and then phenylephrine (and observe the lack of venopressor effect of phenylephrine), but this was not available. The same speculation would hold good for the interplay of yohimbine and BHT 920.

In isolated canine portal veins, α₁-adrenoceptors predominate over α₂-adrenoceptors while in the external jugular vein, femoral vein and saphenous vein, α₂-adrenoceptors predominate. Stimulation of both α₁- and α₂-subtypes constricts the veins. These findings suggest that both α₁- and α₂-adrenoceptor subtypes exist in systemic capacitance vessels, and that stimulation of either α₁- or α₂-subtype receptors will constrict these vessels. In fact, according to studies in which systemic vascular capacity was examined in dogs using the total cardiopulmonary bypass method, both α₂-agonists (UK 14304 and BHT 920) and an α₁-agonist (methoxamine) decreased vascular capacity, suggesting that constriction of systemic capacitance vessels had occurred. We found that the results obtained in our study (Figs. 2 and 5) were in good agreement with the findings reported in the aforementioned studies.

Our results suggest that there are α₁- and α₂-adrenoceptors in the systemic capacitance vessels, and that activation of both with agonists induces their constriction in areflex dogs.

REFERENCES

1. DUBOCOVICH ML, LANGER SZ: Negative feedback regulation of noradrenaline release by nerve stimulation in the perfused cat’s spleen: differences in potency of phenoxycetamine in blocking the pre- and post-synaptic adrenergic receptors. J Physiol 237: 505, 1974


7. SCHÜMANN HJ, LUES I: Postjunctival α-adrenoceptors in the isolated saphenous vein of the rabbit: Characterization and influence of angiotensin. Naunyn-Schmiedeberg’s Arch Pharmacol 323: 328, 1983


10. MEY JD, VANHOUTTE PM: Uneven distribution of postjunctional α₁-adrenoceptors in canine arterial and venous smooth


25. SUPPLE EW, GRAHAM RM, POWELL WJJ: Direct effects of α1-adrenergic receptor stimulation on intravascular systemic capacity in the dog. Hypertension 11: 352, 1988


Japanese Circulation Journal Vol. 54, February 1990