SUMMARY  The involvement of postsynaptic $\alpha_2$-adrenergic receptors in the adrenergic constriction of the capacitance vessels was studied in anesthetized, spontaneously breathing dogs under ganglionic blockade (hexamethonium, 10 mg/kg + 10 mg/kg/hr; methylatropine, 0.5 mg/kg). Effective vascular compliance was measured as an indicator of venous tone (blood volume was varied by ± 4 ml/kg in an 11-minute cycle of infusion, withdrawal, withdrawal, and reinfusion) and was calculated from the correlation between the observed changes in central venous pressure and the changes in blood volume. Sympathetic activity and central venous pressure were lower and effective vascular compliance was higher than values in untreated conscious dogs. The $\alpha_2$-agonist UK 14,304 (5-bromo-6-[[imidazolin-2-ylamino]-quinoxaline; 0.04 and 0.12 $\mu$g/kg/min; $n = 6$) dose-dependently lowered compliance and increased central venous pressure to levels found in conscious dogs, as did the $\alpha_1$-agonist methoxamine (10 and 30 $\mu$g/kg; $n = 6$). Rauwolscine ($\alpha_2$-antagonist), 0.3 mg/kg, significantly attenuated the effects of UK 14,304, but not those of methoxamine, while prazosin ($\alpha_1$-antagonist), 0.12 mg/kg, attenuated the effects of methoxamine, but not those of UK 14,304 ($n = 6$ each). Under $\beta$-blockade (nadolol, 2 mg/kg; $n = 12$) venous tone was increased to about physiological levels by norepinephrine, 0.15 $\mu$g/kg/min i.v., or by neuronal norepinephrine release induced by tyramine, 10 $\mu$g/kg/min i.v. These increases were significantly attenuated by prazosin as well as by rauwolscine and were abolished by a combination of both. These results indicate that postsynaptic $\alpha_2$-adrenergic receptors (in addition to $\alpha_1$-adrenergic receptors) are functional in the venous system in vivo and contribute substantially to adrenergic sympathetic and humoral regulation of venous tone. (Hypertension 8: 1003-1014, 1986)

KEY WORDS  •  total effective vascular compliance  •  venous tone  •  vascular $\alpha_2$-adrenergic receptors  •  capacitance vessel constriction  •  rauwolscine  •  prazosin
it has been hypothesized that postsynaptic α₂-adrenergic receptors in the capacitance vessels are not functional in vivo. Therefore, we studied in vivo the contribution of postsynaptic α₂-adrenergic receptors to capacitance vessel constriction induced by selective α-agonists as well as by exogenously applied and endogenously released norepinephrine. Total effective vascular compliance, as modified from Gauer and co-workers, was measured as an indicator of venous tone in the intact dog with circulatory reflexes inhibited by ganglionic blockade.

Materials and Methods

Animals

Twelve mongrel dogs of either sex, weighing 19 to 47 kg (mean, 29 ± 6 kg), were used repeatedly (4–5 times) in different experimental protocols without repeating the same protocol in any dog. For each protocol, the dogs were anesthetized and acutely catheterized percutaneously, the catheters being removed at the end of the protocol. Intervals of at least 2 weeks were allowed between two consecutive experiments in each dog. The animals were fed a standard diet containing Na⁺, 2 to 4 mEq/kg/day, with free access to tap water. During the experimental period the dogs maintained their body weight and apparently remained in vigorous condition. In an additional two dogs, an inflatable occlusion cuff was implanted around the ascending aorta through a thoracotomy under pentobarbital anesthesia and sterile conditions. The dogs were allowed to recover for 2 weeks from the operation and then were used in similar protocols while under anesthesia (see Experimental Protocols). Seven anesthetized and acutely catheterized dogs underwent a laparotomy for removal of the spleen (3 dogs) or for application of occlusive snares around the arteries of the splanchnic bed (4 dogs). Following closure of the abdomen, these anesthetized dogs were used in protocols as will be described and were killed by an overdose of pentobarbital at the end of the protocol. All studies were performed in accordance with the guiding principles in the care and use of animals, as approved by the council of the American Physiological Society.

Experimental Preparation

For each of the experimental protocols, the dogs were anesthetized with pentobarbital, 27 ± 1 mg/kg plus 2.5 ± 0.5 mg/kg/hr i.v., and breathed spontaneously through an endotracheal tube. Arterial blood gases and pH were measured repeatedly throughout the experiment and kept within the physiological range (carbon dioxide tension, 35–41 mm Hg; oxygen tension, 78–90 mm Hg; pH, 7.36–7.44) by adjusting the infusion rate of anesthesia or by an i.v. infusion of 8.4% sodium bicarbonate, or both, if necessary. Body temperature was kept at 37.0 to 37.5°C by means of a heating pad.

Two peripheral venous lines were inserted for separate injection sites for drugs or i.v. infusions. The femoral artery was catheterized percutaneously for recording of arterial blood pressure and heart rate, for changing blood volume, and for arterial blood sampling. A catheter was placed into the right atrium under fluoroscopic control through the external jugular vein for recording of central venous pressure.

Ganglionic blockade (hexamethonium, 10 mg/kg + 10 mg/kg/hr, methylatropine, 0.5 mg/kg) and β-blockade (nadolol, 2 mg/kg) in Protocols 8 to 12 were administered, and the dogs received heparin, 500 U/kg initially and 250 U/kg/hr maintenance. Dextran (Macrodex®), 4 ml/kg, was exchanged for an equal volume of blood, which was stored in a waterbath at 37.0°C. Following an equilibration period of 45 minutes, the experimental protocols (see Table 1) were started. Saline and dextran were infused continuously during the experiments (2.5 and 1.5 ml/kg/hr in Protocols 1–6, respectively; 4 and 2.5 ml/kg/hr in Protocols 8–12, respectively, after an initial infusion of saline, 10 ml/kg, and dextran, 7 ml/kg).

Measurements

Arterial and venous pressures were measured with Statham P23 pressure transducers (Oxnard, CA, USA) and recorded continuously on a Watanabe Linear-corder (Herrsching, West Germany). Mean central venous pressure was recorded at a sensitivity of 0.17 mm Hg/mm (see Figure 1). Effective vascular compliance was measured as modified by Gauer and colleagues. The stored blood (4 ml/kg) was injected into the abdominal aorta through the femoral artery catheter at a rate of 2 ml/kg/min. After 1 minute the same volume of blood was withdrawn at the same rate, and after a further interval of 1 minute, another 4 ml/kg blood was withdrawn and, 1 minute thereafter, was reinfused in an identical fashion. Thus, the total cycle time was 11 minutes. From the central venous pressure tracings recorded during one such cycle of volume changes, 12 readings at 1-minute intervals (integrating central venous pressure over several respiratory cycles) were obtained according to Figure 1. For each cycle of volume changes a linear regression was calculated, relating the observed central venous pressure values to the induced changes in blood volume. The effective compliance of the total vascular bed was calculated as the inverse of the slope of this regression line (expressed in ml·mm Hg⁻¹·kg⁻¹). In experimental Protocols 1 and 2, cardiac output was measured 10 minutes before every compliance measurement using the dye dilution technique (indocyanine green).

For estimation of norepinephrine release rate, a tracer amount of [³H]norepinephrine was infused intravenously at a rate of 0.5 μCi/min and arterial blood samples were taken at 10-minute intervals. Plasma norepinephrine concentration was quantified by a radioenzymatic technique. [³H]Norepinephrine was separated from its metabolites by column chromatography, and specific activity was estimated by liquid scintillation spectrometry. The release rate of endogenous norepinephrine into the plasma is calculated as ([³H]norepinephrine infusion rate X steady state plasma norepinephrine)/steady state plasma [³H]norepinephrine.
Experimental Protocols

The 12 dogs without the aortic cuff were assigned to 10 experimental protocols as follows (Table 1). Each dog was used once in control experiments with selective α-agonists (Protocols 1 or 2), once in experiments under α₂-blockade by prazosin (Protocols 3 or 4), and once in experiments under α₂-blockade by rauwolscine (Protocols 5 or 6). Seven of these dogs were used in experiments analyzing the effects of changes in intravascular volume (Protocol 7), and each dog was used once in experiments under β-blockade (Protocols 8–10). During each of the experimental conditions listed in Table 1, a cycle of volume changes for analyses of compliance was performed. Additionally, in Protocols 1 and 2, cardiac output was measured before starting the cycle.

The α-adrenergic blockers (Protocols 3–6, 9, 10, and 12) were applied 15 minutes before the compliance measurements were made. The cycle was started 5 minutes after the application of α-agonists. In pilot experiments, rather constant venous and arterial pressure levels were observed following bolus injections of methoxamine for at least 20 minutes, while continuous infusions of UK 14,304, norepinephrine, and tyramine had to be applied for steady state conditions over such a period of time.

After control measurements in Protocol 7, mean central venous pressure was elevated by an infusion of dextran (approximately 50 ml/kg) above those pressure values observed with the highest doses of α-agonists in protocols 1 through 6, and compliance was measured in this state. Then, blood was withdrawn in at least four steps to lower central venous pressure again, and compliance measurements were repeated at each step (i.e., at 4 different central venous pressure values with the last one being lower than the lowest pressure value observed in Protocols 1–6).

The dosages of norepinephrine and tyramine (Protocols 8–10, 12) were adjusted according to pilot experiments to yield a decline in effective vascular compliance at least comparable to that obtained with the higher dosages of the selective α-agonists in Protocols 1 through 6. When the measurements during norepinephrine or tyramine infusions were finished, the infusions were discontinued and intervals of 15 to 20 minutes were necessary for central venous pressure and arterial pressure to return to baseline values. The protocols then were continued.

Protocols 1 through 6 lasted 90 minutes, Protocol 7 lasted 2 hours, and Protocols 8 to 12 lasted 3 hours. After the end of the experimental protocols, all drug infusions were stopped, protamine was given, the catheters were removed, and the animals were allowed to recover (except following Protocol 12; see below).

In three of the 12 dogs two further protocols were studied. With the dogs under pentobarbital anesthesia and ganglionic blockade as already described, plasma norepinephrine concentration and norepinephrine release rate were measured under control conditions for 60 minutes and for a further 60 minutes after the administration of prazosin, 0.12 mg/kg, or rauwolscine, 0.3 mg/kg, respectively.

In the two dogs with chronically implanted pneumatic cuffs around the ascending aorta, experiments (Protocol 11) were performed with the dog under pentobarbital anesthesia, ganglionic blockade, and β-blockade as already described. A second arterial catheter introduced through the femoral artery was placed proximal to the cuff for pressure measurements. After control measurements of compliance, proximal systolic pressure was elevated by inflating the occlusion cuff in a stepwise fashion with increasing volumes, and we repeated the measurements at each obtained level of proximal systolic pressure. The maximal pressure value obtained in this manner was higher than the systolic pressure reached with the highest doses of agonists in the other protocols. Following deflation of the cuff, norepinephrine (0.15 μg/kg/min) was infused and compliance was measured again.

In Protocol 12, the dogs had occlusive snares around the truncus celiacus and the superior and inferior mesenteric arteries. Following compliance measurement under ganglionic and β-blockades, the splanchic arteries were occluded and compliance was measured 3 minutes later. After 15 minutes of splanchic occlusion, the snares were released and the splanchic bed was reperfused; 20 minutes later, basal compliance was measured again. Thereafter, the protocol was executed as listed in Table 1, and arterial blood samples were withdrawn for plasma catecholamine measurement at each step of the protocol. In the three splenectomized dogs, the effects of UK 14,304 (0.12 μg/kg/min), methoxamine (30 μg/kg), and norepinephrine (0.15 μg/kg/min) in the presence of nadolol (2 mg/kg) were studied; thereafter, these dogs were killed by an overdose of pentobarbital.

Calculations and Drugs Used

Mean values ± SEM are presented. For comparison between treatment and control groups (Protocols 1–6) we used Student's t test for unpaired data; for comparison between treatment and control data in Protocols 8 to 10 and 12 we applied Student's t test for paired data with Bonferroni's correction according to the number of comparisons.

The following drugs and solutions were used (dosages refer to the bases of the salts): pentobarbital sodium (Ceva, Bad Segeberg, West Germany), hexamethonium bromide (Sigma, München, West Germany), methylatropine bromide (E. Merck AG, Darmstadt, West Germany), nadolol (Von Heyden, Regensburg, West Germany), heparin sodium (Hoffmann-La Roche, Grenzach-Wyhlen, West Germany), prazosin HCl (Pfizer, Karlsruhe, West Germany), rauwolscine HCl (Roth, Karlsruhe, West Germany), methoxamine HCl (Wellcome, London, England), UK 14,304 (Pfizer, Sandwich, England), indocyanine green (Paeisel, Frankfurt, West Germany), l-norepinephrine HCl (Hoechst, Frankfurt, West Germany), tyramin chloride (E. Merck AG, Darmstadt), protamine HCl (Hoffmann-La Roche, Grenzach-Wyhlen), 8.4% sodium bicarbonate (Delta-Pharma, Pfullinglen, West Germany), dextran 60 (Macrodex; Schiwa, Glandorf, West Germany).
### Table 1. Experimental Protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Methoxamine, 10 μg/kg</th>
<th>Methoxamine, 30 μg/kg</th>
<th>Methoxamine, 100 μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=6)</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>α₁-blockade: prazosin, 0.12 mg/kg</td>
<td>UK 14,304, 0.04 μg/kg/min</td>
<td>Methoxamine, 10 μg/kg, 0.12 μg/kg/min</td>
</tr>
<tr>
<td>3 (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>α₂-blockade: rauwolscine, 0.3 mg/kg</td>
<td>UK 14,304, 0.04 μg/kg/min</td>
<td>Methoxamine, 10 μg/kg, 0.12 μg/kg/min</td>
</tr>
<tr>
<td>5 (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>α₂-blockade: rauwolscine, 0.3 mg/kg</td>
<td>UK 14,304, 0.04 μg/kg/min</td>
<td>Methoxamine, 10 μg/kg, 0.12 μg/kg/min</td>
</tr>
</tbody>
</table>

### Results

**Selective α-Agonists and Effective Vascular Compliance (Protocols 1–6)**

Typical tracings of phasic and mean central venous pressure during cycles of volume changes are depicted in Figure 1. Under control conditions (ganglionic blockage), a slope of 0.22 mm Hg·kg·ml⁻¹ was obtained, yielding an effective vascular compliance (i.e., the inverse of the slope) of 4.55 ml·mm Hg⁻¹·kg⁻¹. During UK 14,304 (0.12 μg/kg/min) administration, central venous pressure was elevated, the amplitude of the changes in central venous pressure during the cycle of volume changes was larger, and consequently, the slope was augmented to 0.47 mm Hg·kg·ml⁻¹, yielding an effective vascular compliance of 2.13 ml·mm Hg⁻¹·kg⁻¹.

The basal hemodynamic parameters in Protocols 1 to 6 before the application of α-agonists are summarized in Table 2. Under ganglionic blockade, effective vascular compliance was higher and central venous pressure was lower than those in untreated conscious dogs. Basal data during prazosin (Protocols 3 and 4) were not different from those in control Protocols 1 and 2. With rauwolscine, a higher mean arterial (p<0.05) and slightly higher central venous pressure

![Figure 1](https://hyper.ahajournals.org/)

**Figure 1.** Effects of volume alterations (∆V) on central venous pressure (CVP) under control conditions and during an infusion of UK 14,304 (0.12 μg/kg/min) in a dog used in Protocol 2 (after starting the infusion of UK 14,304, pressure zero was shifted downward to center the tracing). Arrows indicate sequence of values obtained during the cycle of volume changes. Highly significant correlations between central venous pressure values and volume variations were obtained (r = 0.95 and r = 0.99, respectively).
**Table 1. (continued)**

<table>
<thead>
<tr>
<th></th>
<th>7 (n = 7)</th>
<th>8 (n = 4)</th>
<th>9 (n = 4)</th>
<th>10 (n = 4)</th>
<th>11 (n = 4)</th>
<th>12 (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>β-blockade: nadolol, 2 mg/kg</td>
<td>β-blockade: nadolol, 2 mg/kg</td>
<td>β-blockade: nadolol, 2 mg/kg</td>
<td>β-blockade: nadolol, 2 mg/kg</td>
<td>β-blockade: nadolol, 2 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Stepwise variation of intravascular volume</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Steepwise variation of aortic obstruction</td>
<td>Stepwise variation of arterial occlusion and reperfusion</td>
<td></td>
</tr>
<tr>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline, 10 ml</td>
<td>Prazosin, 0.12 mg/kg</td>
<td>Rauwolscine, 0.3 mg/kg</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Norepinephrine, 0.015 μg/kg/min</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Norepinephrine, 0.15 μg/kg/min</td>
<td>Prazosin, 0.12 mg/kg, + rauwolscine, 0.3 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td>Tyramine, 10 μg/kg/min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Baseline Hemodynamic Data in Protocols 1 Through 6**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ganglionic blockade (1 and 2)</th>
<th>Ganglionic blockade (3 and 4)</th>
<th>Prazosin + Rauwolscine (5 and 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>96 ± 5</td>
<td>96 ± 4</td>
<td>107 ± 5*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>116 ± 5</td>
<td>114 ± 6</td>
<td>116 ± 6</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>-0.3 ± 0.2</td>
<td>-0.2 ± 0.2</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>EVC (ml/mm Hg⁻¹·kg⁻¹)</td>
<td>5.0 ± 0.5</td>
<td>5.3 ± 0.5</td>
<td>4.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; EVC = effective vascular compliance.

* p < 0.05, compared with control values.

(not significant) were observed, while effective vascular compliance tended to be lower (not significant) than that in the control Protocols 1 and 2.

In experiments without α-blockers (Protocols 1 and 2), the α₁-selective agonist methoxamine dose-dependently augmented mean arterial pressure and total peripheral resistance without affecting heart rate, and only a slight, but not significant, decrease in cardiac output was observed at the highest agonist dose (Protocol 1; Table 3). Similar changes were observed with the α₂-selective agonist UK 14,304 (Protocol 2; see Table 3).

The effects of the two selective α-agonists on the capacitance vessels are summarized in Figures 2 and 3. To clarify the demonstration of constrictive agonist effects, data of the slope of the pressure-volume relationship (i.e., the effective vascular stiffness) are presented. The α₁-agonist methoxamine (10 and 30 μg/kg) dose-dependently reduced effective vascular compliance, thus increasing effective vascular stiffness by 0.06 ± 0.03 and 0.24 ± 0.03 mm Hg·kg·ml⁻¹, and increased central venous pressure by 0.53 ± 0.10 and 1.74 ± 0.25 mm Hg. Rauwolscine, 0.3 mg/kg, did not significantly modify these effects, although basal values of central venous pressure and effective vascular stiffness during rauwolscine were slightly higher. Prazosin, 0.12 mg/kg, significantly attenuated the methoxamine-induced increases in effective vascular stiffness and central venous pressure (see Figure 2).

The α₂-agonist UK 14,304 (0.04 and 0.12 μg/kg/}

**Table 3. Hemodynamic Effects of Selective α-Agonists**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Methoxamine, 10 μg/kg</th>
<th>Methoxamine, 30 μg/kg</th>
<th>Control</th>
<th>0.04 μg/kg/min</th>
<th>0.12 μg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>95 ± 6</td>
<td>104 ± 10*</td>
<td>125 ± 12†</td>
<td>98 ± 8</td>
<td>113 ± 7†</td>
<td>134 ± 7‡</td>
</tr>
<tr>
<td>CO (ml·min⁻¹·kg⁻¹)</td>
<td>168 ± 19</td>
<td>168 ± 14</td>
<td>155 ± 11</td>
<td>180 ± 26</td>
<td>181 ± 25</td>
<td>168 ± 18</td>
</tr>
<tr>
<td>TPR (mm Hg·min⁻¹·kg⁻¹)</td>
<td>0.6 ± 0.04</td>
<td>0.63 ± 0.08</td>
<td>0.81 ± 0.14*</td>
<td>0.60 ± 0.05</td>
<td>0.65 ± 0.06*</td>
<td>0.83 ± 0.06†</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>117 ± 6</td>
<td>114 ± 7</td>
<td>115 ± 7</td>
<td>114 ± 7</td>
<td>111 ± 9</td>
<td>110 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; CO = cardiac output; TPR = total peripheral resistance; HR = heart rate.

* p < 0.05, † p < 0.01, ‡ p < 0.001, compared with control values.
min) reduced effective vascular compliance in a dose-dependent manner, thereby increasing effective vascular stiffness by 0.10 ± 0.02 and 0.25 ± 0.02 mm Hg·kg·ml⁻¹, and increased central venous pressure by 0.72 ± 0.16 and 1.31 ± 0.20 mm Hg. These effects were not modified by prazosin but were significantly attenuated by rauwolscine (see Figure 3). Mean arterial pressure was increased by 9 ± 2 and 30 ± 6 mm Hg by the two dosages of methoxamine. This increase was not modified by rauwolscine but was significantly attenuated by prazosin (increase of only 2 ± 3 and 5 ± 5 mm Hg). The two doses of UK 14,304 increased mean arterial pressure by 15 ± 4 and 36 ± 7 mm Hg. Rauwolscine significantly attenuated this effect (increase of only 2 ± 1 and 8 ± 2 mm Hg), but prazosin did not.

Changes in Volume and Afterload

In seven dogs under ganglionic blockade central venous pressure was elevated by an infusion of dextran, 50 ml/kg, to a level of 1.8 ± 0.2 mm Hg, yielding an effective vascular stiffness of 0.29 ± 0.02 mm Hg·kg·ml⁻¹. The effects of changes in central venous pressure induced by variations in intravascular volume on effective vascular stiffness are depicted in Figure 4. Effective vascular stiffness was independent of central venous pressure in the range below 0 mm Hg (0.16 ± 0.03 mm Hg·kg·ml⁻¹). It was augmented significantly by increasing central venous pressure to 0.26 ± 0.05 mm Hg (0.26 ± 0.02 mm Hg·kg·ml⁻¹), and was not modified further by higher values of central venous pressure. Contrasting this relation between central venous pressure and effective vascular stiffness to that obtained with agonist infusion (see Figure 4) indicates that, at least with the higher dose of agonists, the observed augmentation in effective vascular stiffness cannot be secondary to increases in blood volume alone (potentially resulting from agonist-induced splenic contraction or changes in capillary filtration/reabsorption ratio). It should be noted that the increase in effective vascular stiffness induced by the higher concentration of agonists was used for pharmacological characterizations (see Figures 2 and 3).

In four experiments in two dogs with chronically
Table 4. Effective Vascular Stiffness and Increases in Afterload (Protocol 11, n = 4)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Cuff</th>
<th>Cuff</th>
<th>Cuff</th>
<th>Cuff</th>
<th>Norepinephrine, 0.15 µg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal systolic pressure (mm Hg)</td>
<td>120 ± 4</td>
<td>140 ± 2*</td>
<td>154 ± 2*</td>
<td>171 ± 1*</td>
<td>184 ± 2*</td>
<td>180 ± 1*</td>
</tr>
<tr>
<td>EVS (mm Hg·kg·ml⁻¹)</td>
<td>0.47 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.48 ± 0.08</td>
<td>0.61 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. EVS = effective vascular stiffness. *p < 0.01, compared with control values.

Effect of Norepinephrine and Tyramine on Effective Vascular Stiffness

The effects of norepinephrine and tyramine on effective vascular stiffness under β-blockade were studied in 12 dogs in Protocols 8 to 10 and in the four dogs used in Protocol 12, after volume infusion of dextran, 10 ml/kg, and saline, 10 ml/kg, to obtain a basal central venous pressure above 0.3 mm Hg (i.e., in a range where effective vascular stiffness is independent of increasing central venous pressure; see Figure 4). The effects of norepinephrine and tyramine on hemodynamics and on capacitance vessels in Protocols 8 through 10 are summarized in Table 5. The doses chosen augmented effective vascular stiffness and central venous pressure to levels found in untreated conscious dogs. The arterial plasma levels of norepinephrine measured during infusion of tyramine (10 µg/kg/min) and norepinephrine (0.015 and 0.15 µg/kg/min) in dogs under ganglionic and β-blockade (Protocol 12) are depicted in Figure 5 together with the hemodynamic data of this protocol. The tyramine-induced elevation of circulating norepinephrine cannot have contributed to the effects of tyramine on venous tone, as is shown by a comparison with the effects of norepinephrine, 0.015 µg/kg/min, in this protocol. Arterial plasma epinephrine concentration under ganglionic and β-blockade was 22 ± 7 pg/ml and did not change throughout this protocol.

The effects on capacitance vessels induced by norepinephrine (0.15 µg/kg/min) were attenuated significantly by both rauwolscine (0.3 mg/kg) and prazosin (0.12 mg/kg), but not by sham treatment (10 ml saline; Figure 6). Similarly, the effects of tyramine (10 µg/kg/min) on capacitance vessels were significantly attenuated by rauwolscine as well as by prazosin, but not by sham treatment (Figure 7). Following combined application of rauwolscine and prazosin (Protocol 12), the effects of tyramine (10 µg/kg/min) and of norepinephrine (0.15 µg/kg/min) on effective vascular stiffness and on venous pressure were abolished.

The elevation of mean arterial pressure induced by norepinephrine (0.15 µg/kg/min) was attenuated by prazosin to 52 ± 3% of the control response (Protocol 9) and by rauwolscine to 56 ± 6% (Protocol 10). It was abolished by the combination of prazosin and rauwolscine (Protocol 12) but was not significantly attenuated by sham treatment (Protocol 8). Similarly, the tyra-

Table 5. Cardiovascular Effects of Norepinephrine and Tyramine (Protocols 8-10; n = 12)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Tyramine, 0.15 µg/kg/min</th>
<th>Norepinephrine, 0.15 µg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>98 ± 2</td>
<td>157 ± 6*</td>
<td>151 ± 5*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>116 ± 8</td>
<td>117 ± 8</td>
<td>117 ± 8</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>0.6 ± 0.1</td>
<td>3.4 ± 0.4*</td>
<td>3.3 ± 0.4*</td>
</tr>
<tr>
<td>EVC (ml·mmHg⁻¹·kg⁻¹)</td>
<td>4.9 ± 0.3</td>
<td>1.9 ± 0.1*</td>
<td>2.0 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; EVC = effective vascular compliance. *p < 0.001, compared with control values.

Figure 5. Effects of i.v. tyramine and norepinephrine infusions on effective vascular stiffness (EVS), central venous pressure (CVP), arterial plasma norepinephrine (NE), and mean arterial pressure (MAP) in four dogs under ganglionic and β-blockade (Protocol 12). Columns are shown in the sequence in which the measurements were performed; open columns indicate basal conditions. The tyramine-induced augmentation of circulating norepinephrine cannot account for the concomitant increase in venous tone.
Figure 6. Increases in effective vascular stiffness (ΔEVS) and central venous pressure (ΔCVP) induced by norepinephrine (0.15 μg/kg/min) in experimental Protocols 8 to 10. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference between increase in control period and after infusion of rauwolscine, 0.3 mg/kg, or prazosin, 0.12 mg/kg (paired data).

Figure 7. Increases in effective vascular stiffness (ΔEVS) and central venous pressure (ΔCVP) induced by tyramine (10 μg/kg/min) in experimental Protocols 8 to 10. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference between increase in control period and after infusion of rauwolscine, 0.3 mg/kg, or prazosin, 0.12 mg/kg (paired data).

Norepinephrine Release Rate

In six experiments in three dogs under ganglionic blockade, plasma norepinephrine concentration was 75 ± 27 pg/ml and norepinephrine release rate amounted to 2.8 ± 0.2 ng/kg/min. This level is approximately 18% of the release rate measured in conscious dogs at rest (O. Sommer, unpublished observations). Prazosin (0.12 mg/kg) did not significantly modify plasma norepinephrine concentration (from 96 ± 56 to 104 ± 31 pg/ml) or norepinephrine release rate (from 3.2 ± 0.4 to 3.5 ± 0.7 ng/kg/min; n = 3). After application of α2-blockade by rauwolscline, plasma norepinephrine concentration initially increased from 53 ± 2 to 113 ± 45 pg/ml and norepinephrine release rate increased from 2.5 ± 0.2 to 5.9 ± 1.8 ng/kg/min (data obtained 20 minutes after injection of antagonist), but both parameters returned to baseline values within 30 minutes (52 ± 19 pg/ml and 2.3 ± 0.8 ng/kg/min, respectively).

Effect of Temporary Splanchnic Arterial Occlusion on Effective Vascular Stiffness

In the dogs under ganglionic and β-blockade (Protocol 12), occlusion of the truncus celiacus and the superior and inferior mesenteric arteries immediately augmented mean arterial pressure (from 101 ± 7 to 133 ± 7 mm Hg; p < 0.001) and central venous pressure (from 1.7 ± 0.5 to 3.5 ± 0.9 mm Hg; p < 0.05), while effective vascular stiffness was only slightly modified (from 0.27 ± 0.02 to 0.31 ± 0.03 mm Hg·kg·ml⁻¹; not significant). This occlusion-induced increase in effective vascular stiffness amounted to 15 ± 7% of the increase induced by norepinephrine in these dogs later in the protocol and to 15 ± 9% of the increase induced by tyramine. Following 15 minutes of splanchnic arterial occlusion, a transient hypotension was observed with the onset of splanchnic reperfusion, and preclosure hemodynamic values again were reached 4 to 12 minutes thereafter. It should be noted that later during Protocol 12, constrictive responses of the venous and arterial systems to norepinephrine and tyramine were observed similar to those observed in Protocols 8 to 10 in dogs that were not subjected to temporary occlusion.

Effective Vascular Stiffness in Splenectomized Dogs

In three splenectomized dogs under ganglionic blockade, the pressor effects of the agonists on the venous and the arterial systems were comparable in magnitude to the effects observed in normal dogs under ganglionic blockade. Administration of UK 14,304 (0.12 μg/kg/min) augmented effective vascular stiffness (from 0.28 ± 0.04 to 0.50 ± 0.01 mm Hg·kg·ml⁻¹; p < 0.05), central venous pressure (from 1.9 ± 0.2 to 3.5 ± 0.2 mm Hg; p < 0.001), and mean arterial pressure (from 101 ± 7 to 145 ± 11 mm Hg; p < 0.01). The respective augmentations induced by methoxamine (30 μg/kg) were from 0.30 ± 0.04 to 0.49 ± 0.01 mm Hg·kg·ml⁻¹ (p < 0.05), from 1.8 ± 0.1 to 2.7 ± 0.1 mm Hg (p < 0.01), and from 111 ± 9 to 156 ± 6 mm Hg (p < 0.05). Those induced
by norepinephrine (0.15 μg/kg/min) in the presence of β-blockade (nadolol, 2 mg/kg) were from 0.27 ± 0.02 to 0.47 ± 0.01 mm Hg·kg·ml⁻¹ (p<0.001), from 1.7 ± 0.3 to 3.4 ± 0.3 mm Hg (0.1 > p > 0.05), and from 111 ± 9 to 149 ± 9 mm Hg (p<0.01).

Discussion

Critique of the Model

To assess venous tone in vivo, we measured the effective compliance of the total vascular bed by varying the blood volume and relating the induced changes in central venous pressure to the volume changes. The term effective vascular compliance was used by Gauer and co-workers to describe, in the intact circulation, the elastic properties of the entire vascular system (as compared with the “true” vascular compliance in the arrested or experimentally altered circulation). They demonstrated that values of effective vascular compliance are well in agreement with the values of vascular compliance measured with other techniques. Total vascular compliance reflects mainly the compliance of the venous system, as pulmonary compliance is only one sixth to one tenth and compliance of the arterial vascular tree is only 1/30 of the total systemic compliance. Active constriction of the capacitance vessels results in a decrease in vascular compliance (i.e., an increase in vascular stiffness — the inverse of compliance) or in a reduction in unstressed vascular volume, or in both.

We chose to study effective vascular compliance (or stiffness) as a reflection of venous tone in the intact animal because it allows repeated experiments in the same animal and does not require extremely unphysiological conditions (i.e., extensive surgical manipulation, bypasses, reservoirs, and deep anesthesia), as are necessary for the experimental analyses of “true” venous compliance and unstressed vascular volume. On the other hand, the measurement of effective vascular compliance provides, in the intact animal, more direct information on capacitance vessel tone than do measurements of indirect parameters such as central venous pressure and cardiac output. Furthermore, this approach has been applied in conscious animals and in humans, thus, the data from our model can be compared directly with values obtained under strictly physiological conditions.

Nevertheless, several possible limitations of this model must be discussed. Alterations in the filtration-reabsorption equilibrium in the capillaries may occur during the cycle of volume infusion and withdrawal: a tendency toward increased outward filtration (when the volume is expanded) and toward decreased filtration (when the volume is lowered) may slightly modify the actual changes in intravascular volume. To minimize this possible effect, we used the dog’s own blood for infusion (after having exchanged dextran for blood and allowing enough time for mixing) and the volume was altered within only a small range (±4 ml/kg). Thus, the slope of the pressure-volume relation should not be affected substantially by filtration, although a slight hysteresis (see Figure 1) might be due in part to such effects.

Changes in cardiac or vascular function induced by reflexes should not have influenced the data in this study, as the animals were ganglionically blocked. Thus, sympathetic activity was severely attenuated, as reflected by a markedly lowered norepinephrine release rate (less than 18% as compared with normal, conscious dogs). Neither during a cycle of volume variations nor during α-agonist administration (see Table 3) was a change in heart rate observed.

As α-agonists might indirectly increase intravascular volume by causing splenic contraction or by decreasing the capillary filtration/reabsorption ratio (as a result of precapillary constriction), we tested whether such secondary volume effects could account for the observed increase in effective vascular stiffness during administration of α-agonists. Intravascular volume (and thereby central venous pressure) was increased in a stepwise fashion (see Figure 4). We found a low value of effective vascular stiffness independent of central venous pressure at pressure values below 0 mm Hg and a higher value independent of central venous pressure at pressures above 0 mm Hg. This stepwise increase in effective vascular stiffness may have been due to expansion of partly collapsed large veins at this higher filling state (it should be noted that, because of negative intrathoracic pressures, a central venous pressure of 0 mm Hg reflects a positive transmural venous pressure). As the control values for central venous pressure in the experiments with selective α-agonists were slightly below 0 mm Hg, we cannot exclude a secondary volume effect accounting in part for the observed increase in stiffness induced by the low doses of α-agonists. However, such an effect can be excluded for the high doses of α-agonists on which pharmacological characterizations are based. These doses were applied when central venous pressure values were well above 0 mm Hg (i.e., in a range where changes in intravascular volume and central venous pressure, respectively, do not significantly influence effective vascular stiffness; see Figure 4). In the experiments in which norepinephrine and tyramine were used, volume was infused to obtain central venous pressure values well above 0 mm Hg before starting the protocol, thus minimizing any potential secondary volume effects on measured vascular stiffness. Furthermore, the similarity of the responses between splenectomized and normal dogs indicates that volume changes caused by splenic contraction do not substantially modify the measured effective vascular stiffness.

All agonists substantially increased mean arterial pressure (see Tables 3 and 5) and thereby cardiac afterload. The α₁-agonist clonidine is reported to depress ventricular function at high load. It could be argued that this effect would induce some degree of functional cardiac failure, increasing central venous pressure. Thus, as a result of marked afterload dependency, cardiac output would vary inversely with the cycle of volume alterations, thereby amplifying the actual changes in central venous pressure. However, there are strong arguments against this hypothesis. First, the
α-agonists did not significantly decrease cardiac output (see Table 3). Second, the increased central venous pressure values during administration of α-agonists were always well within the normal range found in conscious dogs and were not in a range consistent with cardiac failure. Third, in experiments similar to the ones presented, which were performed in dogs equipped with chronic aortic flowmeters, cardiac output never changed inversely with the cycle of volume changes, neither under control conditions nor during an infusion of norepinephrine, 0.15 μg/kg/min. Fourth, in the dogs with chronically implanted aortic occlusion cuffs, effective vascular stiffness was not affected by increases in proximal systolic pressure of a greater magnitude than those caused by the α-agonists (see Table 4). Therefore, significant changes in effective vascular stiffness caused by agonist-induced increases in afterload (i.e., independent of venoconstrictive vascular stiffness caused by agonist-induced occlusion cuffs, effective vascular stiffness was not affected by increases in volume pressure in a dose-dependent manner to a central venous pressure to about physiological levels.

The tendency of a rauwolscine-induced arterial and venous vasodilation (though not significant in all protocols) might have resulted from a central sympathoexcitation that is not completely suppressed by the ganglionic blockade. This interpretation is supported by the lack of such a pressor effect of rauwolscine in anesthetized dogs with spinal transection and by the transient augmentation of the norepinephrine release rate observed in the present study. However, this pressor effect was too small relative to the effects induced by the agonists to confound the antagonism at the vascular level.

We conclude that functional postsynaptic α2-adrenergic receptors, in addition to postsynaptic α1-adrenergic receptors, exist in the venous system of the intact dog and mediate constriction of the capacitance vessels induced by selective α-agonists. This conclusion is in agreement with findings in the pithed rat in vivo and with a number of in vitro studies on veins. In contrast to our results, however, it was postulated that the "postsynaptic α2-adrenoceptors were not functional in the capacitance vessels of the dog." These authors drew their conclusion from a lack of effect of the α2-agonist BHT-933 (azepexole) on cardiac output in spinalized dogs under ganglionic blockade. Although this approach might yield only indirect and vague information on venous tone, it is unclear why they did not observe an increase in central venous pressure during BHT-933 infusion as we did during UK 14,304 infusion. The breed of dogs might explain some of the differences, as only beagles were used in their study. The number of postsynaptic α2-adrenergic receptors and/or the contractile responsiveness to their activation seem to vary with age, temperature, endothelial influences, and levels of blood pressure (and perhaps also with race) to a greater extent than that of α2-adrenergic receptors.

Adrenergic Control of Capacitance Vessels

The finding of functional postsynaptic α2-adrenergic receptors in the venous system of our model does not yet allow any conclusion on the relative number of either α1-adrenergic receptor subtype in the venous system nor does it answer the question of whether there is a preferentially innervated, intrasynaptic α1-adrenergic receptor and a preferentially humoral, extrasynaptic α2-adrenergic receptor, as has been proposed, but not confirmed, for the arterial system.

In our model under β-blockade, i.v. infusion of norepinephrine increased effective vascular stiffness and central venous pressure to about physiological lev-
els, as did tyramine. These increases were significantly attenuated by prazosin and by rauwolscine, but not by sham treatment (see Figures 6 and 7). The combination of both antagonists abolished the increases induced by tyramine or by norepinephrine. These results demonstrate that both circulating and neurally released norepinephrine constrict capacitance vessels in vivo by summing \( \alpha_1 \)-adrenergic and \( \alpha_2 \)-adrenergic receptor-mediated components of contractile activation.\(^{14} \) Although the mechanisms by which tyramine and sympathetic stimulation induce neuronal release of norepinephrine are not identical, this is not likely to make a difference regarding the access to and activation of postsynaptic \( \alpha_2 \)-adrenergic receptors. We conclude that postsynaptic \( \alpha_2 \)-adrenergic receptors are intrasynaptically located and therefore innervated in the venous system of the dog. Thus, the hypothesis of preferentially innervated vascular \( \alpha_2 \)-adrenergic, and preferentially humoral \( \alpha_1 \)-adrenergic receptors is again not confirmed.

Based on in vitro studies, a predominance of postsynaptic \( \alpha_2 \)-adrenergic receptors in human veins has been postulated.\(^{18, 43} \) These findings, together with the results from our study, indicate that also in humans postsynaptic \( \alpha_2 \)-adrenergic (in addition to \( \alpha_1 \)-adrenergic) receptors contribute substantially to the adrenergic sympathetic and humoral regulation of capacitance vessel tone. This would complement the role of this receptor type in the control of arteriolar tone in humans\(^{13} \) and extend the evidence that sympathetic and humoral adrenergic activation act on two types of vascular \( \alpha \)-adrenergic receptors, of which one (the vascular \( \alpha_2 \)-adrenergic receptor) seems to be preferentially modulated by various physiological and pathophysiological factors.

Venous tone in hypertensive patients and in models of experimental hypertension is augmented.\(^{1, 4-7} \) This augmentation is considered one of the causal factors for the release of the endogenous inhibitor of Na\(^+\), K\(^-\) ATPase in low renin hypertension.\(^5, 44 \) The enhanced \( \alpha_2 \)-adrenergic receptor responsiveness of arteries in hypertension\(^{10, 46} \) and the involvement of \( \alpha_2 \)-adrenergic receptors in sympathetic venoconstriction (indicated in our model) raise the following questions: Is augmented venous \( \alpha_2 \)-mediated constriction the mechanism of the altered venous tone in hypertension? Is a preferential action of calcium antagonists on such an \( \alpha_2 \)-mediated venoconstriction involved in the prevention or correction of hypertension and in the normalization of associated abnormalities in blood cell sodium transport and in plasma proteins\(^{45, 46} \) by those drugs? The extreme heterogeneity of vascular smooth muscle should prevent conclusions from analogy; however, these questions deserve investigation.

In conclusion, our study demonstrates that in dogs the tone of the entire capacitance vasculature in vivo can be augmented by activation of postsynaptic \( \alpha_2 \)-adrenergic receptors as well as of \( \alpha_1 \)-adrenergic receptors. Furthermore, circulating and neurogenic norepinephrine constrict the capacitance vasculature through activation of both \( \alpha \)-adrenergic receptor subtypes.

This constriction augments right ventricular filling pressure and venous tone within the ranges observed in the healthy, conscious dog at rest. Thus, activation of postsynaptic venous \( \alpha_2 \)-adrenergic receptors might be involved in the physiological regulation of central venous pressure by the sympathetic nervous system.

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References

11. Elsner D, Saeed M, Sommer O, Holtz J, Bassenge E. Sympathetic vasoconstriction sensitive to \( \alpha_2 \)-adrenoceptor blocker: no evidence for preferential innervation of \( \alpha_2 \)-adrenergic receptors in the canine femoral bed. Hypertension 1984;6:915–925


41. Medgett IC, Hicks PE, Langer SZ. Smooth muscle alpha-2 adrenoceptors mediate vasoconstrictor responses to exogenous norepinephrine and to sympathetic stimulation to a greater extent in spontaneously hypertensive than in Wistar Kyoto rat tail arteries. J Pharmacol Exp Ther 1984;231:159-165


44. De Wardener HE, Clarkson EM. Concept of natriuretic hormone. Physiol Rev 1985;65:658-758


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D Elsner, D J Stewart, O Sommer, J Holtz and E Bassenge

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