Uneven Distribution of Postjunctional Alpha$_1$- and Alpha$_2$-like Adrenoceptors in Canine Arterial and Venous Smooth Muscle

JO DE MEY AND PAUL M. VANHOUTTE

SUMMARY We studied isolated canine arteries and veins to compare the pharmacological properties of their postjunctional alpha-adrenoceptors. Rings of femoral and splenic arteries and of femoral and saphenous veins were mounted for isometric tension recording in organ chambers filled with Krebs-Ringer bicarbonate solution. The four blood vessels contracted when exposed to methoxamine, norepinephrine, phenylephrine, and tramazoline; clonidine failed to induce contraction only in the splenic artery. The relative sensitivity to methoxamine was comparable in the arteries and veins, but that for phenylephrine was larger in the former. The veins, but not the arteries, were more sensitive to clonidine and tramazoline than to phenylephrine or methoxamine. Phentolamine was a competitive antagonist against norepinephrine in the arteries and the veins. Prazosin was a competitive antagonist only in the arteries. The competitive antagonistic properties of yohimbine were more pronounced in the veins than in the arteries. Verapamil depressed to the same extent the contractile responses of saphenous veins to clonidine and norepinephrine, but reduced the contractions caused by methoxamine more than those due to norepinephrine. These results indicate the presence of both alpha$_1$- and alpha$_2$-like adrenoceptors on venous smooth muscle cells, whereas arterial smooth muscle cells contain mainly postjunctional alpha$_1$-adrenoceptors.


IN adrenergically innervated blood vessels, endogenously liberated or exogenously added norepinephrine can activate both alpha-adrenergic receptors (alpha-adrenoceptors) on the adrenergic nerve endings (prejunctional alpha-adrenoceptors), and on the vascular smooth muscle cells (postjunctional alpha-adrenoceptors). The prejunctional alpha-adrenoceptors mediate feedback inhibition of transmitter release, whereas the postjunctional alpha-adrenoceptors activate the contractile process of the effector cells [e.g., Somlyo and Somlyo (1970), Stjarne and Grippe (1973), Shepherd and Vanhoutte (1975), Starke et al. (1975), Vanhoutte (1978); Dalemans et al. (1979), and Lorenz et al. (1979)]. It is now well established that the pharmacological properties of pre- and postjunctional adrenoceptors in peripheral tissues are not necessarily identical, and the differences in their affinities for alpha-adrenergic agonists and antagonists have lead to the concept that there are at least two subtypes of alpha-adrenoceptors (alpha$_1$- and alpha$_2$-adrenoceptors). The prejunctional alpha-adrenoceptors have been uniformly classified as alpha$_2$-adrenoceptors and the postjunctional alpha-adrenoceptors can belong to either subtype [e.g., Langer (1974), Starke et al. (1975), Starke and Langer (1979), and Wikberg (1979)]. Recent obser-

vations in the intact dog suggest that the pharmacological characteristics of the postjunctional alpha-adrenoceptors mediating constriction of the resistance vessels are not identical in all vascular beds (Drew and Whiting, 1979). We designed the present experiments because no information seems available on the subtype characteristics of postjunctional alpha-adrenoceptors in isolated blood vessels of the dog. Since major differences exist between responses of arterial and venous smooth muscle to alpha-adrenergic agonists in terms of their dependence on the influx of extracellular Ca$^{2+}$ and of their energy requirements [e.g., Detar and Bohr (1968); Godfraind and Kaba (1972), and Vanhoutte (1976); see Shepherd and Vanhoutte (1975), Bohr and Webb (1978), and Vanhoutte (1978)], isolated arteries were compared with isolated veins. Since the degree of adrenergic innervation can affect the apparent sensitivity of vascular smooth muscle to catecholamines [e.g., Bevan and Su (1973), Bevan (1977), Vanhoutte (1978), and De Mey and Vanhoutte (1980)], experiments were performed on both densely (splenic arteries and saphenous veins) and poorly (femoral arteries and veins) innervated preparations.

To judge from the work by others, the distinction between alpha$_1$- and alpha$_2$-adrenoceptors can be made by comparing the responsiveness of the target cells to appropriate alpha-adrenergic agonists and antagonists (Starke et al., 1975; Starke and Langer, 1979). In the present study the agonists used were clonidine, methoxamine, norepinephrine, phenylephrine, and tramazoline; norepinephrine reputedly...
has a similar potency at alpha<sub>1</sub> and alpha<sub>2</sub> receptor sites, methoxamine and phenylephrine activate preferentially alpha<sub>1</sub>-adrenoceptors, and clonidine and tramazoline activate preferentially alpha<sub>2</sub>-adrenoceptors. As antagonists, we used phentolamine, prazosin, and yohimbine. Phentolamine has a similar affinity for both receptor subtypes. The alpha<sub>1</sub>-adrenoceptor is occupied more readily by yohimbine than by phentolamine and has little, if any, affinity for prazosin (Langer, 1974; Starke et al., 1975; Drew, 1976; Cambridge et al., 1977; Doxey et al., 1977; Starke and Langer, 1979; Wikberg, 1979).

**Methods**

The experiments were performed on isolated femoral arteries, femoral veins, saphenous veins, and splenic arteries taken from dogs (15–30 kg) anesthetized with pentobarbital (30 mg/kg, iv).

**Organ Bath Studies**

Rings of arteries and veins (2–10 mm wide) were mounted individually in organ chambers filled with Krebs-Ringer bicarbonate solution (millimolar composition: NaCl, 118.3; KCl, 4.7; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.5; NaHCO<sub>3</sub>, 25; calcium EDTA, 0.026; glucose, 11.1), gassed with a 95% O<sub>2</sub>-5% CO<sub>2</sub> gas mixture and kept at 37°C. The preparations were connected to a strain gauge (Statham P231) and switched to a stimulator (Grass S44). Before the actual experiments, we placed the preparations at the optimal point of their length-tension relationship (Statham UC3) for isometric tension recording. To stimulate the adrenergic nerve endings in the vessel wall, two platinum electrodes were placed parallel to the rings (Vanhoutte et al., 1967); electric impulses (9V, 2msec) were provided by a direct current supply (Siemens AD 149) triggered by a stimulator (Grass S44). Before the actual experiments, we placed the preparations at the optimal point of their length-tension relationship (Vanhoutte and Leusen, 1969; De Mey and Vanhoutte, 1980) using a standard electrical stimulation (15 Hz, for 10 seconds) for the saphenous veins and the splenic arteries, and a standard concentration of norepinephrine (2 × 10<sup>-7</sup> M) for the femoral arteries and veins (Table 1). After this procedure, the rings were allowed to equilibrate for 45 minutes. The preparations were studied in the presence of the neuronal uptake inhibitor cocaine, the inhibitor of extraneuronal uptake 17-β-estradiol and the beta-adrenoceptor antagonist propranolol. Full dose-response curves to the different agonists were obtained and their ED<sub>50</sub>'s (the concentration causing 50% of the maximal response to the agonist) were determined for the individual preparations. The relative sensitivity for the agonists was calculated in each experiment as the ratio of the ED<sub>50</sub> of norepinephrine to the ED<sub>50</sub> of the agonist tested (Furchgott, 1972; Wikberg, 1979). To assess the potency of alpha-adrenoceptor agonists, dose-response curves to norepinephrine were obtained in the absence and in the presence of increasing concentrations of the agonist tested; to correct for changes in sensitivity with time control dose-response curves for norepinephrine were obtained in parallel on preparations from the same arteries and veins. The ED<sub>50</sub> of norepinephrine was determined for each dose-response curve and the logarithm of the ratio of the ED<sub>50</sub> in presence of the antagonist/ED<sub>50</sub> in absence of the antagonist-1; dose ratio-1), was plotted against the logarithm of the concentration of the antagonist (Arunlakshana and Schild, 1959). The p<sub>A2</sub> value for the antagonists was determined as the negative logarithm of the concentration of the drug for which the ratio between the ED<sub>50</sub> of norepinephrine in the presence of the agonist and in control solution equals 2 (Ariens and Van Rossum, 1957; Arunlakshana and Schild, 1959; Furchgott, 1972; Janssens and Vanhoutte, 1978).

**Drugs**

The following pharmacological agents were used: clonidine hydrochloride (Boehringer); cocaine hydrochloride (Bios Coutelier); 1,3,5-(10-estratrien-3,17-β-diol)(17-β-estradiol) (Sigma), verapamil (Knoll); methoxamine hydrochloride (Burroughs Wellcome); norepinephrine bitartrate (Fluka); phentolamine mesylate (Ciba); phenylephrine hydrochloride (Winthrop); prazosin hydrochloride (Pfizer), propranolol hydrochloride (I.C.I.); tramazoline hydrochloride (Boehringer); yohimbine hydrochloride (Sigma). When the drugs were added to the bath solution, they were contained in 0.1 ml of

<table>
<thead>
<tr>
<th>Vessels at Optimal Length*</th>
<th>n</th>
<th>Size of preparation (mm in length axis)</th>
<th>Basal tension (g)</th>
<th>Maximal response to norepinephrine (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saphenous vein</td>
<td>32</td>
<td>2-3</td>
<td>2.03 ± 0.06</td>
<td>19.6 ± 1.7</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>26</td>
<td>8-10</td>
<td>0.82 ± 0.07</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>32</td>
<td>2-3</td>
<td>12.4 ± 1.1</td>
<td>19.4 ± 2.1</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>26</td>
<td>5-6</td>
<td>18.7 ± 0.9</td>
<td>24.3 ± 1.1</td>
</tr>
</tbody>
</table>

* The ring preparations were stretched to their optimal length using a standard electrical stimulation (15 Hz, 10 sec) (saphenous vein and splenic artery) or a standard concentration (2 × 10<sup>-7</sup> M) of norepinephrine (femoral artery and vein). Data are expressed in absolute values and are shown as means ± SEM.
distilled water. All doses are expressed as molar concentrations.

**Statistical Analysis of Data**

For each group of arteries or veins, the number of preparations is also the number of dogs used. To correct for differences in density of smooth muscle population, all data are expressed as percent of the contractile response to a maximal concentration of norepinephrine which, in all vessels, caused the largest contractile responses (Table 1). The data are shown as mean values ± the standard error of the mean (SEM). For the determination of ED₉₀ values, a log-logit transformation of the dose-response data was used, followed by a standard analysis for linear regression. The latter was used also for the determination of pA₂ values (Aliens and Van Rossum, 1957; Arunlakshana and Schild, 1959). For the analysis of the data, Student's t-test for paired and unpaired observations was used. When P was smaller than 0.05, values were considered to be significantly different. Only statistically significant differences will be described and discussed.

**Results**

**Electrical Stimulation**

Arteries and veins from the same dogs (n = 6) were stimulated electrically at increasing frequencies (0.25–32 Hz). All vessels responded to the stimulation with contraction. When expressed as percent of the maximal contractile response to exogenous norepinephrine, the increases in tension caused by the electrical impulses were the largest in the saphenous vein, followed, in descending order, by the splenic artery, the femoral vein, and the femoral artery. In the four vessels, 3 × 10⁻⁶ M phentolamine abolished the contractile responses to 4 Hz (Table 2).

**Alpha-Adrenergic Agonists**

Three rings were prepared from each of six saphenous veins, and were studied in solution containing cocaine (3 × 10⁻⁵ M), 17-β-estradiol (3 × 10⁻⁶ M) and propranolol (8 × 10⁻⁶ M). At first, the response to increasing concentrations of norepinephrine (10⁻⁸ to 10⁻⁴ M) was recorded in the three rings. After washing out and return to baseline tension, the rings were allowed to equilibrate for 30 minutes. The three rings then were exposed in parallel to increasing concentrations of norepinephrine, phenylephrine, and tramazoline, respectively. After washing out, return to baseline tension, and equilibration (30 minutes), the rings were exposed to increasing concentrations of norepinephrine, methoxamine, and clonidine, respectively. A similar protocol was followed for six splenic arteries, six femoral veins, and six femoral arteries of the same dogs.

Norepinephrine caused dose-dependent contractions in both arteries and both veins (Fig. 1). The

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**Table 2 Responsiveness of Canine Blood Vessels to Electrical Stimulation**

<table>
<thead>
<tr>
<th>Blood vessels (n = 6)</th>
<th>Frequency (Hz)</th>
<th>Phenolamine (3 × 10⁻⁶ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>4.2 ± 0.7</td>
<td>11.7 ± 2.1</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.8 ± 0.1</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>3.9 ± 0.8</td>
<td>9.0 ± 3.0</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>0.9 ± 0.1</td>
<td>3.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Increases in tension induced by electrical stimulation (9 V, 2 msec) with increasing frequency expressed as percent of the maximal response to exogenous norepinephrine (see Table 1) and shown as means ± SEM.
ED₅₀ of the catecholamine differed significantly between vessels; it was the lowest in the femoral artery, followed by the saphenous and femoral vein, and was the largest in the splenic artery (Table 3).

Methoxamine caused dose-dependent increases in tension in the four blood vessels (Fig. 1). The maximal response to the drug was significantly smaller than that obtained with norepinephrine; it was the largest in the saphenous vein, followed by the femoral and splenic arteries, and was the lowest in the femoral artery. The ED₅₀ of methoxamine was the lowest in the femoral artery followed by the saphenous and femoral veins, and was the highest in the splenic artery (Table 3). The ratio between the ED₅₀ of norepinephrine and that of methoxamine was comparable in the four vessels (Table 3).

The four blood vessels contracted in a dose-dependent manner when exposed to increasing concentrations of phenylephrine (Fig. 1). The maximal response to the drug was not significantly different from that obtained with norepinephrine in the saphenous vein, and in the splenic and femoral arteries; it was significantly lower in the femoral vein. The ED₅₀ of phenylephrine was the lowest in the femoral artery, followed by the two veins, and was the highest in the splenic artery (Table 3). The ratio between the ED₅₀ of norepinephrine and that of phenylephrine was significantly larger in the femoral artery and vein than in the two veins; no significant difference was observed between the two arteries, whereas the ratio was significantly larger in the saphenous than in the femoral vein (Table 3).

Clonidine caused dose-dependent contractions of the saphenous vein, the femoral artery, and the femoral vein; in these three vessels the maximal response to the drug was significantly smaller than that obtained with norepinephrine. The saphenous vein responded significantly more to clonidine than the femoral artery and vein. In the splenic artery, clonidine caused no significant changes in tension (Fig. 1). The ED₅₀ of clonidine was significantly smaller in the two veins than in the femoral artery (Table 3). The ratio between the ED₅₀ of norepinephrine and that of clonidine was the largest in the femoral vein, followed by the saphenous vein, and was the lowest in the femoral artery (Table 3).

The four blood vessels responded in a dose-dependent manner to tramazoline; the maximal response to the drug was significantly smaller than that to norepinephrine; it was comparable in the saphenous vein and the femoral artery, but significantly smaller in the femoral vein, and even more so in the splenic artery (Fig. 1). The ED₅₀ of tramazoline was significantly higher in the splenic artery than in the three other blood vessels (Table 3). The ratio between the ED₅₀ of norepinephrine and that for tramazoline was similar in the two veins, and significantly larger than in the arteries; it was significantly larger in the femoral than in the splenic artery.

### Alpha-Adrenergic Antagonists

**Unstimulated Preparations**

From 10⁻⁶ to 3 x 10⁻⁶ m phenotamine, prazosin and yohimbine did not significantly affect the basal tension of the different vessels studied (n = 10).

**Norepinephrine**

The effect of the three alpha-adrenergic antagonists on the dose-response curve to norepinephrine was studied in solution containing cocaine (3 x 10⁻⁵ M), 17-β-estradiol (3 x 10⁻⁵ M), and propranolol (8 x 10⁻⁶ M). Four rings were cut out of each of 10 saphenous veins, 10 splenic arteries, 10 femoral veins, and 10 femoral arteries. In the four rings, a control dose-response curve to norepinephrine was obtained. This was repeated three times, at 30-

### Table 3 Sensitivity and Relative Sensitivity of Canine Blood Vessels for Alpha-Adrenergic Agonists*

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Methoxamine</th>
<th>Phenylephrine</th>
<th>Clonidine</th>
<th>Tramazoline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀ (10⁻⁶ m)</td>
<td>ED₅₀NE/ED₅₀</td>
<td>ED₅₀ (10⁻⁶ m)</td>
<td>ED₅₀NE/ED₅₀</td>
<td>ED₅₀ (10⁻⁶ m)</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>1.1 ±0.2 $</td>
<td>7.7 ±0.4 $</td>
<td>5.4 ±0.3 $</td>
<td>1.9 ±0.1 $</td>
<td>0.6 ±0.02 $</td>
</tr>
<tr>
<td>Vein</td>
<td>1.0 ±0.1 $</td>
<td>0.12</td>
<td>0.14</td>
<td>1.0 ±0.3 $</td>
<td>0.1 ±0.2 $</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>4.5 ±0.8 $</td>
<td>32.9 ±4.9 $</td>
<td>9.1 ±0.9 $</td>
<td>0.6 ±0.1 $</td>
<td>0.02 ±0.03 $</td>
</tr>
<tr>
<td>Fetal artery</td>
<td>0.7 ±0.2 $</td>
<td>5.0 ±0.6 $</td>
<td>1.8 ±0.5 $</td>
<td>0.2 ±0.1 $</td>
<td>0.7 ±0.3 $</td>
</tr>
</tbody>
</table>

* Experiments performed in the presence of cocaine (3 x 10⁻⁵ M), 17-β-estradiol (3 x 10⁻⁵ M), and propranolol (8 x 10⁻⁶ M) on blood vessels from the same dogs (n = 6). The ED₅₀ was determined in the individual preparations. Data shown as means ± SEM.

† The ratio of the ED₅₀ of norepinephrine to the ED₅₀ of a particular agonist is calculated as a measurement of the relative sensitivity for the agonist, which allows the best dissociation between alpha₁ - and alpha₂-adrenoceptors (Wikberg, 1979).

‡ The difference between arteries and veins is significant (P < 0.05).

§ The difference in the experiments is significant (P < 0.05).

|| The difference between the veins is significant (P < 0.05).

† The difference with the splenic artery is significant (P < 0.05).
minute intervals, in the absence (time control ring) or in the presence of increasing concentrations \(10^{-8}, 10^{-7}, 10^{-6} \text{M or } 3 	imes 10^{-8}, 3 	imes 10^{-7}, 3 	imes 10^{-6} \text{M; experimental rings}) of phentolamine, prazosin, and yohimbine, respectively.

Phentolamine caused a parallel shift to the right of the dose-response curve to norepinephrine in the arteries as well as in the veins (Fig. 2). For the two arteries and the two veins, the plot of log (dose ratio-1) vs. the logarithm of the concentration of phentolamine was a straight line with a slope not significantly different from 1 (Fig. 3; Table 4). The \(pA_2\) value for phentolamine was not significantly different among the four blood vessels (Table 4).

Prazosin caused a parallel shift to the right of the dose-response curve to norepinephrine in the arteries, but not in the veins (Fig. 2). In the arteries, but not in the veins, the plot of log (dose ratio-1) vs. the logarithm of the concentration of prazosin revealed a straight line with a slope not significantly different from 1 (Fig. 3; Table 4). The \(pA_2\) value for prazosin was comparable in the femoral and splenic arteries (Table 4); it was significantly higher than those for phentolamine and yohimbine.

Yohimbine caused a parallel shift to the right of the dose-response curve to norepinephrine in the four vessels (Fig. 2). In the splenic artery, the femoral vein, and the femoral artery, the plot of log (dose ratio-1) vs. the logarithm of the concentration of yohimbine was a straight line with a slope not significantly different from 1; in the saphenous vein its slope was significantly lower than 1 (Fig. 3; Table 4). The \(pA_2\) value for yohimbine was similar in the arteries but significantly lower than in the two veins (Table 4). In the arteries, but not in the veins, the \(pA_2\) value for yohimbine was significantly lower than that for phentolamine.

**Verapamil**

Six rings of saphenous veins and of splenic arteries taken from the same five dogs were studied in parallel in control solution. Pairs of rings were exposed, four times, to increasing concentrations (2 \(\times 10^{-8}\) to 6 \(\times 10^{-8}\) M) of clonidine, methoxamine,
and norepinephrine, respectively; one ring of each pair remained in control solution and the other was incubated (20 minutes) in increasing concentrations of verapamil (5 × 10⁻⁷ to 5 × 10⁻⁵ M). Verapamil depressed the responses to norepinephrine and methoxamine significantly more in the artery than in the vein. In both vessels, it reduced the response to methoxamine significantly more than that to norepinephrine. In the saphenous vein, verapamil affected the response to clonidine to the same extent as that to norepinephrine; the splenic artery did not contract when exposed to clonidine (Fig. 4).

**Discussion**

Studies reported so far on isolated arteries from species other than the dog suggest that the post-junctional alpha-adrenoceptors of vascular wall belong to the alpha₁-subtype [e.g., Stjärne and Grippe (1973), Starke et al. (1975), Borowski et al. (1977), and Wikberg (1978, 1979)]. However, recent findings in the intact dog indicate that the alpha-adrenoceptors on the smooth muscle cells of precapillary vessels are not necessarily of the alpha₁ subtype (Drew and Whiting, 1979). The goal of the present

**Table 4  Antagonism by Phentolamine, Prazosin, and Yohimbine of Norepinephrine-Induced Contractions in Canine Arteries and Veins**

<table>
<thead>
<tr>
<th></th>
<th>Phentolamine</th>
<th></th>
<th>Prazosin</th>
<th></th>
<th>Yohimbine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>pₐ₂</td>
<td>r</td>
<td>pₐ₂</td>
<td>r</td>
<td>pₐ₂</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>0.98$</td>
<td>0.91 (1.03; 0.79)</td>
<td>7.3 ± 0.3</td>
<td>0.46</td>
<td>0.50 (1.07; -0.08)</td>
<td>0.95$</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>0.97$</td>
<td>0.87 (1.01; 0.72)</td>
<td>7.5 ± 0.3</td>
<td>0.72</td>
<td>0.89 (1.30; 0.47)</td>
<td>0.94$</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>0.97$</td>
<td>0.91 (1.05; 0.77)</td>
<td>7.4 ± 0.3</td>
<td>0.90$</td>
<td>0.96 (1.17; 0.76)</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>0.99$</td>
<td>0.90 (1.00; 0.81)</td>
<td>7.4 ± 0.2</td>
<td>0.92$</td>
<td>1.02 (1.20; 0.84)</td>
<td>7.9 ± 0.3</td>
</tr>
</tbody>
</table>

* Data derived from the plots between the logarithm of the dose ratio minus one and the logarithm of the concentration of the antagonist (Arunlakshana and Schild 1959), and shown as the correlation coefficients (r), the slopes and the pₐ₂ values obtained (Fig. 3).

† Slopes shown with 95% confidence limits between brackets, pₐ₂ values ± SEM.
‡ The correlation is statistically significant.
§ The slope is significantly different from 1.
¶ The difference from the pₐ₂ values obtained in the arteries is statistically significant (P < 0.05).
¶¶ The difference from the pₐ₂ value for phentolamine in the same blood vessel is statistically significant (P < 0.05).
study was to characterize in vitro the pharmacological properties of postjunctional alpha-adrenoceptors in canine arteries and veins. Therefore we compared, in standard conditions (Furchgott, 1966, 1972; Janssens and Vanhoutte, 1978), their responsiveness to appropriate alpha-adrenergic agonists and antagonists (Langer, 1974; Starke et al., 1975; Starke and Langer, 1979; Wikberg, 1979). The pharmacological analysis of our results is complicated by differences in maximal responses to the different agonists within the same vessel, and by differences in maximal responses to the same agonist between different vessels. The proper analysis of a drug-receptor interaction is based on the assumption that the agonists and antagonists used interact with one type of receptor only (Aliens and Van Rossum, 1957; Arunlakshana and Schild, 1959; Furchgott, 1966; Furchgott and Bursztyn, 1967). Apparently, in the present study, this condition is not met in all vessels, since the results suggest the presence of two subtypes of alpha-adrenoceptors, in particular in venous smooth muscle. For this reason, and since no agonists are available which are specific for the receptor subtypes, the analysis of the contractile responses to clonidine, methoxamine, norepinephrine, phenylephrine, and tramazoline consisted mainly of the comparison of ED50’s and maximal responses. In addition, the potencies of the more-or-less selective alpha1- and alpha2-agonists were compared to that of norepinephrine, using the ratio of the ED50 as the index of relative sensitivity which allows the best dissociation between alpha1- and alpha2-adrenoceptors (Wikberg, 1979). The preliminary conclusions from the studies with alpha-adrenergic agonists were confirmed by the determination of the affinity for alpha-adrenergic antagonists in the different vessels.

In the splenic artery, phenylephrine and methoxamine evoked responses that were similar to, or only slightly less in amplitude than, those obtained with norepinephrine. The ED50 of phenylephrine, and that of methoxamine, was significantly lower than that of tramazoline. The relative sensitivities for phenylephrine and methoxamine were similar to those of the postjunctional alpha-adrenoceptors in vascular smooth muscle of the rabbit pulmonary
artery and of the guinea pig aorta, both of which have been subtyped as alpha_2-adrenoceptors [e.g., Starke et al. (1975), and Wikberg (1978)]. Phentolamine, prazosin, and yohimbine fulfilled the criteria of competitive antagonists. When we compared their pA_2 values, the descending order of potency was: prazosin > phentolamine > yohimbine. The actual pA_2 values are comparable to those reported by other in tissues said to contain mainly alpha_2-adrenoceptors (Starke et al., 1975; Wikberg, 1978). Thus, the postjunctional alpha-adrenoceptors of the splenic artery belong to the alpha_2-subtype.

The small increases in tension observed with tramazoline, as well as the relatively weak inhibitory effect of yohimbine in this artery, could illustrate the alpha_1 component of the action of these two drugs. Similarly, it can be concluded that, in the femoral artery, alpha-adrenergic agonists activate mainly postjunctional alpha_2-adrenoceptors. Since the femoral artery responded more than the splenic vessels to tramazoline and clonidine, the possibility also exists that alpha_2-like postjunctional receptor sites can be activated in femoral arterial smooth muscle, although the studies with the alpha-adrenergic drugs demonstrate that such an alpha_2-like component would contribute little to the contractile response to norepinephrine.

The sensitivity of the femoral and saphenous veins for tramazoline and clonidine was higher than that reported for the postjunctional action of these agonists on the rabbit pulmonary artery and the guinea pig aorta (Starke et al., 1975; Wikberg, 1978), but similar to that reported for prejunctional alpha-adrenergic sites (Starke et al., 1975). In both the saphenous and femoral veins, the relative sensitivity for tramazoline and clonidine was higher than that for phenylephrine; such a difference allows dissociation between alpha_2 and alpha_1 adrenoceptors (Wikberg, 1979). These findings indicate that, in the isolated veins, contractile responses can be elicited by activation of alpha_2-like postjunctional receptors. By contrast, the relative sensitivity of the venous smooth muscle cells to phenylephrine and methoxamine were comparable to those of the splenic artery, and corresponded to the values reported for the postjunctional action of these drugs in rabbit pulmonary artery and the guinea pig aorta (Starke et al., 1975; Wikberg, 1978). These experiments suggest that the contractile process in venous smooth muscle can be triggered by activation of both alpha_1 and alpha_2-like adrenoceptors. In both the saphenous and femoral veins, the antagonism by phentolamine of the response to norepinephrine fulfilled the criteria of competitiveness. The same was true for yohimbine in the femoral but not in the saphenous veins. The pA_2 value of phentolamine, in both veins, was comparable to that obtained in the splenic artery. By contrast, that of yohimbine was significantly larger in the veins than in the arteries. This comparison allows us to suggest that part of the contractile response of the isolated veins to norepinephrine could be due to activation of adrenergic sites with alpha_2-like pharmacological properties. In the veins, unlike in the arteries, prazosin caused a nonparallel depression of the dose-response curve to norepinephrine; a deviation from the criteria for competitive antagonism with prazosin in venous smooth muscle also is suggested by studies performed on isolated rat vessels (Cohen et al., 1979). Such a deviation would be predicted if norepinephrine, acting on both alpha_1- and alpha_2-adrenoceptors, was confronted with a competitive antagonist acting on only one of the involved receptor-subtypes (alpha_2), particularly if the two agonist-receptor interactions have different dose-response characteristics. Such a deviation would not be expected with an antagonist which competes for both receptor sites to the same extent, as appears to be the case with phentolamine. The most likely explanation for the noncompetitive nature of the antagonism of prazosin against contractions of isolated veins of the dog caused by norepinephrine is that, in these blood vessels, the lower concentrations of the catecholamine cause mainly activation of alpha_2-like adrenergic receptors, whereas higher amounts of the adrenergic transmitter also recruit alpha_1-adrenergic sites. Since yohimbine has a greater affinity for alpha_2 than for alpha_1 receptors, this interpretation also explains why the slope of the linear correlation between the logarithm of the concentration of yohimbine vs. the logarithm of the ratio between equieffective concentrations of norepinephrine in presence and absence of that antagonist is smaller than 1 in the saphenous vein.

It could be argued that our results do not necessarily imply that arteries and veins have a heterogeneous population of distinct alpha_1- and alpha_2-like postjunctional adrenergic sites, but that each vessel has a homogeneous population of alpha-adrenoceptors with individual pharmacological properties. The latter possibility is made unlikely by the experiments with verapamil. The drug only moderately depressed the contractions induced by norepinephrine in the saphenous vein, confirming earlier observations (Shepherd and Vanhoutte, 1975; Vanhoutte, 1976), but reduced the responses of the splenic artery significantly more; a similar dissociation has been reported with another Ca^{2+} entry blocker, lidoflazine (Vanhoutte et al., 1980). By contrast, verapamil depresses the contractile responses to methoxamine to the same extent in both types of blood vessels. If the inhibitory effect of verapamil is due to alpha-adrenerolytic properties, these observations would imply that the drug should be regarded as an alpha_2-adrenergic antagonist; this suggestion is supported by binding studies on rat brain homogenates (Fairhurst et al., 1980). In that case, the differential effects of verapamil on
responses in the saphenous vein to norepinephrine and methoxamine would reinforce the interpretation that, in this preparation, norepinephrine activates more than one subtype of alpha-adrenergic receptors. An alternative explanation for the differences in sensitivity between arteries and veins could be that, besides inhibition of Ca$^{2+}$ influx [e.g., Golenhofen and Lammel (1972) and Fleckenstein (1977)], verapamil may affect cellular mobilization of Ca$^{2+}$ differently in both types of smooth muscle (Thoresen and Haeusler, 1979). This interpretation also fails to explain the differences in effectiveness to inhibit responses of the saphenous vein to nor- epinephrine (and clonidine), on the one hand, and methoxamine, on the other hand. Differences in sensitivity to drugs such as verapamil or lidoflazine (Vanhoutte et al., 1980) may reflect differences in the pharmacological properties of the calcium channels of the cell membranes of arterial and venous smooth muscle cells. Again, this interpretation does not provide a satisfactory explanation for the larger depression in the saphenous vein of the responses to methoxamine than of those to clonidine and norepinephrine. A more logical explanation would be that contractions of vascular smooth muscle cells caused by alpha$_1$-adrenergic stimuli depend more on the influx of extracellular Ca$^{2+}$ than alpha$_2$-adrenergic activation, which then must cause a relatively greater mobilization of cellular stores of the activator ion [e.g., Somlyo and Somlyo (1970), Shepherd and Vanhoutte (1975), and Bohr and Webb (1978)]. Independently of the mechanism of action of verapamil, the results obtained with the drug support the interpretation that smooth muscle of canine arteries and veins differ in the pharmacological characteristics of their postjunctional alpha-adrenoceptors.

The presence of mainly alpha$_1$-adrenoceptors in arteries, and of both alpha$_1$- and alpha$_2$-type postjunctional adrenoceptors in veins, illustrates the heterogeneous pharmacological behavior of vascular smooth muscle [e.g., Bohr (1965) and Vanhoutte (1978)]. These differences in the distribution of alpha-adrenergic receptor subtypes are not related to the density of adrenergic innervation, since they were observed independently of the magnitude of the contractile response of the arteries and veins to electrical stimulation. Such stimulation causes contraction of isolated canine blood vessels by releasing endogenous norepinephrine (Vanhoutte et al., 1967, 1979).

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Influence of Secobarbital and α-Chloralose, and of Vagal and Sympathetic Interruption, on Left Ventricle Activation after Acute Coronary Artery Occlusion in the Dog

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SUMMARY The purpose of this study was to examine the effect of secobarbital vs. α-chloralose anesthesia, and of tonic autonomic influence, upon ischemia-induced subepicardial and subendocardial bipolar electrogram changes during acute coronary artery occlusion in the open-chest dog. We found that the degree of bipolar electrogram alterations for similar reductions of regional myocardial blood flow was less in dogs anesthetized with α-chloralose than in those anesthetized with secobarbital. We also noted greater electrogram changes when the sympathetic system was unopposed by the vagus nerves. Finally, this study demonstrates the reproducibility of ischemia-induced changes in bipolar electrograms during serial, short term, acute coronary artery occlusions. Circ Res 48: 884-894, 1981

IT IS well known that the autonomic nervous system may influence the development of cardiac dysrhythmias. Experimental observations in dysrhythmias that occur immediately after coronary occlusion have shown that cholinergic influence could retard (Goldstein et al., 1973; Kent et al., 1973; Corr and Gillis, 1974; Harrison et al., 1974; Myers et al., 1974; Kolman et al., 1975; Brooks et al., 1978) and adrenergic influence exacerbate (Hoffman et al., 1955; Harris, 1966) the development of ventricular dysrhythmias. The electrophysiological basis for these autonomic effects has not been elucidated completely. Two studies have shown no or minimal influence of the autonomic nervous system on conduction delays in the acute (Hope et al., 1974) and the subacute phase (El-Sherif, 1978) of coronary artery occlusion in the dog. During preliminary studies on electrophysiolog-
Uneven distribution of postjunctional alpha 1-and alpha 2-like adrenoceptors in canine arterial and venous smooth muscle.

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