Norepinephrine Uptake, Smooth Muscle Sensitivity, and Metabolizing Enzyme Activity in Rabbit Veins

By John A. Bevan, David W. Hosmer, Bengt Ljung, Barbara L. Pegram, and Che Su

ABSTRACT

Some parameters of the adrenergic neuroeffector mechanism were measured in vitro in the central ear (near the base of the ear), common jugular, pulmonary, brachial, femoral, renal, superficial cervical, cephalic, and small saphenous veins, a branch of the deep circumflex iliac vein, the parietal branch of the internal iliac vein, branches of the anterior mesenteric vein, the inferior vena cava (immediately distal to the left renal vein), and a subcutaneous vein of the back. Although extraneuronal uptake of $^3$H-norepinephrine was the same in all of the veins except the mesenteric, the neuronal uptake of norepinephrine varied widely. A number of veins, including the femoral and superficial cervical veins, showed no neuronal uptake, and the uptake of others, including the cephalic and mesenteric veins, was greater than that measured in any previously studied vascular tissue. The median effective dose for the contractile effect of norepinephrine on the veins was on the same order of magnitude as that for the aorta and the ear artery. Catechol-O-methyl transferase, but not monoamine oxidase, activity appeared to be related to innervation density. It is concluded that veins show a remarkable variation in the dimensions of their adrenergic parameters, particularly those related to innervation density.

KEY WORDS

$^{14}$C-sucrose uptake  monoamine oxidase  catechol-O-methyl transferase

innervation density  ED$_{50}$

The term vascular smooth muscle is used to denote a heterogeneous group of muscle tissue found in a variety of blood vessels. The main focus of interest in the last decade has been arterial tissue. With the exception of the portal and superior mesenteric veins, our knowledge of venous smooth muscle has been obtained predominantly from in vivo experiments.

Our understanding of sympathetic nervous system control of venous smooth muscle is especially limited. Extrapolation from knowledge about adrenergic mechanisms in general and these mechanisms in the arterial system in particular is hazardous, since considerable variation in adrenergic parameters such as innervation density (1, 2), innervation pattern (3, 4), neuromuscular cleft size (5), excitation-contraction coupling mechanisms (6, 7), and enzymatic mechanisms (8-10) exists in the vascular tree. The extent of such variation in the venous system is unknown.

The purpose of the present investigation was to survey the vascular smooth muscle of a number of different veins in the rabbit and to characterize the nature of and the extent of variation in adrenergic mechanisms affecting such muscle.

Methods

Albino rabbits were killed by a blow on the head and exsanguinated, and the veins of interest were removed and placed in Krebs-bicarbonate solution bubbled with 95% O$_2$-5% CO$_2$. Extraneous tissue was removed from the veins by dissection under a Zeiss dissecting microscope. The central ear (near the base of the ear), common jugular, pulmonary, brachial, femoral, renal, superficial cervical, cephalic, and small saphenous veins, a branch of the deep circumflex iliac vein, the parietal branch of the internal iliac vein, branches of the anterior mesenteric vein, the inferior vena cava (immediately distal to the left renal vein), and a subcutaneous vein of the back were studied.

UPTAKE OF 3H-NOREPINEPHRINE AND 14C-SUCROSE

The uptake of tritiated l-norepinephrine ($^3$H-norepinephrine) was measured in a manner similar to that previously described by Nedergaard et al. (11). Longitudinal strips of all veins were equilibrated in pairs
for 30 minutes in Krebs-bicarbonate solution at 37°C. During a subsequent 30-minute incubation period, one vein strip from each pair was exposed to cocaine (10^{-6} M). The vein pairs were then incubated in 10^{-5} M \text{H} - \text{norepinephrine} (3.3 \mu \text{g}) and 2 \times 10^{-6} \text{M} \text{14C-sucrose} (20 \mu \text{g}) for 1 hour in the absence or the presence of the cocaine. The strips were subsequently removed from the bath, rinsed for 1 second in Krebs-bicarbonate solution, blotted, and weighed on a Cahn Electrobalance. Each strip was then digested overnight in Soluene 100 (Packard Instrument Co.). Standard liquid scintillation procedures were used to determine the quantity of each isotope present in the strips.

The uptake of \text{H} - \text{norepinephrine} and \text{H} - \text{C-sucrose} was expressed as milliliters of bath fluid cleared per gram of tissue. It was assumed that the uptake of \text{C-sucrose} represented the size of the extracellular space and that \text{H} - \text{norepinephrine} was evenly distributed within this space after 1 hour. The uptake of \text{H} - \text{norepinephrine} in the presence of cocaine—an agent which blocks neuronal uptake—minus the uptake of \text{C-sucrose} was taken as the extraneuronal uptake of the transmitter. The cocaine-sensitive uptake of \text{H} - \text{norepinephrine}—the total uptake minus the extraneuronal and extracellular uptakes—was taken as the neuronal uptake of the transmitter.

**CONTRACTILE RESPONSE TO EXOGENOUS \text{H} - \text{NOREPINEPHRINE}**

Ring preparations of all veins were used to measure the contractile response to exogenous \text{H} - \text{norepinephrine}. Two stainless steel rods were passed through the vessel lumen and supported as described previously (12). Statham G10b (± 0.15 oz) strain gauges and Sargent model SRG recorders were used to monitor contractile responses. Resting tensions of 250 mg or 500 mg were applied to the veins, depending on their dimensions. After the contractile response to 0.12 \mu \text{M} \text{norepinephrine} attained a steady state, the tissue was washed and allowed to rest for 10 minutes. This procedure was repeated until identical responses to this dose of norepinephrine were obtained; the tissue was then assumed to have reached equilibrium. Propranolol (10^{-6} M) and desmethylimipramine (10^{-7} M) were added to the bath and allowed to equilibrate for 30 minutes. Contractile responses to 0.012, 0.036, 0.12, 0.36, and 120 \text{nM} \text{norepinephrine} were then recorded. After each dose of norepinephrine, the tissue was washed and allowed to recover for 10 minutes. The slope of the log dose-probit response curve and the median effective dose (ED_{50}) of norepinephrine were determined.

**ENZYME ASSAYS**

Monoamine oxidase (MAO) activity was assayed in all...
Adrenergic Parameters of Rabbit Veins

<table>
<thead>
<tr>
<th>Vein</th>
<th>Extracellular uptake of $^{14}$C-sucrose (ml/g)</th>
<th>Extraneuronal uptake of $^3$H-norepinephrine (ml/g)</th>
<th>$l$-Norepinephrine $ED_{50}$ (µM)</th>
<th>Slope of $l$-norepinephrine dose-response curve</th>
<th>Monamine oxidase (µmoles/g protein hour$^{-1}$)</th>
<th>Catecho1-O-methyl transferase (µmoles/g protein hour$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial cervical</td>
<td>0.36 ± 0.06</td>
<td>5.38 ± 0.97</td>
<td>0.15 ± 0.04</td>
<td>1.33 ± 0.33</td>
<td>0.42 ± 0.11</td>
<td>5.00 ± 1.34</td>
</tr>
<tr>
<td>Femoral</td>
<td>0.45 ± 0.04</td>
<td>2.94 ± 0.44</td>
<td>0.23 ± 0.07</td>
<td>1.21 ± 0.16</td>
<td>2.75 ± 0.41</td>
<td>8.67 ± 2.69</td>
</tr>
<tr>
<td>Subcutaneous (Back)</td>
<td>0.43 ± 0.04</td>
<td>4.64 ± 0.53</td>
<td>0.12 ± 0.02</td>
<td>1.22 ± 0.27</td>
<td>2.05 ± 0.25</td>
<td>5.33 ± 1.02</td>
</tr>
<tr>
<td>Brachial</td>
<td>0.38 ± 0.04</td>
<td>4.45 ± 0.47</td>
<td>0.22 ± 0.04</td>
<td>1.40 ± 0.25</td>
<td>1.86 ± 0.31</td>
<td>10.17 ± 2.27</td>
</tr>
<tr>
<td>Deep circumflex iliac (Branch)</td>
<td>0.47 ± 0.03</td>
<td>3.58 ± 0.38</td>
<td>0.16 ± 0.06</td>
<td>1.23 ± 0.14</td>
<td>0.89 ± 0.12</td>
<td>3.37 ± 0.79</td>
</tr>
<tr>
<td>Inferior vena cava</td>
<td>0.59 ± 0.05</td>
<td>3.58 ± 0.61</td>
<td>0.14 ± 0.03</td>
<td>1.26 ± 0.14</td>
<td>1.92 ± 0.30</td>
<td>16.17 ± 4.10</td>
</tr>
<tr>
<td>Renal</td>
<td>0.56 ± 0.06</td>
<td>3.28 ± 0.21</td>
<td>0.20 ± 0.05</td>
<td>1.51 ± 0.14</td>
<td>3.45 ± 0.83</td>
<td>19.17 ± 4.62</td>
</tr>
<tr>
<td>Common perforatory</td>
<td>0.60 ± 0.04</td>
<td>3.28 ± 0.49</td>
<td>0.18 ± 0.02</td>
<td>1.61 ± 0.23</td>
<td>1.54 ± 0.28</td>
<td>17.00 ± 5.84</td>
</tr>
<tr>
<td>Central ear</td>
<td>0.43 ± 0.04</td>
<td>3.86 ± 0.52</td>
<td>0.21 ± 0.04</td>
<td>1.38 ± 0.23</td>
<td>1.38 ± 0.23</td>
<td>5.80 ± 2.50</td>
</tr>
<tr>
<td>Parietal branch of internal iliac</td>
<td>0.46 ± 0.04</td>
<td>3.62 ± 0.22</td>
<td>0.08 ± 0.02</td>
<td>1.27 ± 0.07</td>
<td>5.00 ± 0.51</td>
<td>21.67 ± 5.97</td>
</tr>
<tr>
<td>Small saphenous</td>
<td>0.57 ± 0.03</td>
<td>3.11 ± 0.43</td>
<td>0.05 ± 0.01</td>
<td>1.32 ± 0.18</td>
<td>3.07 ± 0.67</td>
<td>18.83 ± 3.64</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>0.52 ± 0.05</td>
<td>4.24 ± 0.72</td>
<td>0.10 ± 0.03</td>
<td>1.26 ± 0.18</td>
<td>1.75 ± 0.32</td>
<td>26.00 ± 11.88</td>
</tr>
<tr>
<td>Cephalic</td>
<td>0.30 ± 0.02</td>
<td>4.79 ± 0.79</td>
<td>0.14 ± 0.03</td>
<td>1.27 ± 0.08</td>
<td>2.80 ± 0.31</td>
<td>15.14 ± 5.38</td>
</tr>
<tr>
<td>Anterior mesenteric branches</td>
<td>0.51 ± 0.05</td>
<td>7.52 ± 1.52</td>
<td>0.06 ± 0.02</td>
<td>1.62 ± 0.09</td>
<td>3.72 ± 0.80</td>
<td>6.83 ± 0.70</td>
</tr>
</tbody>
</table>

All values are means ± SE of six observations.

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Results

Considerable variation in the capacity of veins to accumulate $H$-norepinephrine exists within the cephalic veins. Extraneuronal uptake of $H$-norepinephrine was about 10 times higher in cephalic veins than in other veins. The high extraneuronal uptake of $H$-norepinephrine was indicated by the fluorescence histochemistry and by the accumulation of radioactivity in the venous system. Neuronal uptake of $H$-norepinephrine was about 4 times higher in the cephalic veins than in other veins. The correlation of $H$-norepinephrine uptake with extraneuronal uptake was highly significant (r = 0.83, n = 22). In each vein, extraneuronal uptake (measured with microvascular uptake) ranged from 3.1 to 7.2 ml/100 g tissue.

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Catechol-O-methyl transferase (COMT) activity was measured by the method of Black and Holaday (13). Catechol and formaldehyde were formed by the method of Marchand and Arnaud (14). Catechol was further oxidized by the method of Connolly and Seidler (15), and formaldehyde was measured by the method of Marchand and Arnaud (13). Adenosy!methionine and $i$-norepinephrine form 14C-Tryptamine was converted to 14C-indolylacetic acid in the presence of MAO. The conversion compound was then extracted into toluene. Standard liquid scintillation was used to determine the quantity of 14C-Tryptamine.
The extraneuronal uptake of $^{3}$H-norepinephrine was essentially the same in most of the veins studied. Only the anterior mesenteric branch veins had an unusually high extraneuronal uptake (7.52 ± 1.52 ml cleared/g tissue).

**Contractile Response to Exogenous $\alpha$-Norepinephrine**

All of the veins studied contracted in response to exogenous $\alpha$-norepinephrine. Dose-response relationships were obtained in the presence of the neuronal uptake blocking agent, desmethylimipramine ($10^{-7} \text{M}$), and the beta-receptor blocking agent, propranolol ($10^{-6} \text{M}$). The slopes of the dose-response curves were not significantly different from each other under these conditions. However, there were differences in the ED$_{50}$ for norepinephrine (Table 1). Figure 2 illustrates the inverse relationship between the ED$_{50}$ for norepinephrine and the log neuronal uptake of $^{3}$H-norepinephrine ($r = -0.54$, 95% confidence interval = -0.85 to 0.05). If we assume that neuronal uptake of $^{3}$H-norepinephrine is a measure of the density of the adrenergic plexus (see Discussion), this relationship suggests that the sensitivity of venous smooth muscle to norepinephrine may be higher in veins with a more dense innervation than it is in those with a less dense innervation.

**Monoamine Oxidase and Catechol-O-Methyl Transferase**

Both MAO and COMT activities were present in all veins studied. Although MAO activity varied among the veins, no relationship between MAO activity and neuronal uptake (Fig. 3) or any of the other parameters studied was found. A linear relationship between COMT and log neuronal uptake is evident from Figure 4. The correlation coefficient, $r = 0.84$, indicates the strength of this relationship (95% confidence interval = 0.53 to 0.95). In those veins in which neuronal uptake of $^{3}$H-norepinephrine was extremely low, i.e., the superficial cervical, femoral, back subcutaneous, brachial, and deep circumflex iliac branch veins, a sizable amount of COMT was present.

**Discussion**

The uptake of $^{3}$H-norepinephrine was measured by assaying total tritium activity. However, the presence of the catabolic enzymes MAO and COMT could have compounded this measurement. Thus, uptake values are approximations. The small mass of many of the vein specimens precluded an analysis of the nature of the tritiated material. However, uptake measurements determined under comparable conditions in the rat portal vein have shown that 94% of the tritium activity represents $^{3}$H-norepinephrine (15). Furthermore, it has been reported that the MAO inhibitor pargyline ($10^{-4} \text{M}$) does not affect the uptake by the aorta of $^{3}$H-norepinephrine ($10^{-8} \text{M}$) (16). In this study, the ex-
traneuronal uptake of $^3$H-norepinephrine in all veins was similar despite considerable variation in levels of COMT, an extraneuronal enzyme (17). In the absence of more direct evidence, these observations suggest that MAO and COMT do not appreciably affect the uptake of $^3$H-norepinephrine by the veins.

Because of node-crowding and other effects, the neuronal uptake of $^3$H-I-norepinephrine is only an approximation of the adrenergic innervation density in a blood vessel (2, 18, unpublished results). By and large, however, we feel that adrenergic nerve density parallels the extent of neurogenic control in these veins (unpublished results). Neuronal uptake of $^3$H-norepinephrine determined under similar conditions in arterial tissue is 6.32 ml/g for the aorta (2), 15.32 ml/g for the ear artery (2), and 18.71 ml/g for the proximal saphenous artery (unpublished results). Thus, it appears that the small saphenous, pulmonary, cephalic, and mesenteric veins have a higher $^3$H-norepinephrine binding capacity than does the most densely innervated arterial tissue studied to date. The mesenteric and the pulmonary veins have an uptake capacity greater than that indicated by histochemical observations and responsiveness to neurogenic stimulation (unpublished results); the high uptake values for the cephalic and small saphenous veins may in part be accounted for by the intramedial distribution of their adrenergic neurons. Since the separation of nerve endings within the media tends to minimize the node-crowding effect (2), such a distribution could be associated with greater $^3$H-norepinephrine uptake. Vessel wall thickness is not the same in all veins; therefore, uptake per unit surface area of vessel wall and the nerve plexus density at the plexus-muscle junction cannot be expected to follow the same distribution.

Significant variation in the endogenous content of norepinephrine in several veins, reflecting differences in the density of the adrenergic plexus and in wall thickness, has been reported by others. Mayer et al. (1) have found that the mean norepinephrine content of the brachial, cephalic, and metacarpal veins of the dog is 0.17, 1.30, and 0.47 µg/g, respectively. These data contrast with similar measurements in the dog brachial, ulnar, and metacarpal arteries of 0.41, 1.46, 1.27 µg/g.
If 12.0
II
5 9 0
6.0-
3.0-
0.5
r = 0.84
1.0 3.0 5.0
10.0
30.0 50.0
NEURONAL UPTAKE of 3H-L-norepinephrine (ml cleared/g tissue)
FIGURE 4
Relationship between mean neuronal uptake of 3H-L-norepinephrine (log scale) and mean catechol-O-methyl transferase activity for 13 different rabbit veins. The dots from left to right correspond to the vein sequence in Figure 1, excluding the mesenteric vein; r is the correlation coefficient. In each instance N = 6.

In comparison with the neuronal uptake, the extraneuronal binding capacity for 3H-norepinephrine was remarkably constant among the veins studied. It was similar to that measured in arterial tissues (2). The extraneuronal component is a complex sum of a number of separate entities (22, 23), which in toto appear to have a comparatively small capacity and become saturated relatively rapidly. The functional role of this component is unknown.

The neuronal uptake mechanism is an important determinant of the concentration of exogenous norepinephrine at the alpha receptors. To avoid the unequal influence of this mechanism which results from unequal innervation densities, the sensitivity of the venous smooth muscle cells to norepinephrine was determined in the presence of the neuronal uptake blocking agent, desmethylimipramine. Similarly, any influence of the beta receptors, which may also vary from vein to vein (24, 25), was blocked by propranolol. Under these conditions, a suggestion of an inverse relationship between the ED50 for norepinephrine and the density of the adrenergic plexus was noted (Fig. 2). However, in this study neuronal density was inferred from measurements of neuronal 3H-norepinephrine uptake; the procedure provides only an indirect measurement of this parameter. The venous smooth muscle from densely innervated veins thus appears to be more sensitive to norepinephrine than the smooth muscle from veins with little or no adrenergic innervation. It seems unlikely that these differences are the result of either an insufficient concentration of desmethykimipramine or the increased COMT activity found in more densely innervated veins, since both of these effects would result in the opposite relationship. This observation is contrary to expectations based on studies of other tissues. In the cat nictitating membrane, the sensitivity to norepinephrine was inversely related to innervation density; yet the sensitivity of the smooth muscle...
cells was the same in the denervated inferior and medial muscles (18).

The mean ED$_{50}$ of norepinephrine obtained under similar conditions for both rabbit aorta and ear artery strips is approximately $6 \times 10^{-8}$M (unpublished results). Thus, the sensitivity of these vein and artery preparations is of the same order, although variations in the artery preparations are known to exist (26).

Enzymatic degradation may play an important role in the disposition of norepinephrine in vascular tissue. Both MAO and COMT are present in vascular tissue (17, 27). Osswald et al. (10) have reported that MAO metabolism appears to be important in the dog saphenous vein. This finding contrasts with observations by others that COMT activity is dominant in elastic arteries (8, 9). In the rat portal vein, MAO and COMT appear to play nearly equal roles in the catabolism of released transmitter (28). These dissimilarities illustrate the necessity for caution in extrapolating from one vascular tissue to another. The metabolic enzyme activities found in veins were similar to those in the rabbit aorta (MAO 1.79 ± 0.22 μmoles/g protein hour$^{-1}$, COMT 15.78 ± 2.0 nmoles/g protein hour$^{-1}$) and thus provide no reason for anticipating differences in metabolic patterns between arterial and venous tissue. Clearly, direct studies of the metabolites formed in these tissues are required.

Despite a considerable range in the density of the adrenergic plexus among the veins studied, the presence of large quantities of MAO in vascular muscle cells (17) obviated any correlation between neuronal density and total MAO content. It is quite possible, however, that the importance of intraneuronal deamination of norepinephrine (absent in the present series of veins because of treatment with desmethylimipramine) varies with neuronal uptake in the intact veins. COMT activity appeared to be greater in those veins with a higher neuronal uptake of $^3$H-norepinephrine, but the significance of this relationship is presently obscure. In recent studies Jarrott and co-workers (29, 30) have reported significant decreases in COMT activity in nonvascular tissue after denervation, suggesting a significant neuronal component of the enzyme. In the rabbit aorta, however, COMT activity is predominantly extraneuronal in origin (17). Certainly the present study shows that the noninnervated tissue contains COMT, although the possibility that the increased COMT activity in some veins represents a neuronal component of the enzyme cannot be ruled out at present.

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References


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