Catecholamines in Arteries and Veins of the Foreleg of the Dog

By Howard E. Mayer, M.Sc, Francois M. Abboud, M.D., Dennis R. Ballard, B.A., and John W. Eckstein, M.D.

ABSTRACT

The catecholamine content of various blood vessels of the foreleg of the dog was measured by a fluorimetric method (48 vascular segments from 11 dogs) and also by bioassay (33 segments from 8 dogs). The results obtained by the fluorimetric method indicate that the average concentration of catecholamines in the brachial artery (0.14 µg/g of wet tissue) was significantly lower than that in the ulnar (0.70 µg/g) and metacarpal arteries (0.66 µg/g); the average concentration in the brachial vein (0.17 µg/g) was significantly lower than that in the cephalic (1.30 µg/g) and metacarpal vein (0.47 µg/g). The values obtained by bioassay were generally higher than those obtained by the fluorimetric method, but the differences between the arterial segments and between the venous segments were similar by both methods. There was a close correlation between the concentration of catecholamines in the different vascular segments and the relative responsiveness of these segments to nerve stimulation as reported previously (Circulation Res. 18: 263, 1966). Pretreatment with reserpine decreased markedly the catecholamines in segments of ulnar artery and cephalic vein. Intravenous infusion of norepinephrine in amounts sufficient to raise blood levels of catecholamines five- to tenfold did not alter the content of segments of ulnar artery.

ADDITIONAL KEY WORDS large and small vessels fluorimetric method trihydroxyindole method rat bioassay norepinephrine infusion reserpine vasoconstriction

There have been many reports on the concentration of catecholamines in heart (1-4), spleen (1, 5, 6), nervous tissue (7-9), and skeletal muscle (5, 10, 11). The reported values in some tissues are thought to reflect primarily the catecholamine content of blood vessels, but there have been a few direct measurements of catecholamine concentrations in vessel walls (12-15). In 1948, Schmitz et al (12) extracted pressor and depressor substances from large samples of aortas of cows and reported an amount of pressor substance equivalent to 2 µg of adrenaline per gram of tissue. Von Euler and Lishajko (14) in 1958 reported concentrations of norepinephrine ranging from 0.24 to 0.77 µg/g in bovine splenic vessels, bovine and canine pulmonary vessels, and various other vessels from the dog. Their observations, however, were limited to only one or two measurements in most instances. Burn and Rand (15) extracted a pressor substance from aortas of rabbits and reported values ranging from 0.21 to 0.75 µg of norepinephrine per gram of tissue.

Recent modifications of the trihydroxyindole method for fluorimetric determination of catecholamines permit analyses of minute amounts in small tissue samples (7, 16). Using the improved fluorimetric trihydroxyindole method of Haggendal (16), we measured the catecholamine content of large and small arteries and veins in the foreleg of the
dog. The possibility that the concentration of catecholamines differs in large and small vessels of the foreleg was considered because of the difference in responsiveness of these various segments to nerve stimulation (17, 18), and because Angelakos et al. (19), using fluorescence histochemical techniques, reported a difference in the degree of terminal adrenergic innervation in arteries and veins of various sizes in different organs.

The length of time involved in extraction and purification of the catecholamines for fluorimetric determination led us to consider an alternative method of analysis. In this method the catecholamine content of saline extracts of vessels was estimated using the pressor responses in the anesthetized rat as a bioassay. The sensitivity of the bioassay method proved satisfactory, and the values obtained by the assay were comparable to those obtained by the fluorimetric method.

**Methods**

**FLUORIMETRIC DETERMINATIONS OF CATECHOLAMINES**

Male mongrel dogs weighing 15 to 31 kg were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. Segments of either arteries or veins were exposed in both forelegs of each dog. Segments of the brachial, ulnar, and metacarpal arteries or the brachial, cephalic, and metacarpal veins were excised. Immediately after excision, the vessels were quickly stripped of loose connective tissue, blotted, weighed, and frozen in liquid nitrogen. The weights ranged from 30 to 714 mg, and the time from dissection to freezing was 2 to 3 minutes. The frozen vessels were crushed in a stainless steel pulverizing apparatus that had been cooled in liquid nitrogen. The crushed tissues were homogenized for 2 to 3 minutes with 0.4N perchloric acid and centrifuged (9000 × g) at 0°C for 15 minutes. The extract was passed through a column (3 X 35 mm) of Dowex 50 X 8 at a rate of 10 ml/min with a solution of 0.1% EDTA, phosphate buffer, and water and then eluted with 1.0 N HCl. A 2.8-ml aliquot was collected after the pH of the eluate had fallen to 2.5; this portion of the eluate is known to contain the norepinephrine and epinephrine (16). The adsorption, purification, and elution on the column requires at least 12 hours. The length of time involved may account for the recovery values of 70 to 80% reported by Haggendal (16); our recoveries in four experiments ranged from 73 to 88%. The eluted catecholamines were oxidized with potassium ferricyanide, and the products, adrenochrome and noradrenochrome, were treated with alkali (NaOH) to yield the fluorescent trihydroxyindoles. The fluorescence was stabilized by the addition of BAL-sodium sulfite and read in relation to a reagent blank. Overoxidized or faded tissue blanks and two duplicate standards were routinely analyzed with each sample. Standards were prepared from pure DL-norepinephrine HCl.

Fluorescence was measured with a C. K. Turner Model 111 fluorometer. This was equipped with a High Sensitivity Kit and Blue Lamp (Turner 110-853); linear standard curves were obtained with samples ranging from 0 to 75 ng of norepinephrine. The majority of the analyses were performed in the range of 5 to 30 ng. Fluorimetric readings were taken between 20 and 30 minutes after initiation of the chemical reaction. The fluorescence was relatively stable at this time. To obtain an estimate of the total catecholamine content, a filter system which yields nearly equal readings for the fluorescence of derivatives of both epinephrine and norepinephrine was selected. No corrections were applied to the data for losses in the purification procedures and column recovery.

**DETERMINATION OF CATECHOLAMINES BY BIOASSAY**

Rats (200 to 550 g) were anesthetized with pentobarbital sodium, 35 mg/kg, ip, and given atropine, 2.5 mg/kg, and pentolinium, 5 mg in a water polyvinylpyrrolidone base, subcutaneously. Tracheotomy and bilateral vagotomy were performed, and the right common carotid artery was cannulated. Arterial blood pressure was measured with a small volume-displacement Statham transducer (FP3-De) and recorded continuously. Heparin, 1000 units/kg, was given through a 29-gauge needle in the right external jugular vein. The left external jugular vein was cut and cannulated both upstream and downstream with two short polyethylene cannulas. The two cannulas were connected with a short piece of rubber tubing, and the jugular vein

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1It was important to reduce to a minimum the time from dissection to freezing to limit the loss of norepinephrine. Sedgall (16) found an early loss of norepinephrine amounting to as much as 20% in skeletal muscle 10 minutes after death.

2General Biochemical, Chagrin Falls, Ohio.

3Composed of primary filter (405 m/i) no. 110-312 (405) and secondary filter (495 m/i) no. 110-825 (65A).
Catecholamines in Blood Vessels

Before Blockers

After Blockers

NE (ng)

Before

Blockers

After

Blockers

Pressor responses in the rat to graded ascending doses of norepinephrine (NE) before and after intravenous administration of phentolamine, 0.25 mg, and propranolol, 0.1 mg.

Pressor responses in the rat to aliquots of extracts of brachial (Brae.), ulnar, and metacarpal (Meta.) arteries to norepinephrine (NE) and to angiotensin (Angio) before (top strip) and after (bottom strip) administration of phentolamine (Phent.) and propranolol (Prop.). The blockers antagonized to an equivalent degree both the effect of norepinephrine and the effect of the extracts, but the effect of angiotensin was not altered significantly, suggesting that the pressor effect of the extracts is caused predominantly by their catecholamine content.

Levophed, Winthrop Laboratories, New York, N. Y.

4

Flow was re-established. Injections were made directly into the rubber tubing.

Frozen blood vessels were crushed as described for the fluorimetric determination and homogenized briefly in 2 or 3 ml of isotonic saline. After centrifugation (9000 × g for 15 minutes) at 0°C, pressor responses to aliquots of the saline extracts were compared to those of standard doses of norepinephrine bitartrate. The specificity of the pressor activity of the extracts was tested by injecting the rat intravenously with the alpha- and beta-receptor blocking drugs, phentolamine methanesulfonate, 0.25 mg, and propranolol, 0.1 mg; these doses were sufficient to antagonize the effect of norepinephrine (Fig. 1). In each experiment three doses of norepinephrine (5, 10, and 50 ng of base), two doses of angiotensin (1.5 to 5.0 ng), and 0.1- or 0.2-ml aliquots of extracts of brachial, ulnar, and metacarpal arteries or brachial, cephalic, and metacarpal veins were injected in random order before and after phentolamine and propranolol (Fig. 2). Each extract was administered to one rat only. The rats that were used for bioassay were those in which responses to graded doses of norepinephrine were linear and those in which increases in arterial pressure of at least 1 mm Hg per nanogram of norepinephrine were observed. Responses to the extracts and to norepinephrine obtained before and after the blockers were used to calculate the concentration of norepinephrine equivalents in blood vessels. Angiotensin was used in these experiments as an internal control to test the specificity of the blockade.

Circulation Research, Vol. XXII, November 1968
ARTERIAL SEGMENTS
Analyses were carried out on arterial segments taken from each foreleg of eight dogs.

Fluorimetric Method (Five Dogs).—The catecholamine content averaged 0.14 μg/g in segments of brachial artery, 0.70 μg/g in segments of ulnar artery, and 0.66 μg/g in segments of metacarpal arteries (Table 1). The content of brachial arteries was lower than that of corresponding ulnar arteries in eight of nine forelegs. The content of metacarpal arteries was lower than that of corresponding ulnar arteries in six of nine forelegs.

Bioassay Method (Three Dogs).—The catecholamine content averaged 0.41 μg/g in segments of brachial artery, 1.46 μg/g in segments of ulnar artery, and 0.57 μg/g in segments of metacarpal arteries (Table 2). The content of brachial arteries was lower than that of corresponding ulnar arteries in each of six forelegs. The content of metacarpal arteries was lower than that of corresponding ulnar arteries in four of the six forelegs.

VENOUS SEGMENTS
Analyses were carried out on pooled venous segments from both forelegs in each of 11 dogs.

Fluorimetric Method (Six Dogs).—The catecholamine content averaged 0.17 μg/g in segments of brachial vein, 1.30 μg/g in segments of cephalic vein, and 0.47 μg/g in segments of metacarpal veins (Table 3). The content of cephalic veins exceeded that of corresponding metacarpal and brachial veins in each of the six dogs.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catecholamine Content (Fluorimetric Determination) of Consecutive Arterial Segments from Ten Forelegs of Five Dogs</td>
</tr>
<tr>
<td>Dog no.</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>MEAN</td>
</tr>
<tr>
<td>Mean difference in NE content ± SEM</td>
</tr>
<tr>
<td>P value</td>
</tr>
</tbody>
</table>

*NE represents the concentration of catecholamines calculated in terms of norepinephrine equivalents.
†P values represent the significance of the difference between the concentrations in the arterial segments (Brachial vs Ulnar and Ulnar vs Metacarpal) using Wilcoxon's signed rank test (24). The signed rank test was preferred to the paired t-test because the assumption of normality of distribution of the differences was in doubt and the number of paired observations permitted such an analysis. P values of 0.054 or less were considered indications of statistical significance.
CATECHOLAMINES IN BLOOD VESSELS

Bioassay Method (Five Dogs).—The catecholamine content of segments of cephalic vein averaged 1.96 μg/g and was significantly greater than that of segments of metacarpal veins which averaged 1.04 μg/g (Table 4). Extracts of segments of brachial veins did not cause a pressor response in the rat; instead a depressor response was apparent with four of the five extracts after blockade of alpha and beta receptors. The catecholamine content of extracts of brachial veins was therefore not detectable by bioassay.

CATECHOLAMINE CONTENT OF ARTERIAL SEGMENTS BEFORE AND AFTER INTRAVENOUS ADMINISTRATION OF NOREPINEPHRINE

In each of three dogs, one ulnar artery was excised for fluorimetric determination of its catecholamine content before the infusion of norepinephrine bitartrate, 0.4 μg/kg/min, iv. After a 30-minute infusion, the contralateral ulnar artery was excised for analysis. Levels

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Brachial artery</th>
<th>Ulnar artery</th>
<th>Metacarpal artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (mg)</td>
<td>NE* (μg/g)</td>
<td>Weight (mg)</td>
</tr>
<tr>
<td>1</td>
<td>65</td>
<td>0.97</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>0.00</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>129</td>
<td>0.79</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>2.24</td>
<td>63</td>
</tr>
<tr>
<td>MEAN</td>
<td>101</td>
<td>0.41</td>
<td>97</td>
</tr>
</tbody>
</table>

Mean difference in NE content ± SEM

<table>
<thead>
<tr>
<th>NE content (μg/g)</th>
<th>1.05 ± 0.16</th>
<th>0.19 ± 0.33</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>0.032</td>
<td>&gt; 0.312</td>
</tr>
</tbody>
</table>

*See footnote to Table 1.

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Brachial vein</th>
<th>Cephalic vein</th>
<th>Metacarpal vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94</td>
<td>0.28</td>
<td>365</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>0.83</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>108</td>
<td>0.10</td>
<td>342</td>
</tr>
<tr>
<td>4</td>
<td>210</td>
<td>0.06</td>
<td>518</td>
</tr>
<tr>
<td>5</td>
<td>214</td>
<td>0.10</td>
<td>714</td>
</tr>
<tr>
<td>6</td>
<td>79</td>
<td>0.23</td>
<td>261</td>
</tr>
<tr>
<td>MEAN</td>
<td>135</td>
<td>0.17</td>
<td>438</td>
</tr>
</tbody>
</table>

Mean difference in NE content ± SEM

<table>
<thead>
<tr>
<th>NE content (μg/g)</th>
<th>1.13 ± 0.36</th>
<th>0.83 ± 0.37</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>0.032</td>
<td>0.032</td>
</tr>
</tbody>
</table>

*See footnote to Table 1.

$P$ values represent the significance of the difference between concentrations in the venous segments (Brachial vs Cephalic and Cephalic vs Metacarpal) using Wilcoxon's signed rank test (51). See footnote to Table 1.

Circulation Research, Vol. XXIII, November 1968
TABLE 4

Catecholamine Content (Bioassay) of Pooled Venous Segments from the Forelegs of Five Dogs

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Brachial vein</th>
<th>Cephalic vein</th>
<th>Metacarpal vein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (mg)</td>
<td>NE+ (µg/g)</td>
<td>Weight (mg)</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
<td>---------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>0.00</td>
<td>237</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>0.00</td>
<td>136</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>0.00</td>
<td>245</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>0.00</td>
<td>146</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>0.00</td>
<td>254</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td><strong>1.96 ± 0.42</strong></td>
<td><strong>0.92 ± 0.30</strong></td>
<td><strong>1.96 ± 0.42</strong></td>
</tr>
</tbody>
</table>

*See footnote to Table 1.

fThe paired t-test was used here because Wilcoxon's signed rank test (24) requires at least six pairs of observations to achieve the conventional 0.05 level of significance and there were only five pairs of observations in this group.

The catecholamine content of arterial blood increased five- to tenfold by the end of the period of infusion, whereas the catecholamine content of the ulnar arteries did not increase significantly (Fig. 3). The difference between the concentrations in the right and left ulnar arteries in these dogs was within the range encountered in other dogs without infusion of norepinephrine (Table 1).

CATECHOLAMINE CONTENT OF ULNAR ARTERIES AND CEPHALIC VEINS AFTER RESERPINE

Six dogs were treated with reserpine, 0.175

to 0.437 mg/kg, ip, daily for 2 days. Pooled right and left ulnar arteries from three of the dogs and pooled right and left cephalic veins from the other three dogs were analyzed fluorimetrically. A marked reduction in the catecholamine content of both arteries and veins was apparent when the values were compared to controls (Fig. 4).

FIGURE 3

Concentration of norepinephrine (NE) in plasma and in arterial segments in three dogs after intravenous infusion of norepinephrine, 0.4 µg/kg/min × 30 min. The catecholamine content of segments of ulnar arteries excised before and after the intravenous infusion of norepinephrine was not significantly different.

FIGURE 4

Effect of treatment with reserpine on the catecholamine content of segments of ulnar arteries and of cephalic veins.

Circulation Research. Vol. XXIII, November 1968
compared to those obtained in untreated animals (Fig. 4).

Discussion

The results indicate that there is a significant difference in the concentration of catecholamines in segments of various arteries and veins in the foreleg of the dog anesthetized with pentobarbital. This difference appears to be related to the responsiveness of the vascular segments to nerve stimulation. For example, the ulnar artery contains a larger concentration of catecholamines than the brachial artery. This finding correlates with the greater responsiveness of the ulnar artery to nerve stimulation that we have observed in previous studies (17, 18). Similarly, the cephalic and metacarpal veins have a higher concentration of catecholamines than the brachial vein. This observation is also in agreement with our previous findings concerning the venoconstrictor responses to nerve stimulation (18). Increases in resistance to venous outflow from the paw which is drained by the metacarpal and cephalic veins were much greater than increases in venous resistance in the muscular portions of the foreleg which are drained primarily by the brachial vein (Fig. 5). It appears that there is a close correlation between the catecholamine content and the response to adrenergic stimulation.

There was an unexpected degree of variability from animal to animal in the catecholamine content of the same vascular segment and even in the same animal in contralateral vascular segments. We considered the possibility that these variations may have resulted from wide fluctuations in the levels of catecholamines in blood. Lammerant and DeHerdt (20) found considerable variation in blood levels of catecholamines in anesthetized dogs; they reported values ranging from 0 to 6.9 µg/liter of plasma. In an attempt to determine the degree to which the catecholamine content of vascular segments would be influenced by the catecholamine levels in blood, we infused norepinephrine intravenously in amounts sufficient to raise blood levels five-to tenfold but did not observe a consistent or significant increase in vascular catecholamine content. Wide variations in the content of catecholamines in skeletal muscle have been reported by Sedvall (10). Von Euler and Lishajko (14) have reported concentrations of 0.36 and 0.73 µg of norepinephrine/g in two observations on dog femoral arteries. Davignon et al. (13) analyzed human umbilical arteries and found low values of 0.005 µg of norepinephrine/g of tissue, probably reflecting a lack of adrenergic innervation in these vessels; their single observation on dog femoral artery gave a value of 0.03 µg/g. Therefore, the variability of the catecholamine content of the same vascular segment from animal to animal may reflect a variation in the degree of adrenergic innervation. One would expect a variation in the degree of adrenergic innervation since the response of a vascular segment to nerve stimulation may differ markedly from animal to animal (18).

It was shown as early as 1956 by Bertler et al. (21) and by Holzbauer and Vogt (22) that reserpine depletes rabbit heart (21) and
cat hypothalamus (22) of norepinephrine. Burn and Baird (15) demonstrated depletion of the stores of noradrenaline-like substances in rabbit aorta after reserpine. The present study indicates that reserpine as administered in these experiments, depletes more than 80% of catecholamines in the ulnar artery and cephalic vein of dog. The norepinephrine remaining in the vessel walls after reserpine does not mediate a vasoconstrictor response upon nerve stimulation; doses of reserpine similar to those used in these experiments may cause a complete reversal of the vasoconstrictor response to nerve stimulation in the perfused foreleg (23).

The particular advantage of the fluorimetric method of Haggendal is that it allows measurements to be carried out on small samples of tissue. The column used is small enough to permit elution of a relatively high concentration of catecholamines in a small volume of eluate but the rate of flow through the column is limited to 10 ml/hour, extending the extraction and purification over a 12-hour period. The bioassay method that was used in these experiments had the advantage of requiring less time. Results could be obtained within an hour from the time of dissection of the tissue. With the exception of the analyses on brachial vein segments, the values observed by bioassay were generally higher than those measured by the fluorimetric method. The discrepancy may reflect in part the incomplete recovery of catecholamines from the column when the fluorimetric method is used and possibly a greater loss of catecholamines during the extractions of homogenate before adding it to the column. Another reason may be the fact that different standards were used in the two procedures. Finally, the adrenergic blocking agents used to test the specificity of the pressor activity of the extracts in the bioassay may have antagonized other pressor substances in addition to norepinephrine and epinephrine. Such substances would then be included in the calculation as norepinephrine equivalents. Because of the absence of a pressor response and the frequent small depressor responses to extracts of segments of brachial vein, we reported that the brachial vein content of catecholamines was not detectable by bioassay. Depressor substances were present in amounts sufficient to oppose or reverse the pressor effect of the small quantities of catecholamines which were detected in this segment by fluorimetry. The nature of these depressor substances is not known but they have been reported also in extracts of aortic wall of cattle by Schmitterlow (12).

References
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