Evoked Release of Endogenous Norepinephrine in the Canine Saphenous Vein

Inhibition by Acetylcholine

P.M. Vanhoutte, E.P. Coen, W.J. De Ridder, and T.J. Verbeuren

SUMMARY Most of the information concerning adrenergic neurotransmission in the blood vessel wall has been obtained from isolated blood vessels previously incubated with radiolabeled norepinephrine. In the present study, we determined the tissue content of norepinephrine and dopamine in the dog's saphenous vein using a radioenzymatic assay; tissue content of dopamine was 30 times less than tissue content of norepinephrine, and no epinephrine could be detected. During superfusion of isolated canine saphenous vein preparations, superfusate samples were collected for subsequent radioenzymatic analysis of norepinephrine, epinephrine, and dopamine. The basal efflux of endogenous norepinephrine declined slightly with time. Nerve stimulation caused frequency-dependent increases in tension, paralleled by increases in efflux of endogenous norepinephrine. The changes in tension were correlated significantly with the changes in norepinephrine overflow. Acetylcholine at 5 x 10^-7 M had no effect on basal tension or basal norepinephrine overflow. During nerve stimulation at 2 Hz and at 5 Hz, it significantly depressed the contractile response and the evoked overflow of endogenous norepinephrine. Neither dopamine nor epinephrine was detected during these superfusion studies. The present experiments introduce the means for measuring endogenous norepinephrine overflow in the canine saphenous vein, validate earlier work on this blood vessel, and, in particular, provide direct evidence for the inhibitory effect of acetylcholine on adrenergic neurotransmission in the blood vessel wall.

Circ Res 45: 608-614, 1979

THE canine saphenous vein has been used frequently as a model of adrenergically innervated vascular smooth muscle (Osswald, 1978; Shepherd and Vanhoutte, 1975; Vanhoutte, 1978). Most of the information so far has been derived from studies in which the tissue uptake or the overflow of norepinephrine was measured after incubation with exogenous radiolabeled transmitter. In such studies, the question remains as to whether or not the alterations in overflow of labeled norepinephrine truly reflect the movements of endogenously synthesized transmitter. Therefore, in the present study, we determined tissue content and overflow of endogenous norepinephrine using a sensitive radioenzymatic assay (Peuler and Johnson, 1977).

Methods

All experiments were performed on lateral saphenous veins, taken from mongrel dogs (15-25 kg) anesthetized with pentobarbital (30 mg/kg, iv).

Measurement of Catecholamine Content

Tissue catecholamines were extracted by placing strips of saphenous veins (2 mm wide, 3-4 cm long) in test tubes containing 0.5 ml of ice-cold 1 N acetic acid, 0.95 mg of Na2EDTA, and 0.60 mg of reduced glutathione (Levin, 1973). The test tubes were mixed for 30 minutes in a vortex mixer, and the preparations then were transferred to another set of test tubes containing the same extraction fluid; after another 30 minutes of continuous vortexing, the strips were removed, and the two 0.5-ml portions were pooled and frozen until assayed. Immediately before the assay, the samples were thawed and diluted 100 times with distilled water containing glutathione (1.2 mg/ml). For the blanks, the
same amount of acetic acid and protectives as used to
extract the catecholamines was diluted 100 times.
Earlier work performed on saphenous vein strips
incubated with \(^3\)H-norepinephrine has shown that
the extraction procedure used in the present study
recovers 95% of the total radioactivity taken up by
the tissue (Muldoon et al., 1978).

**Superfusion Studies**
Vein strips (4 mm wide, 10 cm long) were sus-
pended in a tunnel-shaped chamber kept at 37°C
and continuously superfused (1 ml/min) with aer-
ated (95% \(\text{O}_2\)-5% \(\text{CO}_2\)) Krebs-Ringer bicarbonate
solution of the following millimolar composition:
\[
\text{NaCl, 118.3; KCl, 4.7; MgSO}_4\cdot 7\text{H}_2\text{O}, 1.2; \text{KH}_2\text{PO}_4, 1.2; \text{CaCl}_2, 2.5; \text{NaHCO}_3, 25; \text{glucose, 11.1; and CaEDTA, 0.026. To stimulate the adrenergic nerves in the vessel wall, two platinum wires (0.5 mm in diameter, 15 cm long) were placed parallel to the veins (Van-
houtte et al., 1967; Vanhoutte et al., 1973). Electrical
impulses consisted of rectangular waves (9 V, 2
msec) provided by a direct current power supply
and switching transistor (MBLE BD 139) triggered
by a stimulator (Grass Instruments). In some ex-
periments acetylcholine, in a final concentration of
5 \(\times\) 10\(^{-7}\) M, was infused in the circuit upstream from
the roller pump.

The preparations were connected to a force
transducer (Grass FT 03) for isometric tension re-
cording; the initial tension was set at 3 g. After this
initial stretch, tension decreased and stabilized
within 30 minutes; the first superfusate sample
was collected 30 minutes later. The superfusate was
collected at selected intervals during 3 minutes into
cooled test tubes containing 5.7 mg of EGTA and
3.6 mg of reduced glutathione (Peuler and Johnson,
1977). Blanks were obtained by collecting super-
fusate in the cooled test tubes in the absence of vein
strips in the superfusion apparatus. The samples
were frozen at \(-23°C\) until they were assayed; the enzymatic assay always was performed within 3
days after the superfusion experiment.

**Radioenzymatic Assay**
We measured norepinephrine, epinephrine, and
dopamine levels in the superfusate and extraction
samples, as described by Peuler and Johnson (1977),
using a commercially available kit ("Cat-a-kit", Up-
john Diagnostics). Briefly, 100 \(\mu\)l of the thawed sam-
ple was incubated for 1 hour at 37°C in a medium
containing 10 \(\mu\)l of COMT\(^*\) solution, 100 \(\mu\)M Tris
buffer, 10 \(\mu\)M EGTA, 30 \(\mu\)M MgCl\(_2\), 1 \(\mu\)M reduced
glutathione, 0.1 \(\mu\)M benzoylxyamine, and 5 \(\mu\)Ci \(^3\)H-
methyl-S-adenosyl-L-methionine (New England
Nuclear, specific activity, 8.8-11.5 Ci/mmol). The total
incubation volume was 150 \(\mu\)l, and the pH was
8.1-8.3. For each sample, an internal standard was
prepared by adding 100 pg of each of the catechol-
olamines to a duplicate incubation mixture containing
100 \(\mu\)l of sample. All assays were performed in
duplicate. After incubation, the reaction was
stopped by adding 50 \(\mu\)l of boric acid containing
carrier normetanephrine, metanephrine, and 3-
metoxytyramine. The \(^3\)H-O-methylated metabo-
lites were first extracted into toluene:isoamylalco-
hol (3:2) and then reextracted into 0.1 \(\text{n}\) acetic acid.
Ethanol was added, and the O-methylated com-
pounds were separated by thin layer chromatogra-
phy on silica gel. After chromatography, the zones
containing the O-methylated metabolites were
scraped into scintillation vials.

Dopamine was assayed by eluting \(^3\)H-3-methox-
ytyramine with ammonium hydroxide and subse-
quently addition to the vial of 10 ml of a scintillation
mixture (toluene:isoamylalcohol (3:2) containing
7 \(\text{g}/\text{liter of 2-(4-tert-butylphenyl)-5-(4-biphenylyl)-}
1,3,4-oxadiazole (Butyl-PBD) and 0.1 \(\text{g}/\text{liter of 1,4-
bis-[2-(5-phenyloxazolyl)]-benzene (POPOP). Nor-
erpinephrine and epinephrine were assayed by elut-
ing their O-methylated derivatives with ammonium
hydroxide, subsequent conversion of the metabo-
lites to \(^3\)H-vanillin by periodate oxidation, and by
extracting the vanillin into 10 ml of scintillation
mixture (toluene, containing 7 \(\text{g}/\text{liter of Butyl-PBD}
and 0.1 \(\text{g}/\text{liter of POPOP). The radioactivity of the}
samples was measured in a liquid scintillation spec-
trometer (Packard model 2450). The samples were
counted for 10 minutes; corrections for quenching
were made with an external standard.

The linearity of the enzymatic assay was tested by
dissolving increasing amounts of the three cate-
cholamines in Krebs-Ringer bicarbonate solution
containing 1.90 mg of EGTA and 1.20 mg of reduced
glutathione per milliliter. Final concentrations rang-
ing from 1 to 100 pg/100\(\mu\)l, were analyzed. The results
are summarized in Figure 1. In the concentra-
tion range tested, the assay is linear for the three
catecholamines yielding a mean of 22.1 \(\pm\) 0.7
counts/min (CPM) per pg of norepinephrine, 24.9
\(\pm\) 1.3 CPM/pg for epinephrine, and 17.1 \(\pm\) 0.7
CPM/pg of dopamine. When the assay was per-
formed on blanks (Krebs-Ringer solution; \(n=19\)),
the procedure to determine norepinephrine yielded
48.7 \(\pm\) 5.7 CPM, for epinephrine, 37.1 \(\pm\) 3.5 CPM,
and for dopamine, 118.1 \(\pm\) 6.7 CPM. The amounts
of catecholamines for which the yield was twice the
blank were considered to be the limits of sensitivity.
This was 3 pg for norepinephrine, 2 pg for epine-
phrine, and 5 pg for dopamine.

**Calculation of Results**
The concentration of catecholamines present in
the samples was calculated using the formula:
\[
[\text{CPM}_{\text{sample}} - \text{CPM}_{\text{blank}}]/(\text{CPM}_{\text{internal standard}} - \text{CPM}_{\text{sample}}) \times ((100 \times 10^{-12} \text{g})/0.1 \text{ml}) = a \times 10^{-12} \text{g/ml. By taking into account the wet weight}
\text{of the tissue, the molecular weight of the catechol-
amine, and the flow in the superfusion, the results}

\* COMT = catechol-O-methyltransferase.
CONCENTRATION OF CATECHOL AMINE

FIGURE 1 Linearity of the radioenzymatic assay (4) for norepinephrine (○, \( r^2 = 0.99 \)), epinephrine (●, \( r^2 = 1.00 \)), and dopamine (▼, \( r^2 = 1.00 \)); Krebs-Ringer bicarbonate solution was used as a solvent. The concentration of the catecholamines is expressed as µg/ml.

were standardized and converted to moles per milligram of tissue per minute of superfusion.

Statistical Analysis

For each group of preparations, the number of strips reported is also the number of dogs used. The data are expressed as means ± SE. For statistical analysis of the data, Student's t-test for paired or unpaired observations was used. \( P \) values < 0.05 were considered to be significant. Correlations were evaluated by use of linear regression analysis.

Results

Endogenous Catecholamine Content

Six saphenous vein preparations (mean weight: 54.6 ± 3.6 mg) were analyzed. In these strips, the norepinephrine content was 5.77 ± 0.72 µg/g, the dopamine content averaged 0.168 ± 0.042 µg/g, and no epinephrine could be detected.

Overflow of Endogenous Norepinephrine

Twenty-three preparations (mean weight: 185.8 ± 7.9 mg) were studies in the superfusion apparatus.

Unstimulated Preparations

No basal efflux of epinephrine and dopamine could be detected in any of the preparations. During superfusion in basal conditions, detectable amounts of norepinephrine were present in the superfuse samples from all veins (mean basal efflux: 4.21 ± 0.59 × 10^{-15} mol/mg per min). In five saphenous vein strips, the concentration of norepinephrine in basal efflux averaged 3.89 ± 0.76 × 10^{-15} mol/mg per min at the start of the experiment. This efflux slightly declined with time and averaged 2.70 ± 0.77 × 10^{-15} mol/mg per min after 20 minutes, 1.55 ± 0.29 × 10^{-15} mol/mg per min after 40 minutes, and 1.14 ± 0.24 × 10^{-15} mol/mg per min after 60 minutes. The latter value was just above the sensitivity limit of the assay.

Nerve Stimulation

Electrical stimulation at 2 Hz or at 5 Hz was applied for 5 minutes; samples for the analysis of catecholamines were collected before the start and during the last 3 minutes of stimulation. The results are summarized in Table 1. Both frequencies caused an increase in tension and an increased overflow of endogenous norepinephrine; the augmentation of the latter was significantly larger at 5 Hz.

When three successive 5-minute periods of 2-Hz, 5-Hz, and 10-Hz stimulation were applied in the same preparation, increasing stimulation frequencies caused progressive increases in tension and overflow of endogenous norepinephrine (Table 1). When two successive 5-minute periods of 5-Hz stimulation were applied at a 30-minute interval in the same vein, no significant difference in contractile response or evoked release of endogenous norepinephrine could be noted (Table 1).

When saphenous veins were subjected to prolonged (30 minutes) stimulation at 2 Hz and samples for analysis collected after 3, 15, and 27 minutes of stimulation, there was a slight decrease in tension and overflow of endogenous norepinephrine with time (Table 1); the increases in tension and in overflow of endogenous transmitter during the collection of the second sample (15th to 18th minute of stimulation) were not significantly different (94.7 ± 1.5 and 97.9 ± 7%, respectively) from those obtained by averaging the increases observed during the first (3rd to 6th minute) and the third (27th to 30th minute) period of collection.

If, for the 23 strips reported, the overflow of endogenous norepinephrine is plotted against the contractile response, no correlation is found (Fig. 2, upper). However, if changes in tension and in norepinephrine overflow are compared within the same preparations using the first response as control (100%), a linear and significant correlation is found between both parameters (Fig. 2, lower).

Detectable amounts of epinephrine and dopamine were not found in the superfuse samples at any of the frequencies of electrical stimulation.

Effect of Acetylcholine

In five saphenous veins, acetylcholine 5 × 10^{-7} M, was infused during sustained contractions
evoked by a 2-Hz electrical stimulation (Table 1). Acetylcholine caused a decrease in tension (to 49.1 ± 5.1% of control) and in the overflow of endogenous norepinephrine (to 27.7 ± 5.7% of control). These effects of acetylcholine were reversible (Fig. 3).

In four veins, a first response to 5 Hz (5 minutes) was obtained. After 20 minutes, acetylcholine (5 × 10⁻⁷ M) was added to the superfusing solution. Acetylcholine had no effect on basal tension and basal overflow of endogenous norepinephrine, but significantly depressed the contractile response (to 49.6 ± 4.1% of control) and the evoked release of transmitter (to 35.0 ± 1.0%) during 5-Hz electrical stimulation (Table 1). In the concentration used, acetylcholine did not interfere with the radioenzymatic assay.

**Discussion**

The purpose of the present study was to determine the tissue content and the overflow of endogenous norepinephrine in the saphenous vein of the dog in order to validate earlier conclusions reached in preparations for which the efflux of ³H-norepinephrine was followed after incubation with exogenous labeled transmitter (Vanhoutte et al., 1973; Vanhoutte et al., 1977; Muldoon et al., 1976; Vanhoutte and Verbeuren, 1976; Verbeuren et al., 1978). The experimental conditions of superfusion and determination of tissue content of norepinephrine were identical to those used in these studies (Vanhoutte et al., 1973; Vanhoutte et al., 1977; Muldoon et al., 1976; Vanhoutte and Verbeuren, 1976; Verbeuren et al., 1978).

The radioenzymatic assay used in the present study is specific for catecholamines (Peuler and Johnson, 1977). When known amounts of epinephrine, dopamine, and norepinephrine were dissolved in Krebs-Ringer bicarbonate solution, the radioenzymatic assay, which was originally designed for measurement of plasma catecholamines (Peuler and Johnson, 1977), yielded linear relationships for

**Table 1 Effect of Nerve Stimulation on Tension and Norepinephrine Overflow in Dog’s Saphenous Veins**

<table>
<thead>
<tr>
<th></th>
<th>Changes in tension (g)</th>
<th>Norepinephrine overflow (10⁻¹⁵ mol/mg per min)</th>
<th>Differences from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.67 ± 0.74</td>
<td>10.47 ± 1.42*</td>
<td>6.80 ± 1.03</td>
</tr>
<tr>
<td>E.S. 2 Hz</td>
<td>2.82 ± 0.36</td>
<td>23.82 ± 3.91*</td>
<td>18.91 ± 3.55</td>
</tr>
<tr>
<td>Control</td>
<td>4.91 ± 0.94</td>
<td>21.05 ± 1.03*</td>
<td></td>
</tr>
<tr>
<td>E.S. 5 Hz</td>
<td>3.16 ± 0.57</td>
<td>2.07 ± 0.23</td>
<td>4.74 ± 1.09</td>
</tr>
<tr>
<td>Control</td>
<td>2.77 ± 0.31</td>
<td>2.70 ± 0.69*</td>
<td>7.89 ± 1.38</td>
</tr>
<tr>
<td>E.S. 10 Hz</td>
<td>3.10 ± 0.27</td>
<td>2.50 ± 0.95</td>
<td>21.05 ± 1.03*</td>
</tr>
<tr>
<td>Control</td>
<td>5.48 ± 2.81</td>
<td>25.62 ± 5.36*</td>
<td>20.14 ± 4.95</td>
</tr>
<tr>
<td>E.S. 5 Hz</td>
<td>2.13 ± 0.47</td>
<td>3.12 ± 2.16</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.20 ± 0.53</td>
<td>25.17 ± 4.46*</td>
<td>22.05 ± 4.51</td>
</tr>
<tr>
<td>E.S. 8 Hz</td>
<td>2.65 ± 0.42</td>
<td>5.07 ± 1.43</td>
<td></td>
</tr>
<tr>
<td>5 × 10⁻⁷ M ACh</td>
<td>3.18 ± 0.98</td>
<td>17.31 ± 5.27*</td>
<td></td>
</tr>
<tr>
<td>E.S. 5 Hz</td>
<td>1.35 ± 0.32</td>
<td>9.41 ± 3.00*</td>
<td>6.23 ± 2.02*</td>
</tr>
<tr>
<td>Control</td>
<td>3.50 ± 0.88</td>
<td>8.88 ± 1.21*</td>
<td></td>
</tr>
<tr>
<td>E.S. 2 Hz</td>
<td>2.06 ± 0.06</td>
<td>8.25 ± 0.82*</td>
<td></td>
</tr>
<tr>
<td>E.S. 2 Hz</td>
<td>1.73 ± 0.08</td>
<td>7.90 ± 0.90*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.15 ± 0.58*</td>
<td>7.85 ± 0.80*</td>
<td></td>
</tr>
<tr>
<td>E.S. 2 Hz</td>
<td>2.42 ± 0.54</td>
<td>9.78 ± 1.45*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.14 ± 0.46*</td>
<td>3.08 ± 1.12</td>
<td></td>
</tr>
</tbody>
</table>

Values shown as mean ± SE. ACh = acetylcholine. E.S. = electrical stimulation.

* Difference from preceding value statistically significant (P < 0.05; Student’s t-test for paired observations).
† Difference from value at 2 Hz statistically significant (P < 0.05; Student’s t-test for paired observations).
‡ Difference from value at 5 Hz statistically significant (P < 0.05; Student’s t-test for paired observations).
| This response to nerve stimulation was obtained 30 minutes after the previous one.

Downloaded from http://circres.ahajournals.org/ by guest on January 8, 2018
50 TENSION VS OVERFLOW

OVERFLOW OF NOREPINEPHRINE

CHANGES IN TENSION VS CHANGES IN OVERFLOW

FIGURE 2. Upper: No significant correlation was found between tension development and overflow of endogenous norepinephrine (expressed as $10^{-15}$ mol/mg per min) for the 23 saphenous vein preparations used ($r^2 = 0.01$). Lower: Correlation between changes in tension and changes in norepinephrine overflow (expressed as $10^{-15}$ mol/mg per min) due to different stimuli in the same preparation; the first response was used as control (n = 26; $r^2 = 0.89)$. O = responses to nerve stimulation; • = responses to acetylcholine ($5 \times 10^{-7}$M) during nerve stimulation. Tension: 100% = 2.42 ± 0.39 g. Norepinephrine overflow: 100% = 9.27 ± 0.95 $\times 10^{-15}$ mol/mg per min.

the three catecholamines in the range tested. For norepinephrine, the blank value obtained with Krebs-Ringer was higher than that in distilled water or in plasma, which explains why the sensitivity is slightly less than for the determination of plasma norepinephrine levels (Peuler and Johnson, 1977). The use of a larger volume (100 instead of 50 µl in the original technique) did not affect the sensitivity of the assay.

The values for tissue content of norepinephrine obtained in the present study are about twice as high as those reported for the same preparation by others who used a spectrophotometric technique (Brandao, 1976) and approximate those measured in dog's mesenteric arteries (Genest et al., 1969; Garett and Branco, 1977). Other blood vessels of the dog and those of the guinea pig, the rabbit, and the rat contain less norepinephrine (Genest et al., 1969; Garett and Branco, 1977; Euler and Lishajko, 1958; Shore et al., 1968; Faredin et al., 1961; Berkowitz et al., 1971). Thus, the present study confirms that the saphenous vein of the dog is densely innervated (Osswald, 1978; Shepherd and Vanhoutte, 1975; Vanhoutte, 1978).

The tissue content of dopamine is about 30 times less than that of norepinephrine, which is in line with the ratios reported for the dog heart and the dog spleen (e.g., Anton and Sayre, 1964). In none of the experimental conditions imposed could dopamine be detected in the superfusate.

No tissue epinephrine could be detected with the technique used. In no case did epinephrine appear in the superfusate. Thus, the present experiments indicate that, in the saphenous vein of the dog, no further N-methylation of norepinephrine to epinephrine occurs after it has been formed from dopamine.

Despite the absence of inhibitors of the disposition mechanisms for norepinephrine, small but significant amounts of the transmitter were detected in the superfusate from unstimulated preparations. In veins previously incubated with $^3$H-norepinephrine, most of the basal $^3$H-efflux is due to metabolites, but intact $^3$H-norepinephrine accounts for 5-10% of the total (Vanhoutte, 1978; Vanhoutte et al., 1973; Vanhoutte et al., 1977; Muldoon et al., 1976; Vanhoutte and Verbeuren, 1976; Verbeuren et al., 1978). Of the metabolites, 3,4-dihydroxyphenylglycol (DOPEG) is the largest fraction, which prompted the conclusion that the intact norepinephrine present in the superfusate in basal conditions originates from the storage sites and escapes to the extracellular space without being deaminated by intraneuronal mitochondrial monoamine oxidase.

Despite the absence of inhibitors of the disposition mechanisms for norepinephrine, small but significant amounts of the transmitter were detected in the superfusate from unstimulated preparations. In veins previously incubated with $^3$H-norepinephrine, most of the basal $^3$H-efflux is due to metabolites, but intact $^3$H-norepinephrine accounts for 5-10% of the total (Vanhoutte, 1978; Vanhoutte et al., 1973; Vanhoutte et al., 1977; Muldoon et al., 1976; Vanhoutte and Verbeuren, 1976; Verbeuren et al., 1978). Of the metabolites, 3,4-dihydroxyphenylglycol (DOPEG) is the largest fraction, which prompted the conclusion that the intact norepinephrine present in the superfusate in basal conditions originates from the storage sites and escapes to the extracellular space without being deaminated by intraneuronal mitochondrial monoamine oxidase.

FIGURE 3. Effect of acetylcholine on tension development (upper) and norepinephrine overflow (lower; $10^{-15}$ mol/mg per min) evoked by nerve stimulation in a superfused dog's saphenous vein.

5 mm

ACETYLCHOLINE ($5 \times 10^{-7}$ M)

NERVE STIMULATION (2 Hz)
(e.g., Vanhoutte, 1978). The basal efflux of both endogenous norepinephrine (present study) and 3H-norepinephrine (Vanhoutte et al., 1973; Muldoon et al., 1976; Vanhoutte and Verbeuren 1976; Verbeuren et al., 1978) decreases with time. This could be due to the trauma to the preparation which temporarily augments the spontaneous leakage of transmitter. An alternative explanation is that norepinephrine leaking out of the storage sites is displaced by newly synthesized transmitter (Kopin et al., 1968), and that in our experimental conditions no synthesis of new transmitter is possible.

Electrical stimulation augments the overflow of endogenous norepinephrine. Earlier experiments had shown that the contractile response to electrical impulses as used in the present study is abolished by sympathectomy, reserpine, bretylium tosylate, tetrodotoxin, and α-adrenolytic drugs (Vanhoutte et al., 1967; Vanhoutte et al., 1977; Vanhoutte and Verbeuren, 1976; Vanhoutte, 1970; Vanhoutte and Shepherd, 1973). Electrical stimulation also augments the overflow of 3H-norepinephrine in saphenous veins previously incubated with the labeled transmitter (e.g. Vanhoutte et al., 1973; Vanhoutte et al., 1977; Verbeuren et al., 1978). The present study provides direct evidence that the electrical stimulation causes activation of the adrenergic nerve endings in the vein wall, resulting in release of endogenous norepinephrine. Both the increases in tension and the increases in overflow of the neurotransmitter during nerve stimulation were frequency dependent. These responses are reproducible and can be sustained for 30 minutes with only minimal parallel decreases in both parameters with time. When different veins are compared, there is no direct correlation between the absolute increase in tension obtained with electrical stimulation at a given frequency and the amount of norepinephrine overflooding. This may be due in part to the fact that all strips studied were submitted to an identical load, rather than being placed at the optimal point of their length-tension curve (Vanhoutte and Leu- sen, 1969). However, the changes in contractile responses of the same strips to repeated or sustained stimulation are highly significantly correlated to the amount of adrenergic neurotransmitter escaping into the superfusion solution. This correlation validates the conclusion that the amount of endogenous transmitter detected in the superfusate reflects the amount made available to the effector cells during activation of the sympathetic nerve endings.

If, in the same strips, increasing stimulation frequencies (2, 5, and 10 Hz) are applied, and the response to 5 Hz is expressed as percent of that to 2 Hz, the contractile response augments by about 35%, and the overflow of norepinephrine increases by about 140%; if a similar comparison is made between responses to 5 and 10 Hz, the increases approximate 13 and 16%, respectively. In the per-fused cat spleen, previously incubated with 3H-norepinephrine, the perfusate contains progressively more intact 3H-norepinephrine and progressively less norepinephrine metabolites when the sympathetic nerves are stimulated at increasing frequencies (0.5–5 Hz). In samples collected immediately after the stimulations, the pattern of metabolites is the same, regardless of the stimulation frequency applied. The frequency-dependent dissociation between the amounts of intact 3H-norepinephrine and its metabolites disappears after blockade of neuronal uptake (Dubocovich and Langer, 1976). Earlier work in the dog’s saphenous vein supported the premise that the neuronal uptake pump operates mainly between nerve impulses and is switched off during depolarization of the nerve endings to allow for optimal transmitter diffusion (Vanhoutte, 1978; Verbeuren et al., 1978). If this hypothesis is correct, neuronal uptake should be more effective at 2 Hz than at higher frequencies, and hence, less intact norepinephrine should escape into the superfusate, as seen in the present study.

In the dog’s saphenous veins, as in most blood vessels studied (Vanhoutte, 1977), acetylcholine depresses the response to sympathetic nerve stimulation and decreases the overflow of 3H-norepinephrine during activation of the sympathetic nerves (Vanhoutte et al., 1973; Vanhoutte and Verbeuren, 1976; Vanhoutte, 1970; Vanhoutte, 1977; Vanhoutte, 1974; Van Hee and Vanhoutte, 1978). These earlier studies suggested that acetylcholine has an inhibitory effect on the evoked release of norepinephrine. In the present study, acetylcholine, in a concentration that did not interfere with the radioenzymatic assay, and that did not affect the basal efflux of endogenous norepinephrine, caused a marked and reversible inhibition of the release of the latter during sympathetic nerve stimulation.

Thus, the present experiments not only demonstrate the possibility of detecting the overflow of endogenous norepinephrine in blood vessels where the disposition mechanisms for the adrenergic transmitter are operative, but also validate earlier conclusions concerning the effect of acetylcholine. They provide direct evidence that, when the cholinergic transmitter reaches adrennergic nerve endings while they are activated, it causes an abrupt reduction in the release of norepinephrine. This prejunc- tional effect of acetylcholine greatly reinforces the direct inhibitory effect it has on certain vascular smooth muscle cells (Vanhoutte, 1977).

Addendum

After the submission of the present manuscript, a paper appeared by S.M. Muldoon, G.M. Tyce, T.P. Moyer, and D.K. Rorie (Measurement of endogenous norepinephrine overflow from canine saphenous veins. Am. J. Physiol 23B: H263–H267, 1979) which describes in the canine saphenous vein that electrical stimulation
causes the overflow of endogenous norepinephrine measured by high pressure liquid chromatography.

Acknowledgments

We wish to thank P. Bossuyt, F. Jordaens, L. Zonnekeyn, and Y. Onsea for their valuable technical assistance and L. Van den Eynde for typing the manuscript.

References


Garett J, Branco D (1977) Uptake and metabolism of noradrenaline by the mesenteric arteries of the dog. Blood Vessels 14: 43-54


Vanhoutte PM, Lorenz RR, Tyce GM (1973) Inhibition of norepinephrine-H-release from sympathetic nerve endings in veins by acetylcholine. J Pharmacol Exp Ther 185: 386-394


Evoked release of endogenous norepinephrine in the canine saphenous vein. Inhibition by acetylcholine.

P M Vanhoutte, E P Coen, W J De Ridder and T J Verbeuren

Circ Res. 1979;45:608-614
doi: 10.1161/01.RES.45.5.608

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/45/5/608.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/