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Prejunctional and Postjunctional Actions of Endogenous Norepinephrine at the Sympathetic Neuroeffector Junction in Canine Coronary Arteries

Richard A. Cohen, John T. Shepherd, and Paul M. Vanhoutte

From the Department of Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

SUMMARY. The effects of endogenous and of exogenous norepinephrine were studied in isolated rings of canine left circumflex coronary artery and its first ventricular branch. Norepinephrine was released from adrenergic nerve endings by transmural electrical stimulation and by tyramine. In rings contracted with prostaglandin F$_2$α, transmural electrical stimulation resulted in frequency-dependent relaxations which were blocked by propranolol or tetrodotoxin; tyramine and exogenous norepinephrine caused concentration-dependent relaxations which were blocked by propranolol. The tyramine-induced relaxations also were inhibited by cocaine. The left circumflex artery was less sensitive than its branch to β-adrenergic activation; this difference was significant even between rings of the two vessels immediately adjacent to the branching point and was abolished by phentolamine. In the presence of propranolol, transmural electrical stimulation, tyramine and phenylephrine, produced contractions of the left circumflex artery, but not the branch; these contractions were prevented by phentolamine. Phentolamine, but not prazosin, augmented the β-adrenergic response of left circumflex artery to low frequency stimulation; in arteries preincubated with $^3$H-norepinephrine, this was accompanied by an increased overflow of tritiated neurotransmitter. The prejunctional effect of phentolamine was also evident in branch coronary arteries which exhibit no postjunctional α-adrenergic responses. With high frequency stimulation, both α-adrenergic antagonists equally augmented the relaxation of left circumflex artery; the efflux of tritiated norepinephrine was not different from untreated arteries. These experiments demonstrate, in isolated coronary arteries, that the primary adrenergic response to released endogenous norepinephrine is β-adrenergic relaxation. The prejunctional effects of nonspecific α-adrenergic antagonists preclude their use in determining the importance of postjunctional coronary α-adrenergic receptor activation caused by sympathetic nerve stimulation. (Circ Res 52: 16–25, 1983)
tolamine, a greater release of norepinephrine during electrical stimulation could account entirely for the observed augmented β-adrenergic relaxation.

**Methods**

The heart was removed from mongrel dogs (15-25 kg) following anesthesia with sodium pentobarbital (30 mg/kg, iv), anticoagulation with sodium heparin (150 units/kg, iv), and exsanguination. The left circumflex coronary artery from its origin to its first major ventricular branch (approximately 3 cm long and 2.0 mm outside diameter) and the entire epicardial portion of the branch artery (approximately 2.5 cm long and 1 mm outside diameter) were dissected free.

**Organ Bath Experiments**

Rings of vessel, 4 mm long, were placed in organ chambers (25 ml) filled with physiological salt solution of the following millimolar composition: NaCl, 118.3; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25.0; calcium EDTA, 0.026; and glucose, 11.1. The solution was maintained at 37°C and gassed with 95% O2:5% CO2. The rings were connected to a strain gauge (Gould model UC2) for measurement of isometric force.

Before the actual experiments, the rings were placed at the optimal point of their length-tension relationship (Vanhoutte and Leusen, 1969) by repeated 3-minute exposures to 20 mM potassium chloride. Basal tension in the rings was increased gradually over a 90-minute period until contractions were maximal. The optimal basal tension was 14 ± 0.6 g for left circumflex rings and 13 ± 0.4 g for the branch rings (n = 18). This tension was maintained throughout the experiment.

**Drugs**

The following pharmacological agents were used: angiotensin II (Valine®, Ciba-Geigy); cocaine hydrochloride (generic); indomethacin (Sigma); Levo-[7-3H(N)]-norepinephrine, specific activity: 2.7 Ci/mmol (New England Nuclear); l-norepinephrine bitartrate (Sigma); propranolol mesylate (Ciba-Geigy); prazosin hydrochloride (Pfizer); prostaglandin F2α, tetrodotoxin; tyramine hydrochloride (Sigma).

Drugs were dissolved in distilled water such that volumes of less than 0.5 ml were added to the organ chambers. Concentrations of drugs are expressed as final molar bath concentrations (M). Indomethacin was dissolved in 50% ethanol; final bath concentration of ethanol was 4 × 10^-5 M. We added blocking agents to the bath for at least 30 minutes before determining their effect on the response to agonists or electrical stimulation. The response of coronary rings in the presence of antagonists was always compared to that of a ring from the same vessel in which a response in the absence of antagonist was elicited simultaneously.

In order to study relaxation of coronary arteries, rings were contracted with prostaglandin F2α (2 × 10^-6 M). Constrictions reached a maximum in 6-8 minutes, and in time control experiments, the plateau was found to be linear for 40 minutes. Inhibitory responses to electrical stimulation or drugs were elicited after the plateau was reached and were expressed as a percentage of the prostaglandin F2α-induced tension above baseline. In preliminary studies, it was found that successive responses to electrical stimulation and tyramine were diminished and that this could be prevented by indomethacin (Table 1); all subsequent studies were performed in the presence of indomethacin (3 × 10^-5 M).

IC50 and I250 were defined as the concentrations of agonist causing 30 and 50% inhibition of contraction, re-

### TABLE 1

<table>
<thead>
<tr>
<th>Tyramine (10^-5 M)</th>
<th>Incubation time (hrs)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>2.5</th>
<th>3.5</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indomethacin</td>
<td>55 ± 11</td>
<td>12 ± 6.1</td>
<td>9.3 ± 3.8</td>
<td>34 ± 4.2</td>
<td>53 ± 6.9</td>
<td>0.1 ± 1.85</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>43 ± 11</td>
<td>30 ± 6.5</td>
<td>23 ± 3.4</td>
<td>20 ± 4.8</td>
<td>20 ± 5.5</td>
<td>15 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>Cocaine</td>
<td>30 ± 13</td>
<td>26 ± 13</td>
<td>22 ± 5.0</td>
<td>35 ± 5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Electrical stimulation (16 Hz)</strong></td>
<td>Control</td>
<td>55 ± 6.2</td>
<td>46 ± 8.9</td>
<td>37 ± 5.2</td>
<td>40 ± 8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indomethacin throughout</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Control rings were incubated in the presence of ethanol (4 × 10^-5 M) which was present as the indomethacin solvent. In control rings, the response to tyramine was significantly less at 1 and 2 hours. When indomethacin was present throughout, there was no significant difference in the relaxation produced by tyramine or electrical stimulation in the first 2 hours. Sequential additions were made to only control rings following the first 2 hours, while responses to tyramine or electrical stimulation were elicited at the same time for comparison in rings treated with indomethacin (3 × 10^-5 M) throughout. Electrical stimulation parameters were 0.2 msec, 10 V, 16 Hz.

† Incubation for 30 minutes in indomethacin (3 × 10^-5 M) significantly increased the response to tyramine and electrical stimulation.

‡ Incubation for 1 hour with norepinephrine (2 × 10^-5 M) restored the response to tyramine to a value not significantly different from the initial relaxation.

§ Cocaine (3 × 10^-5 M) blocked the response to tyramine.

Values are means ± SEM, n = 6.
Electrical Stimulation

Adrenergic nerve endings of left circumflex and branch coronary rings were electrically stimulated with parallel rectangular platinum electrodes (1 cm², 7-mm spacing), a Grass stimulator (SD9), and a d.c. current amplifier. Since supramaximal transmural stimulation of coronary artery rings results in non-neurogenic inhibitory responses (Brine et al., 1979; Toda, 1981; Rooke et al., 1982), the current threshold for the non-neurogenic response was determined for each experiment. Rings were contracted with prostaglandin 
F₂α (2 × 10⁻⁶ M) in the presence of tetrodotoxin (10⁻⁷ M) and the maximal pulse duration at 10 V and 16 Hz which caused no relaxation was determined (pulse duration: 0.27 ± 0.02 msec, mean current 450 ± 60 mA). Following washout of tetrodotoxin, responses to these stimuli then were elicited under basal conditions or after repeated contraction with prostaglandin 
F₂α (2 × 10⁻⁶ M); relaxations were expressed as a percentage of prostaglandin 
F₂α-induced tension above baseline. Frequency-response curves were obtained by allowing responses to reach maximum before increasing the frequency. The responses in the presence of α-adrenergic antagonists were obtained simultaneously with control responses and were expressed as a percentage of a 16 Hz initial response elicited just prior to a 30-minute incubation with the antagonist or control solution. Neither antagonist, in the concentrations used, had any effect on the prostaglandin 
F₂α-induced contraction.

³H-Norepinephrine Displacement by Tyramine

The left circumflex artery was cut into 1-mm-wide longitudinal strips of 10–20 mg each and incubated in \( ^3 \)H-norepinephrine (10⁻⁶ M) in physiological salt solution maintained at 37° and gassed with 95% O₂-5% CO₂ for 120 minutes. The strips were then washed for 2 hours by serial passage in 6-ml aliquots of physiological salt solution. At the end of the washing period, efflux of tritium was at a constant rate. Strips were subsequently incubated in 2-ml aliquots of physiological salt solution and serially exposed for 10-minute periods each to physiological salt solution, prostaglandin 
F₂α (2 × 10⁻⁶ M) and prostaglandin 
F₂α with tyramine (3 × 10⁻⁵ M) in the presence or absence of propranolol (10⁻⁷ M).

³H-Norepinephrine Release by Electrical Stimulation

Longitudinal strips of left circumflex coronary artery (4–6 cm long, 2–3 mm wide, 47 ± 2.9 mg) were incubated 120 minutes in \( ^3 \)H-norepinephrine (10⁻⁶ M). After incubation, the tissues were rinsed in fresh physiological salt solution and mounted for superfusion (Vanhoutte et al., 1973). The strips were suspended at 4-g tension and were superfused by means of a roller pump at 3 ml/min with oxygenated physiological salt solution at 37°C. For electrical stimulation, two platinum wires (0.5 mm in diameter, 10 cm long) were placed parallel to and in contact with the strips. Except where otherwise noted, electrical impulses were 10 V, 0.2 msec duration.

After an initial washout period of 120 minutes, the superfusate was collected for 2-minute intervals (by means of a fraction collector) for estimation of the efflux of total radioactivity. Strips were electrically stimulated for 4 minutes at 2 Hz or 1 minute at 16 Hz, followed by a 36-minute washout period, during which tritium efflux reached basal levels.
but the left circumflex rings were significantly less sensitive compared to the branch rings (IC\textsubscript{30} = 5.9 × 10\textsuperscript{-8} M and 2.1 × 10\textsuperscript{-6} M, respectively). This change occurred abruptly at the site of branching. The left circumflex ring taken from just proximal to the arterial branching point was significantly less sensitive to tyramine than the branch ring taken from just distal to the branching point, even though the rings were taken from within 2 mm of the branching point (IC\textsubscript{50} left circumflex, 5.4 × 10\textsuperscript{-6} M; branch, 2.1 × 10\textsuperscript{-6} M). In the presence of phenolamine (5 × 10\textsuperscript{-6} M), there was no significant difference between the IC\textsubscript{50} of tyramine for the two vessels (left circumflex, 4.0 × 10\textsuperscript{-6} M; branch, 3.4 × 10\textsuperscript{-6} M). There was no significant difference between the IC\textsubscript{50} of tyramine for the left circumflex rings in the presence—and that for the branch rings, in the absence—of phenolamine.

Coronary rings contracted with prostaglandin F\textsubscript{2α} relaxed in response to exogenous norepinephrine (Fig. 2). There were no significant differences in the IC\textsubscript{50} among the different sites along either the left circumflex or branch vessels, but the left circumflex rings were significantly less sensitive to norepinephrine than the branch rings (IC\textsubscript{50} = 9.8 × 10\textsuperscript{-8} M and 4.4 × 10\textsuperscript{-8} M, respectively). This change occurred at the site of branching (IC\textsubscript{50} = 9.3 × 10\textsuperscript{-8} M and 4.2 × 10\textsuperscript{-8} M just proximal and distal to the branching point, respectively). The difference in sensitivity to norepinephrine between the left circumflex rings and branch rings was abolished by phentolamine, 5 × 10\textsuperscript{-6} M (IC\textsubscript{50} = 5.5 × 10\textsuperscript{-6} M and 5.2 × 10\textsuperscript{-8} M, respectively). There was no significant difference between the IC\textsubscript{50} of norepinephrine in the left circumflex rings in the presence—and that for the branch rings, in the absence—of phentolamine. From 3 × 10\textsuperscript{-8} M to 3 × 10\textsuperscript{-5} M, norepinephrine caused contractions in left circumflex rings (Fig. 2) which were abolished by phentolamine.

Effect of Propranolol and Cocaine on Responses to Electrical Stimulation, Tyramine and Norepinephrine

In the presence of propranolol (10\textsuperscript{-7} M), the relaxation due to 16 Hz electrical stimulation was significantly reduced to 4.3 ± 3.9% in left circumflex rings and to 21 ± 7.6% in branch rings. Tyramine (10\textsuperscript{-7} to 3 × 10\textsuperscript{-6} M) had no significant inhibitory effect on left circumflex or branch rings treated with cocaine (3 × 10\textsuperscript{-5} M) or propranolol (10\textsuperscript{-7} M) in the presence of phenolamine (5 × 10\textsuperscript{-6} M).

In the presence of phenolamine (5 × 10\textsuperscript{-6} M), propranolol (10\textsuperscript{-7} M) resulted in a significant parallel shift to the right in the norepinephrine concentration response curves in both left circumflex and branch artery rings (IC\textsubscript{50} left circumflex, 3.0 × 10\textsuperscript{-7} M; branch 3.0 × 10\textsuperscript{-7} M).

The effect of propranolol (10\textsuperscript{-7} M) on the displacement of norepinephrine by tyramine was determined after incubation with \textsuperscript{3}H-norepinephrine. In the presence of phenolamine (5 × 10\textsuperscript{-6} M), the basal efflux of tritium was unaffected by propranolol or prostaglandin F\textsubscript{2α}. Tyramine caused a significant increase in tritium efflux, which was not significantly affected by the β-adrenergic blocker (Table 2).

Contractions of Coronary Rings Caused by Electrical Stimulation, Tyramine, Phenylephrine, and Angiotensin II

Under basal conditions, electrical stimulation had no significant effect in either left circumflex or branch rings. In the presence of propranolol (10\textsuperscript{-7} M), electrical stimulation caused frequency-dependent contractions in left circumflex rings (Fig. 1) but not in the branch.

In the presence of propranolol (10\textsuperscript{-7} M) under basal conditions, tyramine caused concentration-dependent contractions in rings of both left circumflex and branch arteries (Fig. 3). When expressed as a percentage of the response to prostaglandin F\textsubscript{2α}, the contraction caused by tyramine (10\textsuperscript{-4} M) was significantly greater in left circumflex rings than in branch rings (68 ± 9.3 and 38 ± 7.6%, respectively). Cocaine caused a significant attenuation of the contractile response in the left circumflex but not in the branch.
vessel. Phentolamine (5 × 10⁻⁶ M) abolished the contractile response to tyramine in the left circumflex artery in the presence or absence of cocaine, but had no significant effect on that in the branch.

In the presence of propranolol (10⁻⁷ M) and cocaine (3 × 10⁻⁵ M), phenylephrine caused concentration-dependent contractions of the left circumflex artery reaching a maximal contraction of 9.9 ± 1.6 g. Phentolamine (10⁻⁶ M) and prazosin (5 × 10⁻⁸ M) caused a parallel shift to the right of the concentration-response curve to phenylephrine (ED₅₀: control, 1.2 × 10⁻⁶ M; phenolamine, 1.6 × 10⁻⁶ M; prazosin, 1.1 × 10⁻⁶ M). The response to phenylephrine was not significantly different in the presence of these concentrations of the two α-adrenergic antagonists. Branch rings did not respond significantly to phenylephrine (10⁻⁶ to 10⁻⁴ M).

Angiotensin II caused concentration-dependent contractions of coronary rings which were maximal at 3 × 10⁻⁷ M and averaged 3.6 ± 0.4 and 2.8 ± 0.4 g, in left circumflex and branch arteries, respectively. This represented 33 ± 10 and 32 ± 5% of the maximal contraction of each ring to potassium chloride. The sensitivity to angiotensin II was similar irrespective of the anatomical location of the rings along the left circumflex and branch artery (ED₅₀: left circumflex

<table>
<thead>
<tr>
<th>Percent fractional release*</th>
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<tbody>
<tr>
<td>Physiological salt solution</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Propranolol (10⁻⁷ M)</td>
</tr>
</tbody>
</table>

* Results are given as percent fractional release of tritium during 10-minute incubations. All tubes contained phentolamine (5 × 10⁻⁶ M). Values are means ± SEM of six determinations. Prostaglandin F₂₀⁺ did not significantly affect basal release rate. Tyramine caused a significant increase in release rate which was not significantly affected by propranolol.
ring #1, 6.3 × 10^-9 M; ring #3, 8.1 ± 10^-9 M; branch ring #4, 7.2 ± 10^-9 M; ring #6, 6.6 × 10^-9 M; n = 5; compare to Fig. 2).

Effect of α-Adrenergic Antagonists on Response to Electrical Stimulation

After a 30-minute incubation in control solution, the maximum relaxation of left circumflex rings at 16 Hz was not significantly different from the initial response. In rings incubated for 30 minutes with prazosin (5 × 10^-8 M), the relaxation response was comparable to control from 0.5 to 2 Hz, but significantly greater between 4 and 16 Hz. Relaxation responses in rings preincubated with phentolamine (10^-6 M) were significantly greater than control responses throughout the frequency range. The relaxation in the phentolamine-treated rings exceeded that in the prazosin-treated rings from 0.5 to 8 Hz, but was not significantly different at 16 Hz (Fig. 4). After 30 minutes of incubation in phentolamine, the relaxations of branch coronary arteries exceeded those of control rings from 1 to 4 Hz, and those of prazosin-treated rings at 1 Hz. There were no significant differences between the relaxation response of prazosin treated and control branch coronary rings (Table 3).

When left circumflex coronary strips preincubated with [3H]-norepinephrine were exposed to phentolamine (10^-6 M) and electrically stimulated at 2 Hz, the fractional release of tritiated compounds (Fig. 5) and the [3H]-norepinephrine efflux (Fig. 6) were significantly greater than that in control or prazosin (5 × 10^-8 M) -treated arteries. There was no significant difference in fractional release of tritiated compounds or [3H]-norepinephrine efflux between control and prazosin-treated strips at 2 Hz. The fractional release of tritiated compounds and the efflux of [3H]-norepinephrine evoked by 16 Hz stimulation was not significantly different between phentolamine-treated and control strips (Fig. 7). Increasing the stimulation pulse duration from 0.2 to 2 msec greatly augmented the fractional release and efflux of [3H]-norepinephrine.

Discussion

The optimal basal tension determined in this study is higher than the tensions previously used in studies of coronary vascular smooth muscle (Zuberbuhler and Bohr, 1965; Borda et al., 1977; Toda et al., 1981; Van Neuten et al., 1980; Brazenor and Angus, 1981). From the Laplace relationship, tension (dynes per cm) equals the product of pressure (dynes per cm²) and radius (cm). For a coronary artery with a radius of 0.1 cm and a distending pressure of 100 mmHg (1.3 × 10⁵ dynes per cm²), circumferential wall tension would approximate 1.3 × 10⁵ dynes per cm or 13 g (Burton, 1965). Thus, the optimal tension determined in vitro is in the range of physiological wall tension. Affinity of adrenergic receptors in arterial smooth muscle is influenced by alterations in wall tension (Raffa and Tallarida, 1981; Price et al., 1981).

Transmural electrical stimulation was employed to release norepinephrine from adrenergic nerve en-
TABLE 3
Effect of Prazosin and Phentolamine on Response of Branch Canine Coronary Artery to Electrical Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Prazosin (5 × 10⁻⁸ M)</th>
<th>Phentolamine (10⁻⁶ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>7.4 ± 2.2</td>
<td>6.2 ± 2.2</td>
<td>12 ± 2.7</td>
</tr>
<tr>
<td>1</td>
<td>13 ± 2.6</td>
<td>18 ± 4.0</td>
<td>33 ± 4.7*</td>
</tr>
<tr>
<td>2</td>
<td>31 ± 4.8</td>
<td>43 ± 5.7</td>
<td>46 ± 6.9†</td>
</tr>
<tr>
<td>4</td>
<td>51 ± 6.9</td>
<td>63 ± 7.1</td>
<td>78 ± 8.8†</td>
</tr>
<tr>
<td>8</td>
<td>74 ± 6.6</td>
<td>90 ± 4.4</td>
<td>98 ± 9.8</td>
</tr>
<tr>
<td>16</td>
<td>102 ± 4.5</td>
<td>110 ± 3.0</td>
<td>108 ± 8.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 9. Results are expressed as percentage of initial response to 16 Hz elicited just prior to 30-minute incubation of three rings with prazosin, phentolamine, or control solution. The electrical stimuli were delivered equally and simultaneously to the three vessels. Stimulation parameters were the same as those in Figure 4.

* Relaxation responses in the phentolamine-treated rings were significantly greater than in control or prazosin-treated rings.

† Relaxation responses in the phentolamine-treated rings were significantly greater than in control rings. No significant differences occurred between responses of control and prazosin-treated branch coronary arteries.

In order to compare the responses in left circumflex and branch coronary arteries to endogenous nor epinephrine with those to exogenous norepinephrine, the sympathomimetic amine, tyramine, was used. The prejunctional action of adrenergic blocking drugs used to characterize these responses do not affect pharmacological displacement of norepinephrine by tyramine, but would hinder interpretation of the observed differences in sensitivity to transmural electrical stimulation (Lorenz et al., 1979; Langer, 1981; Vanhoutte et al., 1981). Since blockade of neuronal uptake prevents the indirect sympathomimetic effect of tyramine, cocaine was used to differentiate responses to tyramine which were due to the release of endogenous norepinephrine from those due to its direct effects on the arterial smooth muscle cells (Trendelenberg, 1978; Vanhoutte et al., 1981). Propranolol was employed to establish the β-adrenergic action of norepinephrine released by tyramine. Although β-adrenergic blockers are known to inhibit neuronal uptake mechanisms and thereby decrease displacement of norepinephrine by tyramine (Foo et
The popliteal artery, which is devoid of medial inner-contiguous branches of the limb arteries of the rabbit.

Neurogenic responses. A similar difference in sensitivity occurred abruptly at the branching point of the coronary artery. Smaller branch coronary arteries are less densely innervated than is the proximal left circumflex artery (Denn and Stone, 1976). However, variations in the density of innervation are not likely to explain the difference in sensitivity observed in the present study, since phentolamine abolished the difference without affecting the response of the branch artery. These observations suggest that both endogenous and exogenous norepinephrine have an α-adrenergic vasocostructor action, which attenuates their inhibitory effect in the left circumflex, but not in the branch segment. The absence of response of the branch segment to phenylephrine suggests that it is devoid of functioning post-junctional α-adrenergic receptors. The abrupt decrement in sensitivity observed at the branching point is probably specific for activation of α-adrenergic receptors, since it is not seen with angiotensin II or prostaglandin F₂α. Furthermore, the similarity in β-adrenergic responsiveness in the presence of phentolamine suggests the parity of the β-adrenergic receptor sensitivity in the left circumflex artery and its branch.

In the presence of β-adrenergic blockade, electrical stimulation or tyramine cause contraction of the left circumflex vessel under basal conditions. The antagonism by cocaine of the tyramine-induced response, and the blockade by phentolamine of the contractions due to electrical stimulation and tyramine, indicate that they result from α-adrenergic activation by endogenous norepinephrine. The lack of response to electrical stimulation and tyramine in the absence of propranolol suggests that the primary action of endogenous norepinephrine in the left circumflex artery is β-adrenergic inhibition of contraction which is secondarily opposed by an α-adrenergic component. Thus, although the level of vessel tone may affect the relative size of α- and β-adrenergic responses (Bevan, 1979), the observations under basal conditions taken in conjunction with the experiments in rings contracted with prostaglandin F₂α demonstrate that the β-adrenergic postjunctional action of norepinephrine released from sympathetic nerves predominates in the canine left circumflex coronary artery. This is unlike most other blood vessels, with the exception of the facial vein (Pegram et al., 1976), where vascular β-adrenergic receptors are not functionally innervated, although they can be humorally activated (Russell and Moran, 1980; Cohen and Coffman, 1981; Vanhoute et al., 1981).

Branch vessels treated with propranolol did not contract when electrically stimulated. Since contractions of the branch vessel occurred only at higher concentrations of tyramine and were not antagonized by cocaine or phentolamine, they probably are due to a direct nonadrenergic action of tyramine. In the left circumflex artery, the contraction caused by tyramine in the presence of cocaine was abolished by phentolamine, indicating that it may be due to a direct α-adrenergic contractile action of tyramine. These experiments further differentiate the action of neuronally released norepinephrine in the two coronary seg-

**Figure 7.** Effect of phentolamine on fractional release of tritiated compounds evoked by 16 Hz stimulation in canine left circumflex coronary artery. Stimulation periods were 1 minute long; pulses were 10 V, 16 Hz, mean current 240 ± 20 mA; 0.2 msec duration for the first and second, but 1 msec duration for the third period. In control strips, fractional release and evoked efflux of norepinephrine during the second stimulation period was 4746 ± 990 dpm and increased significantly to 22031 ± 2820 dpm with the third stimulation. In strips treated for 30 minutes prior to the second stimulation period with phentolamine (10⁻⁶ M), fractional release and efflux of norepinephrine were not significantly different from control strips (second stimulation 8416 ± 1929 dpm, third stimulation 30068 ± 3441 dpm).

Propranolol, in the concentration used, did not significantly affect tyramine stimulated efflux of tritium in coronary arteries preincubated with ³H-norepinephrine.

The relaxation responses to electrical stimulation and tyramine of coronary vessels contracted with prostaglandin F₂α diminished with time. Restoration of the diminished response following addition of indomethacin and incubation with norepinephrine suggests that indomethacin may prevent depletion of norepinephrine from nerve endings, thereby preserving a response dependent upon norepinephrine release. Coronary arteries actively synthesize prostaglandins (Gerritsen and Printz, 1981) which can affect coronary artery tone and adrenergic neurotransmission (Hedqvist, 1974; Sjärne, 1975; Kalsner, 1975). It is possible that inhibition of prostaglandin synthesis by indomethacin accounts for its protective action on neurogenic responses.

**Postjunctional Actions of Endogenous and Exogenous Norepinephrine.**

A major finding in this study is the difference in sensitivity to both tyramine and norepinephrine which occurs abruptly at the branching point of the left circumflex artery. A similar difference in sensitivity to exogenous norepinephrine exists between two contiguous branches of the limb arteries of the rabbit. The popliteal artery, which is devoid of medial innervation, is more sensitive to norepinephrine than the adjacent saphenous artery which has a dense medial innervation. It has been postulated that—in the latter—greater neuronal uptake is responsible for a decrease in the effective concentration of norepinephrine (Bevan and Purdy, 1973). A similar difference in innervation might also explain the change in sensitiv-
ments by confirming the absence of $\alpha$-adrenergic receptor-mediated contraction in the branch vessel.

**Prejunctional Actions of Endogenous Norepinephrine**

This study demonstrates that the $\beta$-adrenergic relaxation of canine coronary artery caused by low frequency electrical stimulation is augmented by phenolamine, but not by prazosin. Phenolamine is a relatively nonselective $\alpha$-adrenergic antagonist which—in addition to its postjuncttional $\alpha$-adrenergolytic effect—increases the efflux of norepinephrine from sympathetic nerve endings by blocking prejuncttional $\alpha$-adrenergic receptors, while, by contrast, prazosin has little prejuncttional blocking effect (Davey, 1980; Langer, 1981). The experiments with phenylephrine demonstrated that the two antagonists in the concentrations used have an equal postjunctional blocking potency. Thus, the greater relaxation during low frequency stimulation seen in phenolamine-treated as compared to prazosin-treated left circumflex and branch coronary rings suggests a prejunctional effect in those treated with phenolamine. Direct evidence in support of this interpretation comes from the demonstration that phenolamine, but not prazosin, augments the fractional release of tritiated compounds and the evoked norepinephrine efflux during 2 Hz stimulation of left circumflex arterial strips.

Phentolamine did not augment the norepinephrine release caused by 16 Hz stimulation, confirming the finding that the effect of prejuncttional $\alpha$-adrenergic inhibition is small at high frequencies (Stjärne, 1975; Starke and Docherty, 1980). The failure to increase the efflux of norepinephrine at 16 Hz is not due to the inability for further norepinephrine release, as demonstrated by the experiments where the pulse duration was lengthened. Thus, the greater relaxation seen in left circumflex coronary rings treated with phenolamine, must be due to blockade of the postjunctional $\alpha$-adrenergic activation caused by the large amounts of norepinephrine released at 16 Hz. This interpretation is supported by the comparable relaxation with phenolamine and the selective postjuncttional $\alpha$-adrenergic blocker prazosin, and by the absence of augmentation by either prazosin or phenolamine of the $\beta$-adrenergic response of the branch coronary vessels at the higher frequencies.

It is evident from these studies that norepinephrine released from coronary sympathetic nerves inhibits its own further release, presumably by activating prejunctional $\alpha$-adrenergic receptors. The relative importance of the pre- and postjunctional $\alpha$-adrenergic limitation of coronary sympathetic $\beta$-adrenergic vasodilation should depend upon the frequency of nerve discharges and whether large or small vessels are involved. This is particularly relevant in view of the in vivo studies which have been interpreted as demonstrating a predominant $\alpha$-adrenergic postjunctional constrictor effect of coronary sympathetic nerves, based on decreased coronary resistance following $\alpha$-adrenergic blockade with phenolamine or phenoxy-

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References


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