Intense Sympathetic Stimulation Releases Neuropeptide Y but Fails to Evoke Sustained Coronary Vasoconstriction in Dogs

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We determined whether a 3-minute period of intense cardiac sympathetic stimulation, which is known to release neuropeptide Y (NPY), elicits a sustained postsynaptic coronary vasoconstriction in anesthetized dogs that had received propranolol. We also periodically measured the cardiac chronotropic responses to test vagal stimuli; these responses served as an index of the neuronal release of NPY. In a group of 11 animals, the coronary vascular resistance increased by 14±4% during the sympathetic stimulation. After cessation of stimulation, however, coronary vascular resistance returned rapidly to its control value. The cardiac responses to the test vagal stimuli were attenuated by approximately 40% after cessation of sympathetic stimulation, and this inhibitory effect persisted for approximately 60 minutes. In a second group of eight dogs, we determined whether the intense sympathetic stimulation potentiates the coronary vascular responses to exogenous norepinephrine (NE). Before sympathetic stimulation, standard intracoronary infusions of NE increased coronary vascular resistance by 14±2%. Intense antecedent sympathetic stimulation did not alter the coronary vascular responses to subsequent NE infusions. However, the chronotropic responses to test vagal stimuli were initially attenuated by approximately 30%, and this inhibitory effect persisted for approximately 1 hour. In a third group of four dogs, we found that exogenous NPY significantly potentiated the coronary vasoconstriction evoked by NE infusions. The coronary vascular responses to combined infusions of NE and NPY were consistently greater (by approximately 13%) than the sum of the responses to these substances when they were infused separately. We conclude that, even though sufficient NPY appears to be released from the sympathetic nerve endings to inhibit vagal neurotransmission, the quantity of NPY released into the coronary blood vessels under the conditions of our experiments appears to be insufficient either to elicit a sustained coronary vasoconstriction or to potentiate the vasoconstrictor effects of intracoronary NE infusions. (Circulation Research 1993;72:816–826)

KEY WORDS • autonomic nerves • coronary blood vessels • heart rate • neuropeptide Y • norepinephrine

Neuropeptide Y (NPY), which coexists with norepinephrine (NE) in sympathetic nerve terminals,1 is itself a potent vasoconstrictor,2 and it also potentiates α-adrenoceptor-mediated vasoconstriction.3,4 Systemic administration of NPY raises the arterial blood pressure by increasing total peripheral resistance.5 The vasoconstrictor actions of NPY are resistant to α-adrenergic receptor blockade, and they are evident even in sympathectomized animals. These findings suggest that NPY has a direct effect on vascular smooth muscle.

The coronary vasculature is particularly sensitive to the vasoconstrictor effects of NPY. Infusion of NPY into the coronary vessels in animals causes prolonged vasoconstriction of the small resistance arteries.6–9 Intracoronary infusion of NPY in dogs elicits a pronounced coronary vasoconstriction that persists for 40–60 minutes.8,9 Similarly, NPY produces sustained constriction in isolated human coronary arteries.10 When infused into human coronary arteries in vivo, NPY decreases the coronary blood flow and transiently alters the electrocardiogram.11 The ability of NPY to constrict coronary vessels has led to the hypothesis that NPY mediates coronary spasm.5

Exogenous NPY was used in all the studies described above. The coronary vascular effects of NPY released from sympathetic nerve endings appear not to have been evaluated in vivo. The present study was therefore designed to determine the physiological role of neurally released NPY on coronary vascular resistance in vivo. The principal aims of our study were to determine whether a 3-minute period of intense cardiac sympathetic stimulation will evoke a sustained coronary vasoconstriction and will potentiate the coronary vascular responses to exogenous NE. Such effects would presumably be mediated by the release of NPY from the sympathetic nerve endings in the coronary vasculature.

Materials and Methods

Preparation

Experiments were conducted on 23 mongrel dogs that weighed between 18 and 27 kg. The animals were

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premedicated with morphine sulfate (2 mg/kg) and anesthetized with α-chloralose (75 mg/kg). Anesthesia was maintained by the intravenous infusion of α-chloralose (5 mg/kg per hour). The trachea was intubated, and intermittent positive pressure ventilation was begun. The inspired air was supplemented with oxygen. Arterial blood gas tensions were maintained within the physiological range by adjusting the ventilation volume or rate. Metabolic acidosis was corrected by the intravenous infusion of a sodium bicarbonate solution. Body temperature was maintained at 37°C with a heat lamp.

A femoral vein was cannulated for the administration of drugs and the maintenance of fluid balance. A femoral artery was cannulated for measuring aortic pressure. The cervical vagi were isolated, doubly ligated, and sectioned to interrupt parasympathetic outflow to the heart. Bipolar hook electrodes were inserted into the cardiac end of the right vagus nerve, and the wires were connected to a stimulator (model SD9, Grass Instrument Co., Quincy, Mass.).

The chest was opened transversely at the fourth intercostal space. The right and left stellate ganglia were isolated, doubly ligated, and sectioned to eliminate sympathetic outflow to the heart. The ansae subclaviae were placed over bipolar shielded iridium electrodes (Harvard Apparatus, South Natick, Mass.), and the electrode wires were connected in parallel to an electronic stimulator (model S-4, Grass Instrument). The pericardium was opened and sutured to the chest wall to form a cradle. A bipolar electrode catheter was inserted into the atrial appendage to record an atrial electrogram. The AA interval (cardiac cycle length) was determined from the atrial electrogram by an analog computer (model 580, Electronic Instruments Inc., West Long Beach, N.J.). A micromanometer (model spe-350, Millar, Houston, Tex.) was placed in the left ventricle through the apex for measurement of the left ventricular pressure and its first derivative (dP/dt).

The proximal region of the left circumflex coronary artery was dissected free from the surrounding tissue for a distance of approximately 1.5 cm. The coronary perfusion system (Figure 1) was primed with approximately 60 ml saline solution that contained heparin (10 units/ml). The dogs were anticoagulated by an initial dose of heparin (1,000 units/kg) and by a supplemental infusion of 10,000 units per hour via an intravenous drip. A wide-bore tube was connected to a cannula in the left femoral artery, which was the source of blood for perfusion of the left circumflex coronary artery. The perfusion pump was actuated for several minutes to prime the perfusion apparatus with blood. Then the left circumflex coronary artery was ligated, rapidly cannulated, and perfused at a constant rate by a roller pump (Masterflex 7520, Colporteur, Chicago). An extracorporeal electromagnetic flowmeter (model BL-615, Bio-tronix Laboratory, Silver Spring, Md.) was included in the perfusion tubing to measure coronary blood flow. The perfusion pressure was measured through a side arm of the cannula (Statham I, Gould, Cleveland, Ohio). The pressure drop from the side arm to the cannula tip was measured in vitro at various flows to allow estimation of the actual intra-arterial pressure during the experiments. At the beginning of each experiment, we adjusted the coronary perfusion rate to generate a perfusion pressure equal to the mean aortic pressure. This adjustment was made to minimize flow through coronary collateral channels. The flowmeter zero was checked repeatedly throughout the experiment.

**Experimental Protocols**

We carried out four groups of experimental protocols to answer the following questions: 1) Does intense stimulation of the cardiac sympathetic nerves induce a persistent poststimulatory coronary vasoconstriction, presumably by releasing NPY? 2) Does intense stimulation of the cardiac sympathetic nerves, presumably by releasing NPY, potentiate the coronary vasoconstriction induced by subsequent infusions of NE? 3) Does exogenous NPY induce a dose-dependent constriction of the coronary resistance vessels in our preparation? 4) Does exogenous NPY potentiate the coronary vascular responses to NE infusion?

**Protocol 1: Coronary vascular responses to intense sympathetic stimulation.** The effects of intense sympathetic stimulation (designed to release NPY) on the coronary vasculature were assessed in 11 animals. The animals were subdivided randomly into the following two groups: a Sham/Stim group and a Stim/Stim group. Each experiment, regardless of the group, was divided into two observation periods. In the Sham/Stim group (n=5), the first observation period was a control (sham stimulation) period, and intense sympathetic stimulation was delivered in the second period. In the Stim/Stim group (n=6), intense sympathetic stimulations were delivered in periods 1 and 2.

At the beginning of period 1 in both groups of experiments, we injected propranolol (1 mg/kg) intravenously to circumvent the large changes of myocardial
metabolism that would otherwise be elicited by subsequent sympathetic stimulations. We verified the adequacy of β-adrenergic blockade by the lack of a chronotropic response to stimulation of the right stellate ganglion. We recorded the atrial electrogram, AA interval (cardiac cycle length), left ventricular pressure and dP/dt, coronary blood flow, coronary perfusion pressure, and mean aortic pressure. The coronary vascular resistance was calculated by dividing perfusion pressure by coronary blood flow.

We implemented a modification of Potter’s repetitive vagal stimulation regimen to obtain an index of the release of NPY from the sympathetic nerves to the sinus node region of the heart. This regimen consisted of repetitively stimulating the right vagus nerve for 10 seconds at a frequency between 2 and 5 Hz (1-msec pulse width). We adjusted the frequency to elicit approximately a 100% increase in cardiac cycle length under control conditions.

After we had obtained a few control responses in the animals in the Stim/Stim group, we stimulated both ansae subclaviae supramaximally (14 V, 1-msec pulse width) at a frequency of 20 Hz for 3 minutes; we shall refer to this procedure as the NPY “release” stimulation. Previous studies by others and from our laboratory have shown that such stimulations do release substantial quantities of NPY from the cardiac sympathetic nerve endings. After we terminated the sympathetic release stimulation in the present series of experiments, we resumed the periodic test stimulations of the right vagus nerve. The poststimulatory effects of the release stimulation were assessed by periodic vagal test stimuli for 1 hour. In the animals in the Sham/Stim group, we carried out the identical protocol during period 1, except that we refrained from delivering the intense sympathetic release stimulation.

At the beginning of period 2, regardless of the group, we gave additional propranolol (0.5 mg/kg) to maintain the β-adrenergic blockade. We used the identical vagal test and sympathetic release stimulation protocol that we had used in period 1 in the Stim/Stim group. Comparison of the responses obtained during periods 1 and 2 in the Stim/Stim group provided information about the changes in cardiac responses over time. In the Sham/Stim group, period 1 served as an internal control for any sustained effects that sympathetic release stimulation may have exerted on coronary vascular resistance during period 2.

Protocol 2: Effects of release stimulation on the coronary vascular responses to norepinephrine infusions. In a second series of eight animals, we injected propranolol (2 mg/kg i.v.) and we evaluated the effects of constant infusions of NE on the coronary vasculature. Each experiment was divided into two observation periods; we randomized the order of these periods in each experiment. One period was designed to assess the effects of neurally released NPY on the chronotropic responses to vagal test stimulations. This period was identical to that for the first series, except that the periodic vagal test stimulations were delivered for 30 minutes instead of for 60 minutes after cessation of the sympathetic release stimulation.

The other period was designed to determine the effects of neurally released NPY on the coronary vascular responses to infusions of NE. In this period, instead of the vagal test stimulations, we infused NE (0.68 μg) periodically at a constant rate for 1 minute. We recorded cardiac cycle length, left ventricular pressure and dP/dt, mean aortic pressure, coronary blood flow, and perfusion pressure continuously. We repeated the test NE infusions three times to confirm the reproducibility of the coronary vascular responses. These control test infusions were followed by a 3-minute train of sympathetic release stimulation (14 V, 1 msec, 20 Hz). We then repeated the 1-minute infusions of NE into the coronary artery every 5 minutes until 30 minutes had elapsed.

Protocol 3: Effects of NPY infusions on coronary vascular resistance. In four additional dogs, prepared as described above for the first series, we examined the effects of 1-minute infusions of exogenous NPY on the coronary vascular bed. We infused increasing doses (0.1, 0.2, 1.2, 2.4, and 4.7 nmol) of NPY (Peninsula Laboratories, Belmont, Calif.) dissolved in 1 ml isotonic saline into the left circumflex coronary vascular bed. We first confirmed that the infusion of 1 ml control vehicle (saline) affected the coronary vascular resistance only slightly and transiently. We recorded cardiac cycle length, left ventricular pressure and dP/dt, mean aortic pressure, coronary blood flow, and perfusion pressure before, during, and after each infusion of NPY.

Protocol 4: Effects of NE infusion on the coronary vascular responses to NE infusions. In a fourth series of four dogs, we injected propranolol (1 mg/kg) intravenously, and we gave supplemental doses (0.5 mg/kg) hourly. We divided each experiment into three observation periods. During the first (control) period, we infused NE (0.2, 0.4, and 0.68 μg/ml) at a rate of 1 ml/min for 1 minute. We randomized the order in which we infused the various concentrations. We allowed a 3-minute recovery time between the end of one infusion and the beginning of the next infusion. During the second observation period, we delivered a sham NPY infusion and then repeated the NE infusions in a randomized sequence of concentrations. In the third observation period, we infused NPY (0.3 nmol/ml) at a rate of 1 ml/min for 15 minutes. During the NPY infusion, we determined the coronary vascular responses to the three NE infusions, again administered in a random order. The first NE infusion was begun 3 minutes after the beginning of the NPY infusion. We recorded the atrial electrogram, cardiac cycle length, left ventricular pressure and dP/dt, coronary blood flow, coronary perfusion pressure, and mean aortic pressure throughout all three observation periods.

Data Analysis

Data are presented as mean ± SEM. We used the analysis of variance to determine statistical significance. If the F statistic was significant, we compared differences between groups by Scheffe’s test. We considered a value of p < 0.05 to be statistically significant.

Results

Protocol 1: Persistent Coronary Vascular Responses to Intense Sympathetic Stimulation

Representative experiment. The aim of this experiment was to test the hypothesis that sufficient NPY would be released from the cardiac sympathetic nerves during
intense stimulation to elicit a sustained coronary vasoconstriction. Figure 2 shows a representative example of the coronary vascular response to a 3-minute period of intense sympathetic release stimulation. The coronary perfusion pressure increased to 145 mm Hg from a basal value of 125 mm Hg; blood flow through the left circumflex coronary vascular bed was held constant by our perfusion system. After cessation of stimulation, the coronary perfusion pressure rapidly returned to the control value.

Figure 3 shows the changes in cardiac cycle length evoked by three identical vagal test stimulations. Before the sympathetic release stimulation (Figure 3A), the vagal stimulation increased the cycle length by 840 msec. Five minutes after the cessation of the sympathetic stimulation, the vagal stimulation increased cycle length by only 600 msec, a 30% reduction (Figure 3B). We continued to stimulate the vagus nerve for 10 seconds every 10 minutes for 1 hour. In this dog, 40 minutes after the sympathetic release stimulation, the response to vagal test stimulation had recovered to the control value (Figure 3C).

Composite data. Figure 4A shows the mean changes in coronary vascular resistance for the animals in the Sham/Stim group. During the first observation period, the mean coronary resistance was 5.4 mm Hg/(min/ml) before sham stimulation of the sympathetic nerves. Throughout the subsequent 1-hour period of observation, the coronary vascular resistance did not deviate significantly from the control level.

During the second observation period, the coronary vascular resistance increased by approximately 20% (p<0.05) during the sympathetic release stimulation (Figure 4A). However, after cessation of the sympathetic stimulation, the coronary vascular resistance rapidly diminished. Two minutes after cessation of stimulation, the coronary resistance was slightly, but not significantly, above the control level. Resistance continued to fall until it was slightly below the control value. Thereafter, it rose very gradually and remained almost precisely at the control level during the last 20 minutes of the observation period.

In the Stim/Stim group, sympathetic release stimulation was delivered during periods 1 and 2. The changes in coronary vascular resistance (not shown) paralleled those obtained during period 2 in the Sham/Stim group (Figure 4A). Furthermore, the changes during period 1 in the Stim/Stim group did not differ significantly from those during period 2 in the same group. During sympathetic stimulation, coronary vascular resistance increased by 15–20% (p<0.05), and the resistance returned to values that did not differ significantly from control

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**Figure 2.** Time course showing the effects of a 3-minute period of bilateral stimulation (14 V, 1 msec, 20 Hz) of the ansae subclaviae on coronary artery perfusion pressure and mean aortic pressure (AOP) in an anesthetized dog that had received propranolol (1 mg/kg). The left circumflex coronary artery was perfused at a constant blood flow.

**Figure 3.** Recordings showing the changes in cardiac cycle length induced by vagal test stimulations (4 Hz for 10 seconds) in a representative experiment before (panel A), 5 minutes after (panel B), and 40 minutes after (panel C) sympathetic stimulation of the ansae subclaviae (20 Hz for 3 minutes). Vagal stimulation periods are denoted by the horizontal bars.
FIGURE 4. Time course showing the mean changes in coronary vascular resistance (panel A) and in the chronotropic responses (panel B) to test vagal stimulations in a group of five anesthetized dogs. During the second observation period (P2), the ansae subclaviae were stimulated at 20 Hz for 3 minutes, as denoted by the horizontal bar in panel B. During the first observation period (P1), the same horizontal bar denotes a sham neural stimulation. The changes in coronary vascular resistance (panel A) during P2 that differ significantly from the corresponding changes during P1 are denoted by asterisks. The chronotropic responses to the vagal test stimulations (panel B) are expressed as percent of control. They were calculated as 100(Re-Re)/Re, where Re is the control response (before sympathetic release stimulation or sham sympathetic stimulus) and Rr is the experimental response (after sympathetic release stimulation or sham sympathetic stimulation).

within 5 minutes after sympathetic stimulation. Table 1 shows the hemodynamic changes observed in the Sham/Stim and Stim/Stim groups of animals before and after the sympathetic release and sham stimulations.

Figure 4B shows the mean changes in cardiac cycle length elicited by the vagal test stimulations in the animals in the Sham/Stim group after termination of the sympathetic release stimulation. In the control period (period 1), the chronotropic responses to the vagal test stimulations did not differ significantly from the control level for the entire 1-hour observation period. In period 2, the initial chronotropic response evoked after cessation of the sympathetic release stimulation was attenuated by approximately 30%. The chronotropic response then gradually recovered toward the control level over the next 50 minutes. The chronotropic responses to the vagal test stimulations after the sympathetic release stimulation (period 2) differed significantly from the responses after the sham stimulation (period 1).

### Table 1. Hemodynamic Values Measured Before and 5, 30, and 60 Minutes After Sympathetic Release Stimulation in Two Groups of Dogs

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<tr>
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<th>Sham/Stim group</th>
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<td>Time after sympathetic release stimulation (min)</td>
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<td>Pre 5 30 60</td>
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<tr>
<td>HR (bpm) P1</td>
<td>114±9 114±10 112±8 107±9</td>
<td>114±9 113±9 111±8 111±8</td>
<td>85±5 82±7 80±7 82±4</td>
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<td>P2</td>
<td>104±9 105±9 104±9 104±10</td>
<td>107±8 108±9 107±9 108±9</td>
<td>85±5 82±7 80±7 82±4</td>
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<tr>
<td>LVPSP (mm Hg) P1</td>
<td>98±5 98±5 93±4 91±7</td>
<td>88±4 86±4 80±3 84±3</td>
<td>85±5 82±7 80±7 82±4</td>
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<tr>
<td>P2</td>
<td>91±6 91±7 86±9 80±9</td>
<td>85±5 82±7 80±7 82±4</td>
<td>85±5 82±7 80±7 82±4</td>
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<td>dP/dt max (mm Hg/msec) P1</td>
<td>1.52±0.17 1.55±0.17 1.48±0.16 1.40±0.20</td>
<td>1.57±0.16 1.53±0.19 1.40±0.19 1.40±0.13</td>
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<td>1.26±0.12 1.35±0.16 1.18±0.15 1.18±0.15</td>
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<tr>
<td>MAP (mm Hg) P1</td>
<td>98±5 97±4 91±4 90±7</td>
<td>88±4 83±5 78±4 84±3</td>
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<tr>
<td>P2</td>
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<td>85±5 82±7 80±7 82±4</td>
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Sham/Stim group, dogs that received a control (sham) stimulation during the first observation period (P1) and an intense sympathetic stimulation during the second observation period (P2); Stim/Stim group, dogs that received intense sympathetic stimulations during P1 and P2; Pre, before sympathetic release stimulation; HR, heart rate; bpm, beats per minute; LVPSP, left ventricular peak systolic pressure; dP/dt max, maximum rate of left ventricular pressure development; MAP, mean aortic pressure. Values are mean±SEM.
The chronotropic responses to the vagal test stimulations in the Stim/Stim group (not shown) during periods 1 and 2 paralleled those obtained during period 2 in the Sham/Stim group (Figure 4B). Also, the changes during period 1 in the Stim/Stim group did not differ significantly from those obtained during period 2 in that same group.

**Protocol 2: Effects of Release Stimulation on the Coronary Vascular Responses to Subsequent NE Infusions**

The aim of these experiments was to test the hypothesis that a 3-minute period of intense sympathetic stimulation, which is known to release NPY, would potentiate the coronary vascular responses to subsequent infusions of NE. Figure 5 shows the changes in coronary arterial perfusion pressure induced by NE infusions when the coronary vascular bed was perfused at a constant flow in a representative experiment. During the control period (Figure 5A), the NE infusion increased the coronary perfusion pressure by 14%. After the termination of the NE infusion, the perfusion pressure returned to the control value within 30 seconds.

Sympathetic release stimulation increased the perfusion pressure by 20%, and after cessation of stimulation, the perfusion pressure returned rapidly to the control value (Figure 5B). Five minutes after the cessation of stimulation, an NE infusion identical to that delivered during the control period increased perfusion pressure by 13% (Figure 5C).

Figure 6A shows the mean coronary vascular responses to test infusions of NE in a group of eight dogs. During the control period, we gave three test infusions into the left circumflex coronary artery in each animal. These infusions increased coronary vascular resistance by a mean value of 14±2%. During the sympathetic release stimulation (S in Figure 6), coronary vascular resistance increased by 21±5%. Five minutes after the cessation of sympathetic stimulation, the basal coronary vascular resistance did not differ from the control value. The coronary vascular responses to the NE infusions after the sympathetic release stimulation were not significantly different from the responses obtained before the sympathetic stimulation.

Figure 6B shows the mean chronotropic responses to vagal test stimulations after the cessation of sympathetic release stimulation in this group of animals. Five minutes after the sympathetic release stimulation, the mean chronotropic response to vagal test stimulation was diminished to 63% of the control value (p<0.001), and the chronotropic responses then gradually returned back to the control value in approximately 25 minutes.

**Protocol 3: Effects of NPY Infusions on Coronary Vascular Resistance**

The aim of these experiments was to determine the dose dependency of the coronary vascular response to exogenous NPY in our preparation. We found that intracoronary infusions of various quantities of NPY induced dose-dependent increases in coronary vascular resistance (Figure 7A). The maximal coronary vascular response to each dose of NPY was produced within 60
Protocol 4: Effects of Exogenous NPY on the Coronary Vascular Responses to NE Infusions

In a representative experiment, the infusion of NE (0.4 µg/ml) at a rate of 1 ml/min for 1 minute increased the coronary perfusion pressure by 6 mm Hg (Figure 8A). Other NE infusions (0.2 and 0.7 µg/ml) increased the coronary perfusion pressure by 8 and 11 mm Hg, respectively (not shown in the figure). Subsequently, we began infusing NPY at a constant rate of 0.3 nmol/min. After 7 minutes, the NPY infusion had increased the coronary perfusion pressure by 5 mm Hg (Figure 8B). At this time, an infusion of NE (0.4 µg/ml for 1 minute) concomitant with the NPY infusion increased the coronary perfusion pressure by 15 mm Hg (Figure 8B). Thus, the NE infusion given concurrently with the NPY infusion elicited an increment in coronary perfusion pressure that was approximately twice that elicited by an equivalent infusion of NE before the NPY infusion.

Figure 9 shows the mean changes in coronary perfusion pressure evoked by infusions of NE and NPY, alone and concomitantly, in a group of four animals. The “NE” curve shows the mean changes in coronary perfusion pressure evoked by three different doses of NE. All doses significantly elevated the coronary perfusion pressure, and the response increased ($p<0.05$) as we raised the dose of NE (Figure 9). The responses (not shown) to equivalent NE infusions administered during a sham infusion of NPY were not appreciably different.

FIGURE 6. Time course showing the effects of sympathetic release stimulation on the coronary vascular responses to test infusions of norepinephrine (NE) (panel A) and on the chronotropic responses to test vagal stimulations (panel B) in a group of eight dogs. Mean±SEM changes in coronary vascular resistance elicited by the NE infusions are expressed as a percent of the preinfusion values. The control ($C_1$, $C_2$, and $C_3$) coronary vascular resistances (before sympathetic release stimulation) increased by approximately 15% during the NE infusions. Time 0 represents the end of the 3-minute period of sympathetic release stimulation. During that stimulation ($S$), coronary vascular resistance increased by 21%. From 5 to 30 minutes after sympathetic release stimulation, the NE infusions increased coronary resistance by approximately the same amount as it did during the control period. However, the chronotropic responses to the vagal test stimulations were attenuated after the sympathetic release stimulation, but the responses recovered in approximately 30 minutes.

FIGURE 7. Mean±SEM changes in coronary vascular resistance (panel A), expressed as percent of control, and the duration of those resistance changes (panel B), in minutes, plotted as a function of the dose (in moles) of neuropeptide Y (NPY) infused into the left circumflex coronary artery in a group of four anesthetized dogs.

seconds. Infusion of 0.2 nmol NPY increased coronary vascular resistance by 3.4±1.3%, and the coronary constriction was sustained for approximately 3 minutes. Doses of 1.2, 2.4, and 4.7 nmol NPY increased the coronary resistance by 17.0±3.4%, 23.3±3.0%, and 30.5±6.5%, respectively. Coronary constriction was sustained for approximately 14, 33, and 48 minutes, respectively (Figure 7B). Except for the largest dose, these quantities of NPY did not alter heart rate, left ventricular pressure, maximum $dP/dt$, or mean aortic pressure; the largest dose (4.7 nmol) did increase left ventricular peak systolic pressure and mean aortic pressure slightly.
(p=0.3) from those represented by the NE curve in Figure 9.

The “NPY” curve in Figure 9 shows the increment in pressure evoked by the actual NPY infusion, measured just before the infusion of each dose of NE; note that in each experiment, the order of infusing the different doses of NE was randomized. Thus, the effect of the NPY did not vary appreciably with regard to the various associated NE doses.

To determine whether the NPY infusion potentiated the responses to the various doses of NE, we compared the responses to the concomitant infusions of NE and NPY with the sums of the individual responses. We found that the combined responses were significantly greater (p<0.05) than the sums of the individual responses (Figure 9). Therefore, the NPY infusion did potentiate the coronary vascular responses to the NE infusions; the extent of the potentiation did not vary significantly for the various doses of NE (Figure 9).

Discussion

Our experiments were designed to determine the vasomotor effects of a 3-minute period of intense sympathetic stimulation, which is known to release NPY, on the normal coronary vasculature in vivo. Our data show that sympathetic release stimulation increased coronary vascular resistance by approximately 20% (Figures 4A and 6A) in animals that have received propranolol. After cessation of sympathetic stimulation, however, the coronary vascular resistance rapidly returned to the control value (Figure 4A). Hence, sympathetic release stimulation did not have a sustained effect on coronary vascular resistance in our experiments, nor did it potentiate the effects of NE infusions on the coronary vasculature (Figure 6A). However, exogenous NPY did increase coronary vascular resistance in a dose-dependent manner (Figure 7A), and it did potentiate the responses to exogenous NE (Figures 8 and 9). Furthermore, the vasoconstriction evoked by exogenous NPY was sustained; with the largest dose of NPY, the resistance remained increased for almost an hour (Figure 7B). The cardiac responses to vagal stimulation were attenuated for 30–60 minutes after sympathetic release stimulation (Figures 4B and 6B).

Abundant evidence has established that NPY evokes a direct sustained constriction of vascular smooth muscle that it potentiates the vasoconstrictor effects of NE. However, this evidence was derived almost exclusively from the responses to exogenous NPY or from in vitro experiments. Our responses to sympathetic release stimulation in vivo differ from the results of these previous studies; intense sympathetic stimulation did not evoke a sustained vasoconstriction, nor did it potentiate the responses to subsequent infusions of NE. The discrepant results in our experiments may be attributed to certain potential deficiencies in our preparation, such as destruction of perivascular nerves, abundant collateral coronary circulation, poor vasomotor responsiveness, excessive myocardial oxygen consumption, or inadequate release of NPY from the vascular nerves. Alternatively, the results of the previous experiments that involved in vitro preparations and exogenous NPY might not be applicable to the normal coronary circulation in vivo.
Abundant Collateral Coronary Circulation

The canine heart has a richer collateral coronary circulation than do the hearts of many other mammalian species. An abundant collateral circulation in our preparation may have masked the coronary vascular effects of neurally released NPY. At the beginning of each experiment, we adjusted the coronary perfusion pressure to the mean aortic pressure to minimize the confounding influence of the prevailing collateral circulation. We elected not to use a constant-pressure perfusion system in our experiments, because any reductions in flow induced by our experimental interventions might have been masked by metabolically induced adjustments (e.g., release of adenosine).

During sympathetic stimulation, the perfusion pressure increased by approximately 15 mm Hg, and the mean aortic pressure increased by approximately 8 mm Hg. Thus, the collateral coronary blood flow from the artifically perfused region (left circumflex coronary vascular bed) to the regions perfused by the cognate arteries (left anterior descending and right coronary arteries) may have been augmented. However, the pressure gradient persisted for only 3–5 minutes after cessation of stimulation. The effects of NPY on blood vessels may persist for as long as 60 minutes8,9; our experiments with exogenous NPY confirmed such a sustained vasoconstriction (Figure 7). Hence, if NPY had been released from sympathetic nerve terminals into the coronary vessels in amounts that would elicit substantial vasoconstriction, the effects of NPY would have been masked for only a few minutes.

Furthermore, we observed that exogenous NPY did constrict the coronary vessels in a dose-dependent manner (Figure 7). If the coronary collateral circulation had been great enough to mask any appreciable effect of neurally released NPY, it would also have markedly attenuated the increase in perfusion pressure evoked by sympathetic stimulation or by NPY infusion. Therefore, it is unlikely that an abundant collateral coronary circulation substantially masked the effects of neurally released NPY in our experiments.

Impaired Responsiveness of the Coronary Resistance Vessels

Many investigators have demonstrated that NPY is a potent vasoconstrictor in vivo2-5,9,13,16,24 and in vitro3,4,9,10 and that NPY constricts blood vessels in a dose-dependent manner.7,9,24 The threshold vasoconstrictor doses of NPY injected directly into the coronary arteries of beating canine hearts varied from 0.02 to 0.5 nmol.7,9,24 Our data (Figure 7) confirm the results of these previous investigators; our threshold dose of NPY was 0.2 nmol. These results suggest that the responsiveness of the coronary vessels to NPY in our preparation was similar to that in other investigations.7,9,24

Increased Cardiac Demand for Oxygen

Changes in cardiac oxygen demand may have masked the vasoconstrictor effects of neurally released NPY in our preparation. Sympathetic stimulation increases myocardial oxygen consumption largely by virtue of its positive chronotropic and inotropic effects.23,25 However, in our preparation, we infused propranolol to minimize the anticipated changes in cardiac oxygen

Destruction of Perivascular Nerves

When a β-adrenergic receptor antagonist has been administered to abolish almost completely the metabolic effects of NE on the myocardium, stimulation of the cardiac sympathetic nerves constricts the coronary vasculature.17-23 Our experiments have confirmed this finding. If we had damaged the pericoronary nerves extensively, coronary vascular resistance would not have increased appreciably during the sympathetic release stimulation. However, coronary vascular resistance did increase consistently in our experiments by a mean value of approximately 20% during the sympathetic release stimulation (Figures 4A and 6A). Other investigators, using similar preparations, observed increases in coronary vascular resistance that ranged from 10% to 25%,17-22 even when coronary blood flow was measured by techniques that did not involve perivascular dissection.20,22 Therefore, we believe that destruction of the perivascular nerves during the dissection of the coronary arteries was not a critical problem in our experiments.

FIGURE 9. Mean±SEM changes in coronary perfusion pressure in the left circumflex coronary artery evoked by intra-arterial infusions of norepinephrine (NE) and neuropeptide Y (NPY) during constant-flow perfusion of the left circumflex coronary artery in a group of four dogs. The NE curve represents the changes induced by NE infusions alone, at rates of 0.2, 0.4, and 0.7 µg/min. The NPY curve represents NPY infusion alone. NPY was infused at a rate of 0.3 nmol/min for 15 minutes. The graph labeled NPY denotes the changes in perfusion pressure that prevailed just before the infusions of the various doses of NE. The sum curve represents the sum of the increases in perfusion pressure evoked by NE alone and by NPY alone. The combined curve represents the increases in perfusion pressure evoked by concomitant infusions of NE and NPY. Note that responses to combined infusions exceed the sum of the responses to separate infusions; i.e., NPY potentiates the coronary vascular responses to NE.
consumption, because myocardial oxygen consumption is such an important factor in the control of coronary vascular resistance.22,23

In our experiments, sympathetic stimulation did not change heart rate appreciably, and left ventricular peak systolic pressure and mean arterial pressure increased only slightly (Figure 2). Furthermore, after cessation of sympathetic stimulation, left ventricular and mean arterial pressures returned rapidly to their control values. Therefore, myocardial oxygen consumption after the sympathetic release stimulation probably did not differ appreciably from the prestimulation level in our experiments, because heart rate, left ventricular pressure, and mean arterial pressure after stimulation did not differ from their respective control values (Table 1). Hence, myocardial oxygen consumption probably did not increase sufficiently during sympathetic stimulation to alter the coronary vascular resistance appreciably after the termination of sympathetic stimulation.

**Inadequate Release of NPY from the Vascular Nerves**

Finally, the sympathetic release stimulations may not have released the expected quantities of NPY from the cardiac sympathetic nerve endings in our preparation. As with all neuropeptides, neuropeptide Y may readily be depleted from the nerve endings, because they are synthesized in the cell body and depend on axonal transport to the nerve endings.26,27 It is conceivable, therefore, that NPY may have been depleted from the cardiac sympathetic nerves in our preparations.

In this study we used a modification of Potter's protocol12 to estimate the release of NPY from the cardiac sympathetic nerves in the anesthetized dog. We stimulated the sympathetic nerves at a high frequency (20 Hz) to release substantial amounts of NPY. Potter showed that short periods of intense sympathetic stimulation at frequencies of 16–20 Hz attenuate the vagal effects on cardiac cycle length for as long as 1 hour.12 She adduced convincing evidence that intense sympathetic stimulation releases NPY, which then depresses the chronotropic responses to vagal stimuli by inhibiting the release of acetylcholine from the vagal terminals. Recent studies from our laboratory15,26,29 have confirmed Potter's observations that strong sympathetic stimulation inhibits the vagal effects on cardiac cycle length and have demonstrated that the amount of NPY released from the cardiac sympathetic nerves depends on the frequency and duration of sympathetic stimulation.

Our present experiments confirmed Potter's findings12 and our previous results.15,26,29 Our current results also demonstrated that intense sympathetic stimulation at 20 Hz inhibited the vagal effects on cardiac cycle length for 30–60 minutes (Figures 4 and 6). Therefore, intense sympathetic stimulation apparently did release the expected quantities of NPY. Furthermore, the NPY receptors in the coronary vessels must have functioned normally, because our preparation did respond well to exogenous NPY (Figure 7). Nevertheless, the amount of NPY released from the sympathetic nerve endings in the coronary vessels was evidently not sufficient to constict the coronary vasculature appreciably nor sufficient to potentiate the effects of NE on the coronary vasculature. In our experiments with exogenous NPY (Figure 7), we found that we had to inject at least 1 nmol into the coronary artery to evoke a vasoconstriction that persisted for at least 10 minutes. Therefore, we conclude that the quantity of NPY released by our sympathetic release stimulations must have achieved a concentration in the coronary resistance vessels that was less than the concentration achieved by the intracoronary injection of 1 nmol exogenous NPY.

Therefore, we conclude from our experiments that 1) sufficient amounts of NPY were released in the vicinity of the vagal nerve endings to affect vagal neurotransmission, but 2) the amounts of NPY released in the coronary vasculature were not sufficient to evoke appreciable coronary vasoconstriction or to potentiate the constrictor effects of NE on the coronary vasculature. Our data suggest, therefore, that neurally released NPY probably does not mediate coronary vasospasm when the coronary circulation is normal. However, this neuropeptide might contribute to the development of coronary vasospasm under certain pathophysiological conditions.

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