Neuropeptide Y Modulates Vasoconstriction in Coronary Microvessels in the Beating Canine Heart

Tatsuya Komaru, Kouichi Ashikawa, Hiroshi Kanatsuka, Nobuyo Sekiguchi, Toshimi Suzuki, and Tamotsu Takishima

The purpose of this study was to determine whether neuropeptide Y has a direct vasoconstrictor effect at low doses, mimicking the physiological plasma concentration on the specific site(s) of coronary arterial microvessels in in situ beating canine left ventricles. Coronary microvessels were directly observed by means of an intravital microscope and video system equipped with a floating objective. Epi-illuminated fluorescence coronary microangiography was performed in open-chest anesthetized dogs (n=14) to examine the changes in internal diameter of epicardial coronary arterial microvessels. Flow velocity of fluorescently labeled microspheres in capillaries was also measured (n=6). To eliminate secondary effects of neuropeptide Y on coronary microvessels via autonomic nervous modulation, experiments were conducted under pharmacological blockade of the regional autonomic nervous system by intracoronary injection of propranolol, 50 µg/kg; phentolamine, 100 µg/kg; and atropine, 5 µg/kg. Aortic pressure and heart rate were kept constant during the experiments. Intracoronary infusion of three different doses of neuropeptide Y (1, 10, and 100 pmol/kg/min) for 5 minutes significantly constricted small microvessels (less than 100 µm in diameter) (−5.2±1.4%, −8.5±1.5%, and −14.0±1.7%; p<0.05 versus before neuropeptide Y at each dose), medium microvessels (100–200 µm in diameter) (−5.5±1.6%, −10.6±1.8%, and −16.8±2.1%, p<0.05 versus before neuropeptide Y at each dose), and large microvessels (greater than 200 µm in diameter) (−3.6±0.6%, −5.8±0.8%, and −10.0±1.1%; p<0.05 versus before neuropeptide Y at each dose) in a dose-dependent manner. Capillary flow velocity was reduced by 17.2±3.1% by an intracoronary dose of 100 pmol/kg/min of neuropeptide Y (p<0.05). The present study indicates that low doses of neuropeptide Y exert a homogeneous direct vasoconstrictor effect on various sizes of coronary arterial microvessels and reduce capillary flow velocity. These results suggest that neuropeptide Y may play a physiological role in modulating coronary microvascular tone. (Circulation Research 1990;67:1142–1151)

It has been recognized that neuropeptides form an important component of the autonomic nervous system and that they have much influence on the cardiovascular system. They may influence blood pressure, blood volume, cardiac function, and vasomotion. Neuropeptide Y, discovered in 1982, is a major neuropeptide in the heart, and immuno-histological studies have shown that nerve fibers around the coronary arterial system are rich in neuropeptide Y. Neuropeptide Y is released mainly as a cotransmitter of norepinephrine from sympathetic postganglionic nerve endings and has a potent vasoconstrictor effect on vessels of various organs in vivo and in vitro, including the heart. The plasma concentration of neuropeptide Y increases significantly with physiological stress such as exercise. Accordingly, neuropeptide Y may play an important role in modulating coronary vascular tone. Although several studies have shown indirectly that neuropeptide Y has a vasoconstrictor effect at the microvascular level, it remains to be elucidated how it constricts the coronary microvasculature and especially whether constriction is homogeneous or heterogeneous. Recently, it has become clear that coronary resistance resides in various sizes of microvessels, including relatively large arterial microvessels, and that vasoactive agents often have...
effects that are heterogeneous with respect to coronary microvessel size. Therefore, direct observation of microvessels is a requisite in clarifying the pharmacological effects of vasoactive substances on coronary microvessels. The manner in which neuropeptide Y acts on vessels is complicated. First, it has direct vasoconstrictor effects on vessels in many organs, and this mechanism remains unclear. Second, neuropeptide Y potentiates \( \alpha \)-adrenoceptor–mediated vasoconstriction by its indirect postjunctional effects. Third, it attenuates norepinephrine release presynaptically. Fourth, it inhibits cholinergic transmission in parasympathetic nerve endings. Therefore, in the present study, we investigated the direct effects of neuropeptide Y on coronary microvessels under autonomic nervous blockade to eliminate the many other indirect effects by using a method of direct observation with the floating objective system. Accordingly, the purposes of this study were 1) to determine whether low doses of neuropeptide Y, which mimic physiological plasma concentrations, have direct vasoconstrictor effects on coronary arterial microvessels under pharmacological sympatholysis and parasympatholysis and 2) to determine whether neuropeptide Y acts at specific site(s) in coronary arterial microvessels.

Materials and Methods

General Preparation

Young mongrel dogs of either sex (\( n = 20 \); body weight, 5–9 kg) were premedicated with ketamine (50 mg i.m.) and anesthetized with an intravenous injection of \( \alpha \)-chloralose (60 mg/kg, Wako Chemicals, Osaka, Japan). Additional doses of \( \alpha \)-chloralose were given to maintain anesthesia, if necessary. The animals were mechanically ventilated through a cuffed endotracheal tube with a respirator (Harvard Apparatus, South Natick, Mass.). A positive end-expiratory pressure of 3–5 cm \( H_2O \) was introduced to prevent atelectasis of the lung. Arterial blood gases tensions were kept within the physiological range by adjusting ventilation volume and/or rate. Metabolic acidosis during anesthesia was prevented by an intravenous infusion of sodium bicarbonate to maintain arterial pH at approximately 7.40. Body temperature was maintained at 37° C with a heat blanket. A lead II ECG was monitored. A catheter was placed in the right atrium through the right external jugular vein to administer fluid and anesthetics. A thoracotomy was performed in the fifth left intercostal space, and the heart was suspended in a pericardial cradle. A plastic wrap was used to separate the lung from the anterior aspect of the heart and to prevent the lung from becoming dry. Aortic pressure was measured at the aortic root with a catheter passed through the right carotid artery. A 16-gauge Teflon tube was passed into the left ventricle through the apex for the recording of left ventricular pressure. Pressures were measured with a Statham strain gauge transducer (model P23, Gould, Cleveland). A catheter was introduced into the left atrium for injection of fluorescein isothiocyanate–dextran (MW 154,200, Sigma Chemical Co., St. Louis). A snare was placed around the descending thoracic aorta, and a balloon catheter was placed in the inferior vena cava through a right femoral vein for the control of systemic aortic pressure. Heart rate was kept constant at 140 beats/min by means of left atrial pacing after suppression of the sinus node with a local injection of 10\% formaldehyde (0.3–0.5 ml). The left anterior descending coronary artery was dissected, and a tiny catheter (o.d., 0.67 mm; Surflo, Terumo, Tokyo) was retrogradely inserted by the direct puncture method for intracoronary drug administration. The tip of this catheter was placed in a site proximal to the branching point of the artery that perfused the area of interest. The preparation was kept moist during the experiment by continuously dripping warm Krebs’ Ringer solution ([mM] \( NaCl 118.2, KCl 4.7, CaCl_2 \) 2.5, \( MgSO_4 \) 1.2, \( KH_2PO_4 \) 1.2, \( NaHCO_3 \) 25mM, calcium disodium EDTA 0.026, and glucose 5.5, maintained at 37° C and pH 7.40) onto the cardiac surface. To reduce excessive cardiac movement, two 24-gauge steel needles were inserted horizontally (5–7 mm apart) into the midmyocardium of the left ventricle. Both ends of each needle were fixed to a needle holder held with coil springs. This apparatus allowed the heart to move perpendicularly but limited excessive horizontal movement to hold the area of interest in the microscopic field of view. The ECG, aortic pressure, and left ventricular pressure were recorded on a reograf (type 8k 12-1s-ME, San Ei Sokki, Tokyo) at a paper speed of 50 mm/sec.

Microscopic System

For direct and continuous observation of coronary microvessels in beating left ventricles, we used an intravital microscope equipped with a floating objective system developed in our laboratory. Details of this system have been previously reported. Briefly, a floating objective consists of a pair of convex lenses that transmit the real image to a standard microscope without any change in magnification. That is, the image of an object on the front focus of a convex lens facing the heart is transmitted to the back focus of another convex lens. This transmitted real image is then observed with the objective of a standard microscope. The convex lens facing the heart is permitted to move perpendicularly in unison with the cardiac motion. The distance between this lens and the heart was adjusted to the focal distance of the lens by an arm connected to a needle holder (objective lifter). Therefore, compression of the heart with this lens is avoided by lifting it just above the cardiac surface. For adjustment of focus, the epicardium of the left ventricle is transilluminated with a xenon arc lamp. A light-conducting glass fiber (diameter, 0.6 mm) introduced through the lumen of a 20-gauge stainless steel needle was inserted into the subepicardial muscle layer of the left ventricle with a micromanipulator.
The needle was connected to a needle holder that allows the tip of the needle to move up and down in unison with the cardiac motion.

For the measurement of microvascular diameters and capillary flow velocities, epi-illuminated fluorescence coronary microangiography was performed. A block diagram of the method for obtaining the fluorescence image is shown in Figure 1. The surface of the left ventricle was epi-illuminated by incident light from a mercury lamp (HBO-100EW/2, Nikon Inc., Tokyo). The maximal wavelength of the illuminating light was 495 nm, obtained using a B2 excitation filter (Nikon). The emitted light was then passed through a 510-nm filter. A highly sensitive TV camera (C 1000-12, Hamamatsu Photonics, Hamamatsu, Japan) and video system (VO-2710, Sony, Tokyo) were used for continuous observation and recording of the enhanced vessel images. Spatial resolution of this system was approximately 2 μm. Motion video pictures were taken at 60 frames/sec.

**Microvascular Diameter Measurement**

Fluorescein isothiocyanate–dextran (20 mg/ml in saline, 0.2 ml) was injected via the catheter placed in the left atrium, and the fluorescent vessel images were recorded. Those images were then printed with a videographic printer (UP-811, Sony) at the end-diastolic phase. By these procedures, microvascular images were well enhanced, including the plasma layer, and well contrasted from surrounding tissues (see Figure 3). Arteriolar diameters were measured on the printed images and/or high-resolution monitor (C 1846-01, Hamamatsu Photonics). To compare diameters before and after each intervention, diameters were measured at the same place in the arteri-oles of interest with reference points such as vascular branchings or other vessels.

**Measurement of Capillary Flow Velocity**

In fluorescence images, red blood cells were not identified. Therefore, to measure capillary flow velocity, 5×10⁶ fluorescently labeled latex microspheres (diameter, 2.13 μm; Fluoresbrite carboxylate microsphere, Polyscience, Niles, Ill.) suspended in 0.5 ml saline with Tween 80 were injected into the catheter placed in the right atrium to trap aggregated microspheres in the lung. Also, the microsphere solution was shaken for 5 minutes before injection to minimize aggregation of the microspheres. Fluorescein isothiocyanate–dextran was injected into the left atrium just after the microsphere injection for simultaneous recording of fluorescent microspheres and vessel images. Fluorescence images of the microspheres and capillaries were recorded on a videocassette recorder.

Late diastolic microsphere velocity in capillaries was calculated by frame-to-frame analysis from the distance of microsphere progression on a video screen and the number of frames required. A video manipulator (C 2117, Hamamatsu) was used to follow the motion of microspheres and a reference point of cardiac motion. The distances the microspheres moved in six serial late diastolic frames were calculated. The motion of the reference point caused by cardiac movement was then subtracted from that of the microspheres by vector analysis to obtain the true distance of progression of the microspheres. The mean value of capillary flow velocity was obtained from measured velocities of 30–40 microspheres.
A preliminary study was performed to demonstrate that the velocity of microspheres reflects capillary flow velocity (n=6). Although in the resting condition absolute values of microsphere velocities differed from those of red blood cells measured by means of transilluminated high-speed cinematography (1,111±33 versus 2,412±311 μm/sec, p<0.05), percent changes of the former caused by an adenosine potentiator (dilazep [Kowa, Nagoya, Japan] 50 μg/kg i.v. or diprydamole 400 μg/kg i.v.) were equivalent to those of the latter (23.0±7.2% versus 25.0±3.0%, NS). The results are consistent with the reported data.22

Protocols

Experiments were performed approximately 30 minutes after the surgical preparation and instrumentation, when all monitored variables were stable. Effects of neuropeptide Y on coronary microvessel diameter were assessed in 14 dogs. Adrenergic and muscarinic nerves were regionally blocked by the intracoronary injection of propranolol chloride (50 μg/kg, ICI, Osaka, Japan), phenolamine mesylate (100 μg/kg, CIBA-Geigy, Takarazuka, Japan), and atropine sulfate (5 μg/kg, Tanabe Pharmaceutical, Osaka, Japan) into the left anterior descending artery to minimize the systemic effects of these agents. Additional phenolamine and atropine were injected every 10 minutes to maintain the effects of these agents during the experiment. The doses of these autonomic nervous blockers were determined on the basis of previously published data.23–25 Before and after the autonomic nervous blockade, diameter measurements were performed. After these procedures, three different doses of neuropeptide Y (human neuropeptide Y, Peptide Institute, Osaka, Japan) were administered by intracoronary infusion in doses of 1, 10, and 100 pmol/kg/min for 5 minutes in a cumulative fashion. At the end of each neuropeptide Y infusion, diameter measurements were repeated. Neuropeptide Y was dissolved in buffered physiological salt solution including 1% albumin (pH 7.4). The rate of neuropeptide Y infusion was less than 0.3 ml/min. After neuropeptide Y infusion, papaverine (4 mg, Wako Chemicals, Osaka, Japan) was administered by intracoronary injection, and diameters of microvessels were measured in nine dogs.

Fluorescent microsphere velocity measurements were done for six dogs. After blockade of the autonomic nervous system in the same manner as in the diameter measurement, neuropeptide Y (100 pmol/kg/min) was administered by intracoronary infusion. Fluorescence images were obtained after the autonomic nervous blockade and 5 minutes after the onset of neuropeptide Y infusion. In four dogs, fluorescent microsphere velocity before autonomic nervous blockade was also measured.

During both microvascular diameter and capillary flow velocity measurements, heart rate was kept constant (140 beats/min) and systemic blood pressure was adjusted to a control level with a balloon catheter placed in the inferior vena cava or by a snare around the descending thoracic aorta.

At the end of the protocol, fluorescein isothiocyanate–dextran was administered by intracoronary infusion at the same rate of drug infusion to confirm whether drugs reached the area of interest. In 14 dogs, the left anterior descending coronary artery was ligated just proximal to the tip of the intracoronary cannula, and Evans blue dye was injected into the cannula used for drug administration to identify the perfusion area. The heart was then excised, and the weight of the left ventricle and the perfusion area (stained area) were determined.

Validation Study

In this study, we retrogradely inserted a cannula (o.d., 0.67 mm) into the proximal portion of the left anterior descending coronary artery for intracoronary drug administration. This procedure may have caused pressure drop on insertion of the cannula. Therefore, we examined the effects of an inserted cannula. Experiments were performed in an additional five dogs. Experimental preparation was the same as above except for the insertion of an additional catheter into a branch distal to the cannula used for drug administration. Differences between aortic and distal coronary arterial pressures were measured at three different aortic pressures (high pressure, 122.2±6.1 mm Hg; medium pressure, 95.6±2.3 mm Hg; low pressure, 67.8±3.6 mm Hg in mean value). In four of five dogs, the pressure differences were also measured 5 minutes after the onset of neuropeptide Y infusion (100 pmol/kg/min). The coronary artery was then ligated at a site proximal to the cannula for drug administration, and 2.5% glutaraldehyde dissolved in 0.1 M phosphate buffered saline was perfused from the cannula at a perfusion pressure of 100 mm Hg. After excision of the vessels to which cannulas were inserted, luminal diameters of vessels were microscopically measured.

Statistical Analysis

All data are presented as mean±SEM. Statistical analysis of data was made by means of one-way analysis of variance for repeated measurements to evaluate the changes in variables caused by interventions.26 One-way analysis of variance was also used for evaluation of percent change in microvascular diameter among vessel groups. When significant values were obtained, Student's t test for paired or unpaired samples (corrected for multiple comparisons with Bonferroni inequality adjustment) was used to determine which measurements differed significantly from one another.26 Differences were considered significant for values of p<0.05.
Results

Blood Gas Tensions and the Weights of the Left Ventricle and Perfusion Area

Blood gas tensions and pH were maintained in normal ranges during the experiments (Table 1). The weights of left ventricles and perfusion areas are shown in Table 1. The weight of the perfusion area was about one third of the left ventricular weight in each case.

Effect of Intracoronary Cannula

A simultaneous record of the pressures of the ascending aorta and coronary artery (cross-sectional area, 0.6±0.1 mm²) distal to the cannula used for drug infusion is shown in Figure 2. The phasic pattern of the distal pressure was almost the same as that of aortic pressure. The pressure differences at high, medium, and low aortic pressure levels were 2.6±0.8, 1.8±0.6, and 0.6±0.4 mm Hg, respectively. Accordingly, the cannula for drug administration was considered not to disturb the perfusion of coronary microcirculation of interest. This pressure difference did not change after neuropeptide Y infusion. The mean cross-sectional area of coronary arteries where the intracoronary cannulas were inserted was 2.1±0.1 mm². The percentage of cross-sectional area of inserted cannula to cannulated vessel was 17.2±1.0%.

Hemodynamics During Experiments

Hemodynamic data during the experiments are summarized in Table 2. Systolic and diastolic arterial pressures were almost the same as the control levels at all doses of neuropeptide Y. Left ventricular end-diastolic pressure increased after autonomic nervous blockade, but it did not significantly change after infusion of any dose of neuropeptide Y. Heart rate was kept constant (140 beats/min) by left atrial pacing.

Responses of Coronary Arterial Microvessels to Neuropeptide Y

Coronary microvascular images obtained with fluorescence microangiography before and during infusion of neuropeptide Y (100 pmol/kg/min) are shown in Figure 3. Responses of all size vessels to neuropeptide Y are summarized in Figure 4. Arterial microvessels were divided into three groups according to their diameters under pharmacological autonomic denervation, that is, small (71.6±5.5 μm in diameter; range, 24–93 μm; n=15), medium (153.8±8.7 μm in diameter; range, 112–196 μm; n=10), and large (237.3±11.9 μm in diameter; range, 205–283 μm; n=8) microvessel groups. Arterial microvessels of all groups significantly constricted at any dose of neuropeptide Y compared with the diameters after autonomic nervous blockade (p<0.05, Figure 4). The 100 pmol/kg/min neuropeptide Y infusion did not produce a plateau in the dose-response curve in any of the microvessel groups. The percent reduction in diameters of

![Figure 2. Simultaneous recording of aortic pressure and distal coronary arterial pressure measured distal to a cannula used for drug administration. The intracoronary cannulation did not cause pressure difference.](http://circres.ahajournals.org/)}
arterial microvessels caused by neuropeptide Y was compared among the three groups (Figure 5). At a dose of 1 pmol/kg/min, the percent changes were -5.2±1.4%, -5.5±1.6%, and -3.6±0.6% in the small, medium, and large microvessel groups, respectively. At a dose of 10 pmol/kg/min, percent changes in diameters were -8.5±1.5%, -10.6±1.8%, and -5.8±0.8%, respectively. At a dose of 100 pmol/kg/min, the percent changes in diameters were -14.0±1.7%, -16.8±2.1%, and -10.0±1.1%, respectively. The dose dependency of the percent change in diameter was statistically significant in each group. There were no significant differences in the magnitude of vasoconstriction among the different vessel groups at any dose of neuropeptide Y. Papaverine dilates small (34.8±5.4%, 11 vessels), medium (29.9±6.4%, six vessels), and large (16.2±2.2%, two vessels) microvessels.

**Microsphere Velocities During Neuropeptide Y Infusion**

Microsphere velocity in capillaries was significantly reduced during neuropeptide Y infusion at the dose of 100 pmol/kg/min compared with that under pharmacological autonomic denervation (865.5±43.9 versus 1,028.1±40.9 μm/sec, p<0.05) (Figure 6). The percent change in the velocity was 17.2±3.1%. In four of six dogs used for velocity measurement, the effect of autonomic nervous blockade on microsphere velocity was examined. Microsphere velocity was reduced after blockade from 1,260.9±56.3 to 1,020.5±58.8 μm/sec (19.0±3.8%, p<0.05).

**Discussion**

This study is the first report to directly demonstrate the effects of neuropeptide Y on coronary microvasculature in beating hearts. There are two important observations in this study. 1) Low doses of neuropeptide Y have a direct vasoconstrictor effect on coronary arterial microvessels in a dose-dependent manner under pharmacological denervation of autonomic nerves. 2) The vasoconstrictor effect of neuropeptide Y is homogeneous over microvessels ranging from 24 to 283 μm in diameter and reduces capillary flow velocity.

**Critique of Methods**

Blood gas tensions, pH, and hemodynamics were maintained at control levels during the experiments.

**Table 2. Hemodynamic Change on Intracoronary Infusion of Neuropeptide Y for 5 Minutes**

<table>
<thead>
<tr>
<th>Blockade</th>
<th>NPY (pmol/kg/min)</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Systolic AOP (mm Hg)</td>
<td>124.8±2.6</td>
</tr>
<tr>
<td>Diastolic AOP (mm Hg)</td>
<td>91.1±3.9</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.8±0.4*</td>
</tr>
</tbody>
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Values are mean±SEM. Blockade, autonomic nervous blockade; NPY, neuropeptide Y; AOP, aortic pressure; LVEDP, left ventricular end-diastolic pressure. *p<0.05 vs. after autonomic nervous blockade.

**Figure 3.** Fluorescent images of arterial microvessels under autonomic nervous blockade (before neuropeptide Y infusion, panel A) and during infusion of 100 pmol/kg/min of neuropeptide Y (panel B). Neuropeptide Y markedly constricted various sizes of coronary microvessels under the nervous blockade.
and a validation study showed that the intracoronary cannula used for drug infusion did not reduce the perfusion pressure in the area of interest.

A floating objective system developed in our laboratory was used in this study to observe microvascular responses. This system allows coronary microvessels to be directly and continuously observed in situ beating hearts.2021 In our experimental procedures, mechanical factors that might affect the coronary microcirculation were eliminated to the extent possible. The light-conducting needle inserted into the myocardium for transillumination may cause microtrauma and change in intramyocardial pressure. Other investigators, however, have suggested that this problem is of little importance.27 The needle was inserted into the deeper layer because transillumination was only for focusing, and light power required for transillumination was low. Previously reported data from our laboratory have demonstrated that microvascular reactivity to various vasoactive agents is well preserved in this preparation.20,21,28 In the present experiments, papaverine markedly dilated coronary arterial microvessels at the end of the experiments. The result supports our belief that vasomotion of microvessels was preserved.

Exposure to ultraviolet light used for taking motion video pictures might have caused microvascular damage. The exposure period, however, was about 15 seconds for each measurement. Reed and Miller20 have shown that intermittent exposures to the light for short periods does not lead to any microvascular injury, while continuous exposure for a long period evokes microvascular injury such as macromolecular leakage and stasis of capillary blood flow. No thrombus formation or stasis of blood flow was observed in the present study.

**Direct Vasoconstrictor Effect of Neuropeptide Y**

Coronary vascular tone is regulated by many factors, for example, metabolic factor, neurohumoral factors, perfusion pressure, and tissue pressure.30 In the present study, oxygen consumption was not measured, but heart rate and aortic pressure were kept almost constant, and left ventricular end-diastolic...
pressure also did not significantly change with neuropeptide Y infusion. Therefore, change in metabolic demand is considered to have been minimal. Also, metabolic vasomotion in coronary arterial microvessels is known to be heterogeneous.\textsuperscript{16} Accordingly, metabolic factors cannot explain the homogeneous vasoconstriction throughout the arterial coronary microvessels observed in the present study.

It is known that neuropeptide Y may change vascular tone by neuromodulation of the autonomic nervous system through 1) potentiation of α-receptor–mediated vasoconstriction in subthreshold doses,\textsuperscript{17} 2) presynaptic inhibition of sympathetic tone,\textsuperscript{18} and 3) presynaptic inhibition of parasympathetic tone.\textsuperscript{19,31} Therefore, neuropeptide Y might cause vasoconstriction via potentiation of α-constriction, attenuation of β-receptor–mediated vasodilation, and/or attenuation of acetylcholine-induced vasodilation. To eliminate effects of regional modulation of the autonomic nervous system on microvessels and to observe the direct pharmacological effects of neuropeptide Y on coronary microvessels, the present experiments were performed under autonomic nerve blockade. It has been reported that neuropeptide Y may also potentiate the vasoconstrictor effects of histamine and prostaglandin F\textsubscript{2α}.\textsuperscript{32} However, histamine markedly dilates the coronary arterial system in dogs.\textsuperscript{33} Rioux et al\textsuperscript{34} have shown that the effect of neuropeptide Y is not blocked by H\textsubscript{1}- or H\textsubscript{2}-blocker, or indomethacin, a cyclooxygenase inhibitor. Therefore, the microvascular vasoconstrictor effect of neuropeptide Y was not thought to be via either the histamine receptors or the prostaglandin receptors.

Perfusion pressure remained almost constant in this study, but microvascular pressure in arterial microvessels might have decreased by the constriction of larger arterial microvessels. However, in in vivo experiments, decreasing perfusion pressure results in vasodilation rather than vasoconstriction in microvessels less than 100 μm in diameter.\textsuperscript{35} Thus, it is not conceivable that homogeneous microvascular vasoconstriction during neuropeptide Y infusion resulted from change in intraluminal pressure.

The effect of tissue pressure change was considered to be minimal in the present study, because left ventricular end-diastolic pressure remained constant, and the microvessels observed were in the subepicardial region. Accordingly, microvascular vasoconstriction observed in the present study was the direct effect of neuropeptide Y.

In support of the concept that neuropeptide Y leads to microvascular vasoconstriction, several immunohistochemical studies have demonstrated that neuropeptide Y–immunoreactive nerve fibers form a network around arterial microvessels, including the coronary arterial system.\textsuperscript{36,38} Moreover, an electron microscopic study\textsuperscript{36} has shown that nerve terminals outside the media of arterioles of skeletal muscle contain large dense core vesicles, which are considered to contain neuropeptide Y.

In a clinical study, it has been reported that intracoronary infusion of a low dose of neuropeptide Y caused myocardial ischemia in half of the cases without obstruction of epicardial large coronary arteries.\textsuperscript{13} The authors have speculated that obstruction of small vessels that could not be seen in coronary angiography occurred. Maturi et al\textsuperscript{39} have recently reported that intracoronary neuropeptide Y causes an increment of total coronary vascular resistance while large coronary vascular resistance remained unchanged and that neuropeptide Y caused myocardial ischemia. Kanatsuaka et al\textsuperscript{40} have recently reported that coronary arterioles less than 100 μm in diameter actively dilate for autoregulation when perfusion pressure is lowered. In the present study, the vasoconstrictor effect of neuropeptide Y on arterial microvessels was observed in all sizes of vessels despite reduction of capillary flow velocity. These results indicate that neuropeptide Y produces such potent constrictor effects on coronary microvessels that it overcomes autoregulatory vasodilation.

**Dose of Neuropeptide Y**

The doses of neuropeptide Y used in the present study were relatively low. Coronary volume flow was not measured in this study. Coronary blood flow in the resting condition is usually estimated to be approximately 0.8 ml/min/g of left ventricle in open-chest anesthetized dogs.\textsuperscript{36} Therefore, the plasma concentration of neuropeptide Y in the microvessels observed seemed to be on the order of 100 pM to 100 nM for the three doses used. The plasma concentration of neuropeptide Y in mammals is considered to be 20–70 pM, except in rats\textsuperscript{37} and, under various kinds of stress, which stimulates sympathetic nervous tone, the plasma level of neuropeptide Y increases from several to several hundred times, according to the intensity of stress.\textsuperscript{11,37,38} Thus, the doses used in the present study appear to be within physiological range.
Clinical Implication

It is accepted that the sympathetic nervous tone is activated by myocardial ischemia. Potent stimulation of the sympathetic nervous system results in neuropeptide Y release. Furthermore, a recent study has suggested that platelets can be a major source of neuropeptide Y in thrombotic diseases. Accordingly, it is possible that myocardial ischemia is impaired by microvascular constriction caused by neuropeptide Y release in patients with ischemic heart disease. Furthermore, neuropeptide Y might be a candidate for a producer of pathophysiological microvascular spasm.

In summary, the present study indicates that low concentrations of neuropeptide Y directly constrict coronary arterial microvessels in a dose-dependent manner and reduce capillary flow velocity. Neuropeptide Y homogeneously constricts all sizes of arterial microvessels less than 300 μm in diameter, and it may overcome autoregulation. Neuropeptide Y may play an important role in modulating coronary microvascular tone in physiological and pathological conditions.

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Key Words • coronary microcirculation • intravital microscope • fluorescence angiography • autonomic nervous blockade • peptide
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