Nonadrenergic Noncholinergic Nerves Regulate Basal Coronary Flow via Release of Capsaicin-Sensitive Neuropeptides in the Rat Heart

Hiroyuki Yata, Eiichi Sato, Michiko Kawaguchi, Tomiyoshi Saito, Kazuhira Maehara, Yukio Maruyama

**Abstract** Nonadrenergic noncholinergic nerve fibers supposedly modulate basal coronary flow by releasing capsaicin-sensitive neuropeptides, but the physiological effects of this intrinsic action have not been clarified. We investigated the intrinsic function of nonadrenergic noncholinergic innervation in modulating basal coronary flow in rats. We administered capsaicin to 44 rats to deplete neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P and administered inert vehicle to 60 control rats. Four days later, we measured the coronary pressure-flow relation in the basal state and during maximal coronary vasodilation induced by intracoronary adenosine administration using Langendorff’s method. Changes in basal coronary flow prompted by intracoronary infusion of CGRP or substance P and their antagonists were measured in 54 and 30 rats, respectively. Capsaicin-treated rats showed a $31.5 \pm 0.9\%$ (mean±SEM) reduction ($P<.01$) of basal coronary flow in the range of perfusion pressures between 60 and 140 mm Hg compared with untreated control rats, but the maximal coronary flow after adenosine was similar between the two groups. Although basal coronary flow was reduced in capsaicin-treated hearts, left ventricular contractile force and myocardial oxygen consumption did not fall significantly. CGRP increased the coronary flow, but substance P did not. CGRP(8-37), a CGRP receptor antagonist, reduced basal coronary flow by $24.5 \pm 2.1\%$ ($P<.01$), but FK888, a substance P antagonist, had little effect on it. Thus, capsaicin-sensitive neuropeptides in the rat heart modulate basal coronary flow, providing $\approx 30\%$ of it. Among the capsaicin-sensitive neuropeptides released from nonadrenergic noncholinergic nerve fibers, CGRP appears to be the one mainly involved in basal coronary flow modulation. (Circ Res. 1994;75:780-788.)

**Key Words** • capsaicin • nonadrenergic noncholinergic nerve • calcitonin gene-related peptide • substance P • basal coronary flow

Nonadrenergic noncholinergic nerves are known to exert cardiovascular actions by releasing neuropeptides stored in their nerve endings.1 These endogenous neuropeptides are continuously transported from nerves to nerve endings,2,3 thus suggesting biosynthesis and release. Under laboratory conditions, these peptides are forcibly released by the administration of capsaicin,4 a peptidergic neurotoxin of nonadrenergic noncholinergic sensory fibers. Thus, nonadrenergic, noncholinergic, nerve-mediated cardiovascular actions have been assessed by exogenously administering capsaicin5 or these capsaicin-sensitive neuropeptides6 or by electrical nerve stimulation.7 Capsaicin evokes transient but marked coronary vasodilation in the heart, suggesting that the nervous system may play a role in coronary flow regulation under physiological conditions. Among capsaicin-sensitive neuropeptides, calcitonin gene-related peptide (CGRP) and substance P are known to cause coronary vasodilation,8-10 although the actions of substance P seem to differ not only among species10,21 but also among various organs.10,21,22 However, it is not possible to relate the magnitude of a cardiovascular effect evoked by extrinsically driven stimulation to that evoked under intrinsic circulatory regulation.

We used nonadrenergic noncholinergic innervation to assess the role of capsaicin-sensitive neuropeptides in modulating basal coronary flow in rats. To this end, we undertook to deplete these neuropeptides stored in nonadrenergic noncholinergic nerve fibers, thus abolishing the intrinsic vasodilation of endogenous neuropeptides. Four days later, when acutely released neuropeptides including CGRP and substance P had disappeared from the tissue, we used Langendorff’s method to measure coronary hemodynamics. Changes in these coronary hemodynamics resulting from our interventions would, we assumed, reflect a deficit in the intrinsic function of nonadrenergic noncholinergic nerve fibers, in which putative neurotransmitters seem to show a potent vasodilatory action.10 We also used selective antagonists23-27 to representative neuropeptides such as CGRP and substance P in specifying the cardiovascular actions induced by capsaicin-sensitive neuropeptides.

**Materials and Methods**

**Animals**

We used a total of 236 male Wistar rats (12 weeks old) weighing 220 to 240 g each. These experiments were approved by the Fukushima Medical College Ethics Committee.
Measurements of Coronary Pressure-Flow Relation

We studied the effects of capsaicin treatment on coronary circulation in six experimental groups (total, 104 rats). In 44 rats, we subcutaneously administered vehicle containing 125 mg/kg capsaicin. This dose of capsaicin leads to a significant reduction in cardiac CGRP and substance P contents in adult rats. Rapid overflow of tachykinins from nerve endings prompted by a bolus dose of capsaicin might cause exaggerated cardiovascular and bronchial actions, so we first injected a small amount of capsaicin (1.25 mg/kg) into the animals twice in the time interval of 1 hour, and then doses were gradually increased (2.5, 5, 10, 20, 40, and 45 mg/kg) every hour. After treatment, the activity levels of these rats did not change. In 60 control rats, we injected only the inert vehicle. The rats had free access to food for 4 days, after which we measured their coronary hemodynamics. For hemodynamic measurements, we anesthetized the rats by intraperitoneal injection of 40 mg/kg sodium pentobarbital and excised the hearts. Quickly connecting the isolated rat hearts to Langendorff's apparatus, we perfused them with modified Tyrode's solution containing oxygenated bovine red blood cells and 15 g/L bovine serum albumin. The perfusate pH, PO2, Q2, saturation, Pco2, and hematocrit just before the experiment were 7.4±0.02, 143±5.3 mm Hg, 99±0.1%, 29±1.6 mm Hg, and 32±0.3%, respectively. Details of the methodology for measurement of coronary hemodynamics in the present study have been reported previously.29

The coronary pressure-flow relation was measured in decrements of 10 mm Hg, ranging from 140 to 30 mm Hg in mean perfusion pressure. In 64 of these 104 rats, coronary flow in the basal state and during 10-5 mol/L adenosine infusion was measured at a constant heart rate during right atrial pacing (pacing rate, 250 beats per minute; output, 1 V). Because electrical stimulation of rat atria at an output >3 V may evoke release of neuropeptides,7 we adhered to an output level of 1 V for atrial pacing. We chose a pacing rate of 250 beats per minute because it was >10% above the spontaneous rate (ie, 220 to 230 beats per minute; data are shown later) and seemed capable of obtaining a constant heart rate in all rats tested.

Of these 64 rats, 24 had emptied left ventricular cavities (vehicle-treated rats, n=12; capsaicin-treated rats, n=12). In the other 40 rats (vehicle-treated rats, n=20; capsaicin-treated rats, n=20), a latex balloon catheter was inserted into the left ventricle through the left atrium before coronary hemodynamics were measured. The balloon was filled with a volume of saline sufficient to produce a left ventricular end-diastolic pressure (LVEDP) of ≈5 mm Hg. In 16 of those 40 rats with a latex balloon in the left ventricle (vehicle-treated rats, n=8; capsaicin-treated rats, n=8), we inserted a small catheter into the right atrium and placed it near the coronary sinus. We took blood samples at perfusion pressures of 140, 120, 100, 80, and 60 mm Hg and then monitored left ventricular systolic pressure (LVSP), maximal value of the first derivatives of left ventricular pressure (±dP/dt), and myocardial oxygen consumption (maintaining the above-mentioned constant intraventricular saline volume during coronary hemodynamic measurements). We determined the left ventricular developed pressure by subtracting LVEDP from LVSP.

Because of the possible influence of electrical atrial pacing on the release of neuropeptides from the peptide-rich atria,7 we also measured coronary hemodynamics in the basal state at a spontaneous rhythm (228±1.5 and 230±2.9 beats per minute in vehicle- and capsaicin-treated rats, respectively) in 40 of the 104 rats (vehicle-treated rats, n=28; capsaicin-treated rats, n=12). After the measurement of basal coronary flow in these 40 hearts, we infused 10-5 mol/L adenosine into the coronary artery. Because adenosine sometimes reduces heart rate, we measured the coronary pressure-flow relation under maximal coronary vasodilatation during right atrial pacing (pacing rate, 220 beats per minute; determined to match roughly the average of spontaneous heart rates found in this experimental subgroup).

At the end of the study, all wet hearts were weighed and then dehydrated on a hot plate for 3 days to permit measurement of dry weight.

Effects of Neuropeptides on Basal Coronary Flow of Isolated Rat Hearts

In 54 control rats, we administered CGRP, substance P, and substance P(4-11), an octapeptide of substance P, to assess their effects on basal coronary flow in isolated working rat hearts with emptied left ventricular cavities. With the rat hearts at a constant perfusion pressure (80 mm Hg) and a constant heart rate (250 beats per minute), we selected one of nine different doses of each agonist (equivalent to 10-11, 10-12, 10-13, 10-16, 10-9, 10-8, 10-7, 10-6, and 10-5 mol/L of perfuse). In each rat, we infused identical doses of these three peptides of CGRP, substance P, and substance P(4-11) one by one for 10 minutes. For each dose, the order of peptide infusion was randomized. To eliminate the residual effect of each previous peptide infusion, we allowed a 20-minute washout period between infusions and continuously monitored the coronary flow during infusions and washout periods. Substance P has been reported to alter coronary flow soon after administration,18 so we carefully monitored the coronary flow during the infusion. To confirm that endothelium-dependent vasodilation was preserved in the rat hearts, at the end of the study, we administered 10-8 mol/L acetylcholine into the coronary artery. All peptides were freshly dissolved in isotonic saline just before the study, and new batches of agonists were used in each experiment.

Intracoronary Administration of Neuropeptide Antagonists

To determine whether endogenous CGRP or substance P is involved in the modulation of basal coronary flow induced by capsaicin treatment, we infused CGRP(8-37) at 10-8 and 3×10-9 mol/min for 10 minutes into the coronary arteries of 14 capsaicin-untreated and 4 capsaicin-treated isolated hearts with emptied left ventricular cavities and followed that with an infusion of isotonic saline vehicle for a 20-minute washout period. The reason for choosing these doses in this experiment is reported in “Discussion.” During the infusion of CGRP(8-37), the mean perfusion pressure was kept constant at 80 mm Hg, with atrial pacing at a rate of 250 beats per minute (output, 1 V). These doses of CGRP(8-37) led to an initial CGRP(8-37) concentration of ≈3.3×10-9 and 3×10-9 mol/L in the perfuse for a heart with a wet weight of ≈1 g. During these infusion periods, we continued to monitor changes in coronary flow. In 5 other control hearts, we infused only inert vehicle and similarly monitored the coronary hemodynamics.

In a manner similar to the infusion of a CGRP antagonist (with a fixed heart rate of 250 beats per minute and a fixed perfusion pressure of 80 mm Hg in hearts with emptied left ventricular cavities), we administered intracoronary FK888, a selective NK-1 receptor antagonist, for 10 minutes in 6 vehicle-treated and 6 capsaicin-treated rat hearts to assess the potency of the endogenous action of substance P. The doses we used 3×10-12 to 3×10-9 mol/min, were determined on the basis of the concentration used in other tissues.25,26 To confirm the antagonistic effect of FK888 in the concentrations we used, we administered 10-9 to 10-6 mol/L substance P(4-11) into 5 control hearts pretreated with 10-8 mol/L FK888 at 3×10-9 mol/min.

Histopathology and Immunohistochemistry

We studied 22 rats histopathologically to confirm the depletion of CGRP in the heart and an absence of vascular damage after capsaicin treatment. In age-matched vehicle- and capsaicin-treated rats (6 per group), we examined the myocardial structure and coronary vasculature by conventional histopa-
thology and by transmission electron microscopy. In 10 other rats, we stained CGRP-like immunoreactivity in the heart by use of an immunohistochemical method. For immunostaining, we perfused the beating heart with Zamboni’s solution\(^{29}\) at a perfusion pressure of 100 mm Hg after the inferior vena cava had been severed. Two days later, we sectioned the specimen (200-μm thickness) on a vibrating microtome (Vibratome series 1000, TPI). After washing the sections with 10⁻² mol/L phosphate-buffered saline (PBS), we pretreated them with 4% normal goat serum (NGS) at room temperature, floated them in PBS containing 2% NGS, 0.3% Triton X, and rabbit anti-rat CGRP antibody (diluted 1:3000) at 4°C, and shook them for 48 hours. We washed the sections again with PBS and added a second antibody (biotinylated anti-rabbit IgG diluted 1:200 with PBS containing 2% NGS). To visualize the specific antibody-binding sites, we used the avidin-biotin-peroxidase complex method. Five capsaicin-treated hearts were examined immunohistochemically, and five normal rat hearts were simultaneously stained as controls. The cross-reactivity of the first antibody against rat CGRP to calcitonin was <1%.

### Measurement of Tissue Water Content

To assess whether capsaicin treatment per se causes myocardial edema, we measured the myocardial content of water in 16 age-matched rats treated with vehicle or capsaicin and expressed it as water content percentage of the wet weight of the heart. For hearts excised from rats anesthetized with sodium pentobarbital, both wet weight and dry weight were determined, as described earlier.

### Drugs

Capsaicin (M-2028, 8-methyl-N-vanillyl-6-nonenamide) and adenosine (A-9251, 9β-β-rifoburanosyladenine) were purchased from Sigma Chemical Co. The antagonists against rat CGRP (CA-08-220) were purchased from Cambridge Research Biochemicals. The anti-rabbit IgG antibody kit (PK-4001) and the diaminobenzidine substrate kit (SK-4100) were purchased from Vector Laboratories, Inc. Rat CGRP (4163-s), substance P (4014-s), and human CGRP(8-37) (4232-s) were purchased from Peptide Institute, Inc. CGRP(8-37) was synthesized by conventional solution procedures, and the purity, as detected by current gradient high-performance liquid chromatography, was >99%. Substance P(4-11) (05-23-0608) was purchased from Novabiochem. FK888 was a gift from Fujisawa Pharmaceutical Co, Ltd.

### Data Analysis

Coronary flow rate was expressed as mean±SEM. The coronary pressure-flow curves in the basal state were fitted to the following equation: CF = 10⁻⁰·₃₂₆⁺0·₀₀₉·P, where CF is coronary flow rate (in milliliters per minute per gram of heart), a and b are constants, and P is coronary pressure (in millimeters of mercury). Then, ANCOVA and two-way ANOVA of pressure-flow data were followed by Tukey’s individual comparison if the F test result was <.05.

### Results

#### Histopathologic Findings

Conventional histopathology and transmission electron microscopy revealed no significant changes in myocardial structure or coronary vasculature in capsaicin-treated hearts compared with vehicle-treated hearts. There was no edema in the interstitial space, nor were there any ultrastructural changes in the capillary or in the endothelium, elastic membrane, and smooth muscle cells of the tunica media and tunica adventitia of the intramyocardial coronary artery. Immunohistochemistry of capsaicin-treated hearts, however, failed to demonstrate CGRP-like immunoreactivity in the pericor-

### Myocardial Water Content

Myocardial water content was 80.0±0.004% and 80.9±0.004% in vehicle- and capsaicin-treated animals, respectively; there was no significant difference between these values.

#### Coronary Pressure-Flow Relation

In 24 paced hearts with emptied left ventricular cavities (heart rate, 250 beats per minute), there was a significant reduction in basal coronary flow of 34.9±1.6% (mean of average reduction in perfusion pressures in which significant \(P<.05\) or \(P<.01\) flow reduction was present) in 12 capsaicin-treated rats compared with 12 vehicle-treated rats. Significant basal coronary flow reduction was observed at perfusion pressures ranging from 140 to 70 mm Hg in these 24 rats.

In 40 paced rats in which a latex balloon was inserted into the left ventricular cavity (vehicle-treated rats, \(n=20\); capsaicin-treated rats, \(n=20\)) to measure left ventricular performance and to compare metabolic demand with and without capsaicin treatment, coronary pressure-flow relations in the basal state were fitted as \(CF = 10⁻⁰·₃₂₆⁺0·₀₀₉·P\) (\(r=.99, P<.01\)) in vehicle-treated rats and as \(CF = 10⁻⁰·₄₇₄⁺0·₀₀₉·P\) (where asterisk indicates \(P<.01\)) in capsaicin-treated rats. Coronary flow was reduced by 26.6±1.2% at perfusion pressures ranging from 140 to 70 (\(P<.01\)) and 60 (\(P<.05\)) mm Hg in capsaicin-treated rats compared with vehicle-treated rats. Despite significant basal coronary flow reduction in capsaicin-treated rats, left ventricular developed pressure, peak values of left ventricular +dP/dt and −dP/dt, and myocardial oxygen consumption did not change significantly in the range of the perfusion pressures measured compared with those in vehicle-treated rats (Table). Maximal coronary flow during adenosine infusion in these 40 rats significantly increased in the range of perfusion pressures tested (140 to 40 mm Hg), and there was no difference in maximal coronary flow between vehicle-treated and capsaicin-treated rats. In subgroups (\(n=8\), each group) shown in the Table, the maximal coronary flow during adenosine infusion was slightly lower in capsaicin-treated rats compared with vehicle-treated rats, which was somewhat in contrast with data on 64 rats shown later (Fig 1). However, this difference of maximal coronary flow in 16 rats was statistically not significant.

Between paced rats with emptied left ventricular cavities (\(n=24\); vehicle-treated rats, \(n=12\); capsaicin-treated rats, \(n=12\)) and those with balloon-inserted left ventricular cavities (\(n=40\); capsaicin-treated rats, \(n=20\); vehicle-treated rats, \(n=20\)), there was a similar trend of changes in coronary pressure-flow relations with (\(n=32\)) and without (\(n=32\)) capsaicin treatment. Therefore, coronary pressure-flow relations of those 64 paced rats were summarized in Fig 1. Basal coronary flow in capsaicin-treated rats (\(CF = 10⁻⁰·₃₄₁⁺0·₀₀₉·P\) [where asterisk indicates \(P<.01\) compared with each
Coronary Hemodynamics, Left Ventricular Performance, and Myocardial Oxygen Consumption in Vehicle-Treated and Capsaicin-Treated Rats in Basal (Without Adenosine) and Maximally Vasodilated (With Adenosine) States

<table>
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<th>PP, mm Hg</th>
<th>CF, mL · min⁻¹ · wet g heart⁻¹</th>
<th>LVSP/LVEDP, mm Hg/mm Hg</th>
<th>LVDP, mm Hg</th>
<th>+dP/dt, mm Hg/s</th>
<th>−dP/dt, mm Hg/s</th>
<th>MVO₂, μL O₂ · min⁻¹ · wet g heart⁻¹</th>
<th>AVO₂, μL O₂ · mL perfusate⁻¹</th>
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PP indicates perfusion pressure; CF, coronary flow; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; +dP/dt and −dP/dt, maximal positive and negative values, respectively, of the first derivatives of left ventricular pressure; MVO₂, myocardial oxygen consumption; AVO₂, arteriovenous oxygen difference. Values are means±SEM.*P<.01 and †P<.05 compared with vehicle-treated rats in basal state at each corresponding PP value; ‡P<.01 and §P<.05 compared with each corresponding value with/without capsaicin treatment in the basal state at a given PP value.

Vehicle-treated (n=8) and capsaicin-treated (n=8) rats were paced at a rate of 250 beats per minute. Including both paced and nonpaced rats (n=104; vehicle-treated rats, n=60; capsaicin-treated rats, n=44), there was a significant reduction of basal coronary flow (P<.01) at perfusion pressures from 140 to 60 mm Hg.

Effects of Intracoronary Neuropeptides on Basal Coronary Flow

Intracoronary administration of rat CGRP at a concentration of 10⁻⁹ mol/L or higher increased coronary flow (P<.01). At 10⁻⁴ mol/L, an increase in coronary flow from baseline values was maximal (104.9±12.5% increase, filled circles in Fig 2). Substance P, however, never increased coronary flow in the concentration tested. Rather, at a high concentration of 10⁻³ mol/L, substance P slightly but significantly (P<.05) decreased coronary flow (13.9±4.7% decrease at 10⁻³ mol/L, open

Corresponding constant in vehicle-treated rats; r=.99, (P<.01) was reduced by 29.0±1.7% (P<.01) compared with that in vehicle-treated rats (CF=10⁻³mol/L+0.01-P⁰ [r=.99, P<.01]) at perfusion pressures ranging from 140 to 60 mm Hg, whereas maximal coronary flow did not differ between the two groups (Fig 1). In 40 nonpaced rats with emptied left ventricles, there were no differences in the beating rate between vehicle-treated (n=28) and capsaicin-treated (n=12) groups (228±1.5 and 230±2.9 beats per minute at perfusion pressures from 140 to 30 mm Hg, respectively). There was a significant reduction of basal coronary flow in capsaicin-treated rats (31.7±4.3%) at perfusion pressures from 140 to 70 mm Hg compared with the flow in vehicle-treated rats. This value of basal coronary flow reduction did not differ from that in paced rats described above.
circles in Fig 2). Also, substance P(4-11) decreased coronary flow at concentrations lower than those of substance P (filled triangles in Fig 2). At a substance P(4-11) concentration of 10^-5 mol/L, coronary flow was reduced by 13.9±4.5%. There was no difference in maximal value of coronary flow reduction induced by substance P and that induced by substance P(4-11).

After we completed the substance P infusion, these hearts responded to intracoronary administration of acetylcholine, which increased the coronary flow by 23.1±4.6% (P<.01).

**Effects of Intracoronary Antagonists of Peptides on Basal Coronary Flow**

Coronary flow rate in 14 control rats was reduced by 24.5±2.1% (P<.01) 10 minutes after intracoronary infusion of CGRP(8-37) at 3×10^-7 mol/min (open circles in Fig 3, left). During the vehicle-infusion washout period, the reduced coronary flow rate began to increase at 10 minutes; at 20 minutes, the coronary flow rate had recovered to 88.1±4.8% of the value before CGRP(8-37) infusion (Fig 3, left). CGRP(8-37), however, did not change coronary flow in capsaicin-treated rats (n=4, filled circles in Fig 3, left). As shown in Fig 3, right, Δ10^-7 to 10^-6 mol/L FK888 at 3×10^-12 to 3×10^-9 mol/min did not change basal coronary flow significantly in either vehicle-treated rats (n=6, open circles in Fig 3, right) or capsaicin-treated rats (n=6, filled circles in Fig 3, right) in each of the 10-minute periods tested. However, when other control rats were used, Δ10^-6 mol/L FK888 prevented the reduction of coronary flow induced by substance P(4-11). In five FK888-pretreated hearts, coronary flow changes induced by 10^-9 and 10^-8 mol/L substance P(4-11) were not significant (0±0% and -4.7±2.0%, respectively) compared with the value of coronary flow reduction induced by substance P(4-11) without FK888 pretreatment (Δ13.8±0.8% and -14.9±1.2%, respectively; Fig 2), although the concentration of 10^-6 mol/L FK888 was not enough to block coronary flow reduction at higher concentrations of substance P(4-11) [i.e., -9.3±2.4%, -11.4±3.3%, and -13.4±4.1% coronary flow changes at 10^-7, 10^-6, and 10^-5 mol/L substance P(4-11), respectively].

**Discussion**

In our isolated rat heart model, coronary flow rate in the basal condition fell by about 30% four days after systemic treatment with capsaicin; intracoronary CGRP(8-37) virtually reproduced this phenomenon. In general, capsaicin evokes a marked transient outflow of neuropeptides, such as CGRP, substance P, and neurokinin A, from nonadrenergic noncholinergic nerve endings, followed by final depletion of these peptides from neurons. Our histopathologic study revealed that capsaicin at the dose used did not induce myocardial edema, a possible cause of reduced coronary flow. Ultrastructural observation revealed no organic changes in myocardial structure or coronary vasculature after the capsaicin treatment, and immunohistochemistry failed to demonstrate CGRP-like immunoreactivity in the heart. However, vehicle-treated and capsaicin-treated hearts showed significant differences in the coronary pressure-flow relation in the basal state, but both groups displayed a similar maximal coronary flow during the infusion of adenosine. These results suggest that treatment with capsaicin caused a functional reduction in basal coronary flow via the depletion of endogenous neuropeptides.

The autonomic control of coronary circulation is well documented. Although cardiovascular actions evoked by neuropeptides stored in nonadrenergic noncholinergic nerve fibers have been reported, the magnitude of intrinsic coronary vasoaction via this nonadrenergic noncholinergic innervation has not been clarified. Capsaicin induces a Ca^{2+}-dependent release of neuropep-
tides beyond the physiological range of chemostimulation under steady-state in vivo conditions. Studies assessing nonadrenergic noncholinergic nerve function in the heart have focused on the exaggerated cardiovascular action evoked just after the administration of capsaicin. This foreign chemical–induced reaction was dose-dependently potentiated; i.e., an 10% and 60% relaxation in rat coronary arterial diameter was elicited with 10−5 and 3 × 10−5 mol/L capsaicin, respectively.12 Such cardiovascular responses to neuropeptides that are released acutely and massively by this exogenously administered chemical may not correspond to the response found in the resting state in vivo. Moreover, a simultaneous neurohormonal alteration that accompanies the release of neuropeptides with acute capsaicin treatment may modify the results attributable to capsaicin itself. This alteration, namely, an increase in circulating blood levels of catecholamines, other peptides, and cortisol, may exert chronotropic and inotropic effects that affect the coronary circulation via changes in metabolic demand as well as in coronary vascular tone. Thus, in the previous studies, it is difficult to assess how nonadrenergic noncholinergic nerve fibers govern basal coronary flow intrinsically.

To assess precisely the effect of nonadrenergic noncholinergic nerve function in modulating basal coronary flow, we depleted neuropeptides contained in the nonadrenergic noncholinergic nerve fibers by treating animals with capsaicin. The dose of capsaicin used reportedly leads to a depletion of endogenous CGRP and substance P in various vascular beds and in the heart.4,28 Because CGRP has a relatively long half-life51 and capsaicin-evoked neurohormonal changes are known to persist for 2 days in the pig,32 we measured coronary hemodynamics 4 days after the administration of capsaicin. Therefore, it is probable that the significant reduction in coronary flow observed reflected the intrinsic function of vasoactive neuropeptides, which may be continuously released in the in vivo condition as described later and which regulate basal coronary circulation. Accordingly, our results seem to elicit the magnitude of the intrinsic modulation of basal coronary flow by capsaicin-sensitive nonadrenergic noncholinergic nerve fibers in the rat.

In general, CGRP exerts positive inotropic and chronotropic effects, in addition to being a potent vasodilator.1,3-10,12 Although the ventricles may lack a CGRP-evoked inotropic response,11 it was possible that reduced contractile force could play a part in the reduction of basal coronary flow in animals depleted of endogenous CGRP. To test this possibility, we assessed the effects of capsaicin treatment on heart rate and on left ventricular performance, including myocardial oxygen consumption in subgroups of animals. We found that heart rate did not differ significantly in vehicle- and in capsaicin-treated animals. In subgroups of 16 rats, capsaicin treatment did not significantly reduce left ventricular developed pressure, peak values of left ventricular +dP/dt and −dP/dt, and myocardial oxygen consumption, although these values tended to decrease compared with vehicle-treated rats. These results make it unlikely that capsaicin-evoked changes in heart rate or myocardial metabolic demand caused a significant reduction of basal coronary flow. The major mechanism of coronary flow reduction seems to be diminution of the vasodilating effect induced by depletion of capsaicin-sensitive neuropeptides.

CGRP and substance P are representative capsaicin-sensitive neuropeptides. Endogenous CGRP and substance P are continuously transferred from neuron cell bodies to their sensory terminals at transport rates of 1 mm/h (Reference 2) and 6 mm/h (Reference 3), respectively, thereby suggesting a continual release of these peptides from the nerve endings.33 Because CGRP or substance P is locally released at the outer layer of the tunica media of the coronary arteries, local peptide concentrations at such sites presumably increase more than does CGRP or substance P concentration in the general circulation. As a result, the conventional methods used to assess the pharmacologic effects of exoge-
nously administered agonists such as substance P and CGRP may not permit a precise assessment of the physiologic effects of those endogenous neuropeptides. In vivo assessment of the vasoaction of CGRP(8-37), a CGRP antagonist, is suitable for clarifying the intrinsic action of endogenous CGRP. Intravenous CGRP(8-37) in rats reduced dermal, renal, and mesenteric blood flow by ~50%, 30%, and 50%, respectively. Results obtained in vivo in the closed circuit model suggest that endogenous CGRP regulates regional blood flow in various vascular beds. Although there is an essential difference between in vivo hearts and our Langendorff’s open circuit heart model, the concentrations of CGRP(8-37) used in the in vivo study were substantially similar to those used in the present study. The intravenous administration of CGRP(8-37) (3 × 10^{-8} mol/kg per minute) for 10 minutes significantly increased resistance in peripheral vascular beds, as mentioned above. In that study, the cumulative concentration of CGRP(8-37) in the circulating blood (~24.5 to 31.5 mL of volume per 350 to 450 g of body weight) 10 minutes after the beginning of the peptide infusion was calculated to be 4 × 10^{-6} mol/L if the biologic half-life of this antagonist was ignored. At the beginning of intracoronary infusion of CGRP(8-37) in our study, its calculated initial concentration in the perfusate was ~10^{-5} mol/L. This concentration of a CGRP antagonist may be sufficient to block both endogenous CGRP localizing in the tunica media of the arterial wall and circulating CGRP. As a result, it is likely that the magnitude of reduction of basal coronary flow after the administration of CGRP(8-37) to capsaicin-untreated rats was nearly identical to that induced by capsaicin treatment, whereas CGRP(8-37) did not reduce basal coronary flow significantly in capsaicin-treated rats, thus eliminating the possibility of nonspecific action of this CGRP antagonist. These results suggest that depletion of endogenous CGRP may contribute to the reduction of basal coronary flow that we observed in capsaicin-treated rats.

Substance P displays a dense distribution in the pericoronary artery. The coronary-dilating action of this peptide varies among species. In dogs, substance P seems to evoke opposite effects in conductive and resistance coronary vessels; an increase in coronary flow by exogenous substance P was followed by a long-lasting decrease in the flow. In rats, substance P significantly decreases vascular resistance in the peripheral circulation. In rat hearts, however, results seem to differ. Studies of the effects of substance P have been designed primarily to observe the vascular tone of the coronary rings in this species, and in these studies, substance P dilated conductive coronary vessels. When administered into the coronary artery of isolated hearts, however, this peptide does not increase the coronary flow; substance P and the octapeptide substance P(4-11), an active fragment of substance P, decreased the coronary flow by 10% to 15% in the working rat heart. Our results pertaining to the vasoaction of substance P were consistent with this previous report. Because the hearts we used responded well to the administration of acetylcholine, possible diminution of endothelial function in coronary vessels secondary to the probable vascular damage resulting from an excised perfused condition may be ruled out. From those previous reports and our present data, one explanation may be that substance P has opposite actions on resistance and on conductive coronary vessels in rats, as reported in dogs. To test this hypothesis, however, simultaneous measurements of coronary flow and conductive vessel diameter are needed.

To confirm endogenous actions of substance P in the rat heart, we used FK888, one of the most potent and selective substance P receptor antagonists. Among several antagonists available, a classic peptidic agonist was not suitable for use in our experiment because of an excessive histamine-releasing action. Recently, potent and selective tachykinin-receptor antagonists have been introduced. Among those agents, CP-96,345 has the highly specific antagonist action of an NK-1 receptor. However, a suppressive cardiovascular action of this nonpeptidic antagonist not related to an antagonistic effect has been reported. Therefore, we used a newly developed, potent, and selective substance P antagonist in our experiment. The dosages of this agent were determined on the basis of dosages used in other tissues. In another study, a concentration of 10^{-6} mol/L. FK888 antagonized a coronary flow-reducing action of substance P(4-11) at concentrations of 10^{-6} and 10^{-5} mol/L. Because of the limited fusibility of this antagonist in the vehicle, the assessment of vasoaction of substance P(4-11) at relatively high concentrations (~10^{-7} mol/L) is limited in the present study. As a result, FK888 did not change coronary flow in both vehicle- and capsaicin-treated rats (Fig 3, right). Therefore, our limited study using a new substance P antagonist in rats did not verify the assumption that endogenous substance P may modulate basal coronary arterial tone, and it is likely that substance P may not contribute to the 30% coronary flow reduction that we observed in capsaicin-treated rats.

 Autoradiographic mapping revealed that CGRP receptors in guinea pigs and humans were mainly distributed over large epicardial and medium-sized intramyocardial coronary arteries, but those receptors were relatively sparse in vessels <100 μm in diameter. Accordingly, it is quite reasonable that a marked dilation of epicardial coronary arteries by CGRP was consistently observed in pigs and humans. In contrast, there are reports that exogenously administered CGRP caused increases of 33.3% and 58.8% in the coronary flow of rats and pigs, respectively. Moreover, even a small amount of intracoronary CGRP in humans resulted in a significant increase in coronary sinus oxygen saturation, thus producing indirect evidence of reduced vascular resistance. It is considered reasonable that CGRP causes significant dilation of small and large coronary arteries. If CGRP-functioning sites correspond to the distribution of CGRP receptors, those data suggesting vasodilation in resistance vessels seem to correspond to the findings of autoradiographic mapping of CGRP receptors, because it has been reported that coronary resistance largely resides in small vessels <200 μm in diameter, and CGRP receptors are mainly found in arterial vessels >100 μm. Thus, it is probable from the present data that compared with adenosine, CGRP is a modest coronary vasodilator of small vessels.

Capsaicin-sensitive nonadrenergic noncholinergic nerves are sensory in nature. The physiological and teleological implications of basal coronary flow regula-
tion by sensory afferent fibers remain to be determined. The beating heart is the tissue in which blood flow regulation must occur in a second-to-second response to changes in myocardial oxygen demand. Although nonadrenergic noncholinergic sensory nerve fibers show dense distribution in pericoronary arteries, to what kinds of stimulations derived from the beating heart these nonadrenergic noncholinergic nerve fibers intimately correspond has not been determined. It is possible, however, that a change in demand causes some stimulus that affects the release of neuropeptides from nerve endings, taking certain time for the response. Also, the interrelation between afferent and efferent fibers is not characterized on vasoaction induced by neuropeptides, namely, the axon reflex hypothesized in other organs.\textsuperscript{42,43} To test this working hypothesis, further investigations will be needed in a system that allows researchers to control each of mechanical, chemical, and thermal stimulants in the working heart.

Our results demonstrate that capsaicin-sensitive nonadrenergic noncholinergic nerve fibers modulate basal coronary flow via the coronary-vasodilating action of neuropeptides. Among them in the neuronal system, at least, CGRP is involved for coronary flow regulation. There are, however, methodological limitations to the assessment of the intrinsic action of nonadrenergic noncholinergic nerve fibers. Several tachykinins and opioids are stored in this nervous system, but we did not examine the cardiovascular actions of those peptides in the present study. Autonomic nerves also interact with non-adrenergic noncholinergic nerve function,\textsuperscript{44,49} and it is likely that these neurogenic regulators of vascular tone interact with nonadrenergic noncholinergic nerve function in vivo. In the isolated heart model we used, however, autonomic and nonadrenergic noncholinergic neurotransmission between intracardiac fibers and upper neurons was interrupted. Consequently, our results mainly reflect control by local peptidergic innervation. Possible cardiovascular actions by central tachykinins\textsuperscript{46} cannot be estimated in our model. In addition, data obtained in the present study should be carefully interpreted in light of species differences, because the cardiovascular actions of CGRP or substance P differ among species,\textsuperscript{6,9,23,47} and within one species, the magnitude of circulatory regulation under nonadrenergic noncholinergic control differs between vascular beds.\textsuperscript{21,23} Further in vivo investigations in a variety of species are needed to characterize adequately the intrinsic function of capsaicin-sensitive nonadrenergic noncholinergic innervation and to clarify the role of endogenous CGRP and neuropeptides released from nonadrenergic noncholinergic nerve fibers in regulating the basal coronary flow.

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References

Nonadrenergic noncholinergic nerves regulate basal coronary flow via release of capsaicin-sensitive neuropeptides in the rat heart.

H Yaoita, E Sato, M Kawaguchi, T Saito, K Maehara and Y Maruyama

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