Peptidergic Innervation of Human Epicardial Coronary Arteries

S. Gulbenkian, O. Saetrum Opgaard, R. Ekman, N. Costa Andrade, J. Wharton, J.M. Polak, J. Queiroz e Melo, L. Edvinsson

The peptidergic innervation of proximal (internal diameter, >0.8 mm) and distal (internal diameter, <0.8 mm) regions of human epicardial coronary arteries was investigated by means of immunohistochemical, chromatographic, radioimmunological, and in vitro pharmacological techniques. The use of an antisera to the general neuronal marker protein gene product 9.5 revealed that the proximal part of epicardial arteries possessed a relatively sparse supply of nerve fibers forming a loose network in the adventitia. The perivascular network increased in density as the vessels were followed distally. In both proximal and distal regions, the majority of nerve fibers possessed neuropeptide Y and tyrosine hydroxylase immunoreactivity. Calcitonin gene–related peptide (CGRP)– and substance P–immunoreactive nerve fibers were very sparse in the proximal region of the arteries and increased in number distally. Only a few scattered vasoactive intestinal peptide (VIP)–immunoreactive nerve fibers were detected in both arterial regions. The use of high-performance liquid chromatography and radioimmunoassay revealed that the immunoreactive material present in coronary artery extracts closely resembled synthetic peptides. An in vitro pharmacological method demonstrated that neuropeptide Y elicited no detectable response in either proximal or distal arterial segments. In contrast, CGRP, substance P, and VIP all produced a concentration-dependent relaxation of both arterial regions. CGRP and substance P were stronger and more potent than VIP. CGRP and substance P induced a more potent response in distal compared with proximal regions of the arteries. These results suggest that the peptidergic nerves supplying human large epicardial coronary arteries may be predominantly involved in mediating vasodilation. (Circulation Research 1993;73:579–588)

KEY WORDS • human coronary innervation • neuropeptides • chromatography • radioimmunoassay • immunohistochemistry • in vitro pharmacology

Although the control of coronary blood flow and coronary vascular resistance is regulated primarily by small intramyocardial arteries, epicardial coronary arteries may also contribute to total coronary resistance.1,2 Furthermore, the observation that the vasospasm of epicardial coronary arteries is a major factor in the development of angina pectoris, myocardial infarction, and sudden death reveals that these conducting arteries have the capacity to both contract and relax.3–5 Coronary artery tone is controlled by myogenic and neurohumoral mechanisms, and it is generally considered that the neural regulation depends on the release of noradrenaline and acetylcholine from sympathetic and parasympathetic nerve terminals, respectively.6–8 However, it is now recognized that, in addition to classical transmitters, the autonomic nervous system also contains other putative transmitters, including several vasoactive peptides that may have a role in regulating the coronary as well as the systemic circulation. The main peptides identified in nerves associated with coronary blood vessels are neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), calcitonin gene–related peptide (CGRP), and tachykinins such as substance P and neuropeptide K.9,10 Recently, it has been demonstrated that a number of vasoactive neuropeptides are present within the human heart11–13 and, on coronary infusion, induce potent local responses.14–17 Few studies have, however, attempted to correlate the distribution of peptide-containing nerves and the action of neuropeptides on human epicardial coronary arteries.

In the present study, we have investigated the pattern of perivascular innervation and the effect of neuropeptides on proximal (internal diameter, >0.8 mm) and distal (internal diameter, <0.8 mm) regions of human epicardial coronary arteries. The innervation was visualized using immunohistochemical techniques on both cryostat sections and whole-mount preparations. The nature of neuropeptide immunoreactivity was examined in tissue extracts subjected to reverse-phase high-performance liquid chromatography (HPLC) and radioimmunoassay. Pharmacological studies were also performed on coronary artery preparations using a sensitive in vitro system.
Radioimmunoassay

boiled in 0.5 M NaOH; weighed, and homogenized in Polytron (Polytron homogenized to serum, pH 7.2). The homogenate was centrifuged at 2000g for 15 minutes. The supernatants were collected and lyophilized. Freeze-dried material was dissolved in 3 mL phosphate buffer (0.05 M, pH 7.5) containing 0.25% human serum albumin and centrifuged at 2000g for 15 minutes.

Immunoreactive NPY was quantified using a rabbit antiserum at a final dilution of 1:4000. This antiserum showed a 30% cross-reactivity with peptide YY but did not cross-react with bovine pancreatic polypeptide, VIP, or peptide histidine isoleucine.20 The detection limit with this assay was 25 pmol/L. Immunoreactive VIP was quantified using rabbit antiserum No. 7852 (1:72,000 dilution, MILAB, Malmö, Sweden). This antiserum recognized the NH2-terminal region (15 amino acid sequence) of the VIP molecule and did not cross-react with peptide histidine isoleucine, secretin, glucagon, gastrin inhibitory peptide, or cholecystokinin.21 With this assay, a minimum of 4 pmol/L could be detected. Immunoreactive substance P was quantified using a rabbit antiserum (SP-2, 1:500,000 dilution) that did not recognize any known tachykinin besides substance P.22 The detection limit with this assay was 10 pmol/L. Immunoreactive CGRP was quantified using a rabbit antiserum (R-8429, 1:37,500 dilution) that was raised against synthetic rat CGRP and that did not cross-react with any of the above peptides.23 The detection limit with this assay was 30 pmol/L. Each tissue extract was assayed using serial dilutions in the respective neuropeptide assay.

Materials and Methods

Immunofluorescence Staining

Epicardial segments from the left anterior and posterior descending coronary artery and its branches were obtained from postmortem cases without cardiac complications (n=4; age range, 22 to 53 years; delay between death and sampling, 1 to 3 hours). The collection of these tissues followed the ethical standards of the institutes from which they were obtained. Immediately after excision, vessel segments were fixed by immersion for 16 hours at 4°C in Zamboni’s fixative.24 After rinsing in several changes of phosphate-buffered saline (0.01 M, pH 7.2) containing 15% sucrose and 0.1% sodium azide, tissues were processed for cryostat sectioning or whole-mount preparations. Cryostat sections (15 μm thick) were immunostained using an indirect immunofluorescence method. A modified indirect immunofluorescence method was performed on whole-mount preparations of the arteries as previously described.18 Briefly, after immersion in phosphate-buffered saline containing 0.2% Triton X-100 for 2 hours, the preparations were stained with pontamine sky blue (BDH, Poole, UK) for 30 minutes to reduce background fluorescence. Preparations were then rinsed in buffer, incubated in diluted primary antiserum (Table 1) overnight at room temperature, rinsed again, and incubated with fluorescein isothiocyanate–labeled goat anti-rabbit immunoglobulin G (diluted 1:100; Sigma Chemical Co, St Louis, Mo) for 1 hour at room temperature. The preparations were finally examined using an Olympus BH-2 microscope equipped for fluorescence epi-illumination. Control conditions included the omission of the primary antiserum, replacing the primary antiserum with preimmune serum, and preabsorbing the antisera with their respective antigens (10−3 to 10−6 M).

Radioimmunoassay

Samples of the left anterior descending coronary artery were obtained post mortem (n=5; age range, 51 to 75 years; delay between death and sampling, 24 to 48 hours). Immediately after removal, the tissues were frozen to −70°C for storage. Tissue samples were weighed, boiled in 0.5 M acetic acid for 15 minutes, homogenized (Polytron for 1 to 2 minutes), and centrifuged at 2000g for 15 minutes. The supernatants were collected and lyophilized. Freeze-dried material was dissolved in 3 mL phosphate buffer (0.05 M, pH 7.5) containing 0.25% human serum albumin and centrifuged at 2000g for 15 minutes.

Immunoreactive NPY was quantified using a rabbit antiserum at a final dilution of 1:4000. This antiserum showed a 30% cross-reactivity with peptide YY but did not cross-react with bovine pancreatic polypeptide, VIP, or peptide histidine isoleucine.20 The detection limit with this assay was 25 pmol/L. Immunoreactive VIP was quantified using rabbit antiserum No. 7852 (1:72,000 dilution, MILAB, Malmö, Sweden). This antiserum recognized the NH2-terminal region (15 amino acid sequence) of the VIP molecule and did not cross-react with peptide histidine isoleucine, secretin, glucagon, gastrin inhibitory peptide, or cholecystokinin.21 With this assay, a minimum of 4 pmol/L could be detected. Immunoreactive substance P was quantified using a rabbit antiserum (SP-2, 1:500,000 dilution) that did not recognize any known tachykinin besides substance P.22 The detection limit with this assay was 10 pmol/L. Immunoreactive CGRP was quantified using a rabbit antiserum (R-8429, 1:37,500 dilution) that was raised against synthetic rat CGRP and that did not cross-react with any of the above peptides.23 The detection limit with this assay was 30 pmol/L. Each tissue extract was assayed using serial dilutions in the respective neuropeptide assay.

High-Performance Liquid Chromatography

Fractionation of NPY-, VIP-, substance P-, and CGRP-immunoreactive material from human epicardial coronary arteries (n=5 for each peptide) was carried out on an HPLC system (model 204, Waters Chromatography Division, Milford, Mass) equipped with a U6K injector, an absorption detector (No. 441), and two pumps (No. 6000 A and M-45 [radiant former]), and an automated gradient controller. A reverse-phase C18 μBondapack column (0.78×30 cm, Waters) was used. The samples were eluted with acetonitrile (CH3CN) and 0.08 M trifluoroacetic acid (vol/vol), pH 2.5, using various gradients. A linear gradient of 28% to 58% for 1 hour was used for NPY and CGRP, and a linear gradient of 28% to 35.2% for 35 minutes followed by a gradient of 35.2% to 58% for 40 minutes was used for substance P and VIP. The flow rate was 1 mL/min, and fractions of 0.5 mL were collected and lyophilized. The dried residues were redissolved in the radioimmunoassay buffer and arranged for the respective peptide-like immunoreactivity.

In Vitro Pharmacology

Epicardial segments from the left anterior and posterior descending artery and its branches were obtained post mortem (n=5; age range, 24 to 86 years; delay between death and sampling, ≤5 hours). Tissue was also obtained from a 57-year-old patient undergoing heart transplantation (for left ventricle malfunction with recurrent cardiac arrhythmia). Circular arterial segments 1 to 2 mm in length and an internal diameter of 0.3 to 2.0 mm were excised and mounted in a temperature-controlled tissue bath (37°C) containing a buffer solution comprising (mM) NaCl, 119; NaHCO3, 15; KCl, 4.6; CaCl2, 1.5; H2PO4, 1.2; MgCl2, 1.2; and glucose, 11. The buffer solution was bubbled continuously with a
mixture of 95% O₂-5% CO₂, giving a pH of approximately 7.4 as previously described. The transducer was connected to a transducer (model FT-03, Grass Instrument Co, Quincy, Mass) for continuous recording of isometric tension on a Grass polygraph. In some trials, the transducer signals were amplified by a Transbridge TBM4 amplifier, digitized by a Maclab TM analog-digital converter, and recorded by a Macintosh Plus computer. The resting tension of the vessel segments was adjusted by varying the distance between the holders, and depending on the vessel size, a tension of 1.5 to 8 mN was applied. Because of initial spontaneous relaxations of the vessel segments, it was necessary to make several adjustments to the metal holders in order to maintain a stable resting tension. After approximately 1 hour, when the tension had stabilized at the desired level, each vessel segment was exposed to a buffer solution containing 60 mM KCl, obtained by substituting equimolar concentrations of NaCl for KCl in the previously described buffer solution. Only those vessel segments that responded to a K⁺-induced contraction, which was reproducible after washing out with the normal buffer solution and showed a variation of <10% between the two contractions, were used for investigation. To study relaxant responses, prostaglandin F₂₀ (PGF₂₀) was used to induce a precontraction of the vessels. Because the length and thickness of the vessel segments varied, the tension induced by PGF₂₀ was set arbitrarily at 100% and used as an internal standard.

The vessel segments were divided into two groups according to their internal diameter. One group included vessels with an internal diameter <0.8 mm; the other group included vessels with an internal diameter >0.8 mm. To study the dilatory responses induced by CGRP, substance P, and VIP, vessel segments were precontracted with 3×10⁻⁶ M PGF₂₀, inducing a tension of 7.9±1.0 mN (n=18), 10.3±1.1 mN (n=19), and 12.5±2.6 mN (n=13) for CGRP, substance P, and VIP, respectively (mean±SEM). The tension induced by K⁺ in the same vessels was 14.3±1.6, 14.1±2.2, and 20.4±5.9 mN for CGRP, substance P, and VIP, respectively. The effect of cumulative concentrations of NPY was examined on vessel segments that were not precontracted. The K⁺-induced contraction for these vessels was 12.8±2.1 mN (mean±SEM, n=21). To further test the capability of the vessels to contract in response to stimulation of different receptors (which would strengthen the assumption of still functionally viable vessels obtained at postmortem), the contractile responses to noradrenaline and serotonin were also tested on vessel segments that were not precontracted. The K⁺-induced contraction of the vessel segments was 13.2±1.2 mN (n=33) and 11.5±1.6 mN (n=17) for noradrenaline and serotonin, respectively.

Analysis of In Vitro Data

The maximum relaxant effect obtained (Iₘₐₓ) and the negative logarithm of the concentration of agonist that elicited the half-maximum effect (pD₂) were derived from concentration-response curves on each vessel segment. Values are given as mean±SEM. The Mann-Whitney U test was used to determine statistical significance with respect to differences in Iₘₐₓ and pD₂ values. Statistical significance was assumed at P<0.05.

Drugs

The following pharmacological agents were used: human α-calcitonin gene–related peptide (α-hCGRP), NPY, substance P, VIP, noradrenaline (norepinephrine), serotonin, and cocaine (all purchased from Sigma); propranolol (Inderal, ICI-Pharma, UK); and PGF₂₀ (Amoglandin, Astra, Sweden). To minimize oxidation and prevent peptides from sticking to solid surfaces, all agents were dissolved and diluted in saline containing 10⁻⁴ M ascorbic acid and 1% bovine serum albumin.

Immunohistochemistry

Immunofluorescence staining with the antiserum to the general neuronal marker protein gene product 9.5 (PGP 9.5) showed that the proximal region (internal diameter, 0.8 to 2.0 mm) of human epicardial coronary arteries possesses a relatively sparse supply of nerve fibers and fascicles, forming a loose network in the adventitia and at the adventitial-medial border (Fig 1, a and b). The distribution pattern of the perivascular innervation was found to be similar in equivalent regions of the vessels examined. The majority of the nerve fibers displayed tyrosine hydroxylase and NPY immunoreactivity (Fig 1, c through d). The relative number and distribution of NPY-immunoreactive nerves was similar to that of nerves containing immunoreactivity for the catecholamine synthesizing enzyme tyrosine hydroxylase (Fig 1, c and d). In contrast, only a few scattered nerve fibers were detected displaying CGRP (Fig 1, e), substance P (Fig 1, f), and VIP (Fig 1, g) immunoreactivity.

In the more distal region of the epicardial coronary arteries (internal diameter, <0.8 mm), PGP 9.5 immunoreactivity was present in a more dense network of nerve fibers localized in the adventitia and at the adventitial-medial border (Fig 2, a and b). The number of nerve fibers containing tyrosine hydroxylase, NPY, CGRP, and substance P immunoreactivity also appeared to increase with declining vessel caliber (Fig 2, c through f). In contrast, nerve fibers containing VIP immunoreactivity were relatively less numerous (Fig 2, g). All perivascular nerve populations displayed an orientation that was mainly parallel to the long axis in the distal region of the vessels (Fig 2, b through g).

HPLC and Radioimmunoassay

HPLC analysis of extracts of the epicardial coronary arteries revealed that NPY-immunoreactive material eluted as one major component corresponding to that of synthetic human NPY. Some additional lower molecular weight components eluted earlier than the marker, indicating less hydrophobic fragments of NPY (Fig 3, a). VIP-immunoreactive material exhibited a complex chromatographic profile, with one small peak appearing at the same elution position as synthetic VIP (Fig 3, b). Substance P–immunoreactive material eluted as two peaks, comprising a major peak with the same retention as synthetic substance P and a smaller one with the same
elution profile as oxidized substance P (Fig 3, c). CGRP-immunoreactive material eluted in one major peak close to the elution position of synthetic α-hCGRP. Additional peaks of CGRP-immunoreactive material eluted earlier, indicating the presence of peptide fragments with less hydrophobicity than native α-hCGRP (Fig 3, d). The concentration of NPY, VIP, substance P, and CGRP immunoreactivity detected in coronary artery extracts was 8.1±3.1, 0.5±0.4, 0.3±0.1, and 1.1±0.8 pmol/g wet wt, respectively (mean±SEM, n=5). In two patients, concentrations of NPY, substance P, and CGRP immunoreactivity were determined in proximal (internal diameter, >0.8 mm) and distal (internal diameter, <0.8 mm) arterial segments; however, no significant differences were detected between the two arterial regions (data not shown).

In Vitro Pharmacology

To further clarify the constrictive potential of the artery segments obtained post mortem, the responses to serotonin and noradrenaline added in cumulative concentrations from $10^{-10}$ to $10^{-4}$ M were investigated on resting vessel segments. Serotonin induced a contraction of all the proximal (n=4; internal diameter, >0.8 mm) and distal (n=13; internal diameter, <0.8 mm) vessel segments tested. The contractions started at $10^{-7}$ M and reached the maximum at $10^{-5}$ and $10^{-4}$ M for distal and proximal segments, respectively. The maximum constrictive effect obtained ($E_{max}$), measured in percentage of the potassium-induced contraction, was 16.2±3.1 (mean±SEM) for distal segments and 35.9±9.9 for proximal segments, and the pD2 values were 6.55±0.08 and 5.17±0.18 for distal and proximal segments, respectively. Noradrenaline induced a contraction in 8 of 15 distal and 10 of 18 proximal vessel segments tested. The contractions started at $10^{-7}$ and $10^{-6}$ M in proximal and distal segments, respectively, and reached the maximum at $10^{-5}$ M in both proximal and distal segments. The $E_{max}$ values were 4.5±1.1 for distal segments and 21.8±6.0 for proximal vessel segments, and the pD2 values were 5.64±0.33 and 5.57±0.20 for distal and proximal segments, respectively. To reduce the neuronal reuptake of noradrenaline and an eventual β-receptor–induced dilatation, $10^{-6}$ M cocaine and $10^{-7}$ M propranolol were added to 11 of...
the above mentioned distal segments and to 10 of the proximal segments; however, no significant differences were observed when compared with vessel segments not incubated with cocaine and propranolol.

A total of 13 distal and proximal vessel segments (internal diameter, from 0.3 to 2.0 mm) were exposed to NPY in cumulative concentrations from $10^{-11}$ to $10^{-6}$ M, and 8 additional vessel segments were tested with concentrations up to $10^{-7}$ M. NPY elicited no detectable response in any of the 21 vessel segments examined (12 proximal segments with an internal diameter $>$0.8 mm and 9 distal segments with an internal diameter $<$0.8 mm).

The exposure of vessel segments precontracted with $3 \times 10^{-9}$ M PGF$_{2\alpha}$ to cumulative concentrations ($10^{-11}$ to $3 \times 10^{-7}$ M) of CGRP (n=18), substance P (n=19), and VIP (n=13) elicited a concentration-dependent relaxation. For substance P, the relaxation started at $10^{-10}$ M and reached its maximum at $10^{-7}$ M. For CGRP and VIP, relaxation started at $10^{-10}$ M and reached maximum at $3 \times 10^{-7}$ M. CGRP (pD$_2$, 8.30±0.23) and substance P (pD$_2$, 8.77±0.34) were equipotent, whereas VIP had a lower potency (pD$_2$, 7.52±0.24; Mann Whitney U test; $P<0.05$). Comparison of proximal (internal diameter, $>$0.8 mm) and distal (internal diameter, $<$0.8 mm) arterial segments revealed that CGRP and substance P elicited a more potent relaxation in distal than in proximal arterial segments (Table 2). For VIP, no significant differences in pD$_2$ value was observed between proximal and distal arterial segments. A tendency for CGRP, substance P, and VIP to induce stronger responses in distal segments was observed, although no significant differences were detected. $I_{max}$ and pD$_2$ values were derived from individual concentration-response curves (Fig 4 and Table 2).

**Discussion**

The present study indicates that human epicardial coronary arteries are supplied by numerous peptide-containing perivascular nerve populations. It was also observed that the number of peptide-containing nerve fibers varied with vessel caliber, with the proximal region of epicardial coronary arteries being more sparsely innervated than the distal segments. HPLC and radioimmunoassay analysis of the immunoreactive ma-

---

**FIG 2.** Cryostat section (a) and whole-mount preparations (b through g) of a human left anterior descending coronary artery (internal diameter, 0.5 mm) immunostained for protein gene product 9.5 (PGP, a and b), tyrosine hydroxylase (TH, c), neuropeptide Y (NPY, d), calcitonin gene-related peptide (CGRP, e), substance P (SP, f), and vasoactive intestinal peptide (VIP, g). a shows PGP-immunoreactive nerve fibers (arrows) localized in the adventitia (a) and at the adventitial-medial (m) border. LA indicates longitudinal axis of the vessel. Bar=50 µm.
FIG 3. High-performance liquid chromatography profiles of extracts of the human left anterior descending coronary artery (for details see “Materials and Methods”). The respective elution position of synthetic neuropeptide Y (NPY, A), vasoactive intestinal peptide (VIP, B), substance P (SP, C), or human α-calcitonin gene–related peptide (hCGRP, D) is indicated by an arrow, and the gradient is depicted by the broken line in the respective graphs.

Distribution and Action of NPY

NPY-immunoreactive nerve fibers were the most abundant of the peptide-containing nerve populations identified in human epicardial coronary arteries and have a distribution pattern broadly similar to that of nerves containing the catecholamine-synthesizing enzyme tyrosine hydroxylase. It is now generally considered that most NPY-containing nerves in the heart represent postganglionic sympathetic neurons originat-

**TABLE 2. Relaxation in Human Epicardial Proximal and Distal Coronary Arterial Segments Induced by Calcitonin Gene–Related Peptide, Substance P, and Vasoactive Intestinal Peptide**

<table>
<thead>
<tr>
<th></th>
<th>Proximal coronary artery</th>
<th>Distal coronary artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Imax (％)</td>
</tr>
<tr>
<td>CGRP</td>
<td>10</td>
<td>52.3±6.5</td>
</tr>
<tr>
<td>SP</td>
<td>12</td>
<td>49.9±8.6</td>
</tr>
<tr>
<td>VIP</td>
<td>7</td>
<td>22.8±7.4*</td>
</tr>
</tbody>
</table>

n, Number of vessels; Imax, maximum relaxant effect obtained; pD₂, negative logarithm of the concentration of the agonist that elicited the half-maximum effect; CGRP, calcitonin gene–related peptide; SP, substance P; VIP, vasoactive intestinal peptide. Values are mean±SEM. Imax values represent percentage of prostaglandin F₂α–induced contraction.

A Mann Whitney U test was used for statistical data. There was no difference in maximum responses between proximal and distal coronary arterial segments.

*VIP elicited significantly smaller responses in both types of arterial segments than those induced by CGRP or SP (P<.05).

†For proximal segments, only SP was more potent than VIP (P<.05).

‡SP and CGRP were more potent dilators of distal as compared with proximal coronary arterial segments (P<.05), whereas there was no difference for VIP.

§There was no difference in potency between SP and CGRP, but these were more potent than VIP in distal segments (P<.05).
ing in the stellate and other paravertebral ganglia, where numerous NPY-immunoreactive cell bodies have been identified. The apparent lack of NPY-immunoreactive ganglion cells in the human heart and the loss of immunostained nerve fibers in the extrinsically denervated cardiac allografts further support an extrinsic origin for this cardiac neuropeptide.

NPY has generally been regarded as a vasoconstrictor peptide; however, in this study it failed to induce an increase in the tone of epicardial coronary artery segments. This is in concert with findings by Tippins et al., in which NPY failed to induce the contraction of isolated human epicardial coronary arteries. Franco-Cereceda and Lundberg originally showed that NPY potently constricted isolated human epicardial coronary arteries; however, in a more recent study, NPY was found to have no effect on large epicardial arteries and only a weak vasoconstrictor effect on small coronary arteries. A similar response has also been found in vivo, with the local intracoronary infusion of NPY in humans inducing a constriction of intramyocardial resistance vessels rather than epicardial arteries. The action of NPY has been shown to be independent from the presence of α-adrenoceptors and is presumed to be mediated via specific receptors, namely, Y₁ and Y₂ receptors. The fact that all the vessel segments in our study responded to serotonin indicates that the tissue preparations and the 5-hydroxytryptamine (serotonin) receptors mediating the contractile effect are still functionally viable. Although this is no evidence for the presence of intact NPY receptors, it seems reasonable to assume that the failure of NPY to induce vasoconstriction is not related to tissue postmortem damage. On the other hand, the relatively poor contractile effect induced by noradrenaline suggests that the α-adrenoceptor constrictive mediated responses in human coronary arteries are of minor importance for the regulation of epicardial coronary vascular tone. Similar results were observed in a previous study on isolated guinea pig coronary arteries, in which noradrenaline induced no contractile response in either epicardial (n=6) or intramyocardial (n=6) coronary arteries and in which NPY did not induce any contraction in epicardial arteries (n=6) and induced only a weak contractile response in two of six intramyocardial arteries. The effects of NPY are complex, including the presynaptic inhibition
of noradrenaline release as well as the potentiation of noradrenaline-induced vasoconstrictor responses. The constrictive effect of NPY has been shown to be endothelium independent. The functional significance of NPY in the epicardial coronary vasculature remains to be established, but it may have a role modulating the action of other vasoactive agents.

Distribution and Action of CGRP and Substance P

Previous immunohistochemical studies have demonstrated that CGRP and substance P are often found together in afferent nerve fibers supplying the mammalian cardiovascular system, since these nerves are sensitive to the sensory neurotoxin capsaicin. We have demonstrated that both peptides are colocalized in the same secretory vesicles in perivascular nerve varicosities. They also occur together in nerve fibers supplying the human atrial appendage and may coexist in nerve fibers supplying epicardial coronary arteries.

CGRP and substance P both induced a potent vasodilator response in segments of human epicardial coronary arteries that was greater than that elicited by VIP. When comparing the response in distal and proximal regions of the arteries, CGRP and substance P showed a significantly higher pD2 value for distal segments. The finding that substance P and CGRP induce a strong vasodilator response in human epicardial coronary arteries is consistent with several other in vitro studies. Furthermore, it has been shown in vivo that substance P and CGRP both induce a potent vasodilator effect on epicardial arteries and only a weak effect on intramyocardial resistance vessels.

In coronary arteries, substance P, via neurokinin receptors localized on the endothelial cells, induces an endothelium-dependent relaxation of the vascular smooth muscle. It is generally assumed that substance P, after being released from perivascular nerves, may diffuse through the medial layer and activate receptors on the endothelial cells, which leads to the release of relaxing agents such as endothelium-derived relaxing factor (EDRF) or nitric oxide. Alternatively, it has been suggested that substance P and other vasoactive substances may be released from endothelial cells and act on their receptors on endothelial cells to cause the release of EDRF or prostaglandins. In contrast, the vasodilatory action of CGRP in human, bovine, and porcine coronary arteries is generally regarded as being endothelium independent. The demonstration of specific receptors for CGRP in the smooth muscle layer of human epicardial arteries further supports the hypothesis that CGRP acts directly on vascular smooth muscle rather than through the release of EDRF. It should be noted, however, that in a recent study of rat coronary arteries by Prieto et al, CGRP was shown to induce vasodilation by endothelium-dependent as well as endothelium-independent mechanisms. Although the physiological function of substance P and CGRP remains to be established, our observations that a network of substance P- and CGRP-immunoreactive nerve fibers is present in the adventitia and adventitial-medial border of human epicardial arteries prompt speculation that these neuropeptides may regulate epicardial coronary vascular tone when released in response to nervous stimulation.

Distribution and Action of VIP

Nerve fibers displaying VIP immunoreactivity were also observed in the adventitia of human epicardial coronary arteries but had a more limited distribution compared with other peptide-containing nerve fibers. The demonstration of VIP-immunoreactive nerve fibers supplying the human heart has previously been reported and is presumed to represent postganglionic parasympathetic or intrinsic cardiac neurons. However, the origin of VIP-immunoreactive cardiac nerves is uncertain because, although the presence of VIP-positive intracardiac neurons has been demonstrated in the dog, they have not been identified in the human heart.

The present findings demonstrate that VIP induces a potent vasodilator response in precontracted human epicardial coronary arteries in vitro. A higher Imax value and a slightly higher pD2 value was observed for VIP in distal compared with proximal regions of the epicardial arteries, but neither of these differences was significant. The finding that VIP induces vasodilation of human epicardial coronary arteries is consistent with a previous report by Franco-Cereceda and Rudyehl demonstrating that the infusion of VIP to the human left coronary artery induced direct coronary vasodilation. The demonstration of specific receptors for VIP in bovine coronary arterial membranes further supports the hypothesis that VIP is implicated in the control of coronary vasomotor tone. The vasodilator effect of VIP in isolated pig, bovine, and human coronary arteries is not dependent on the presence of an intact endothelium, thus suggesting that EDRF may not be a mediator of VIP-induced coronary vasodilation. However, in other blood vessels, such as the rat aorta and bovine pulmonary artery, VIP causes vascular dilatation by an endothelium-dependent mechanism.

Although only a few scattered VIP-immunoreactive nerve fibers were found supplying human epicardial coronary arteries, this neuropeptide may also have a role in the regulation of epicardial coronary vascular tone.

In conclusion, the results indicate that human epicardial coronary arteries are supplied by a network of neuropeptide-containing perivascular nerve fibers located in the adventitia and at the adventitial-medial border. Our results also indicate that the predominant neuropeptide-mediated response in human large epicardial coronary arteries appears to be vasodilation.

Acknowledgments

This study was supported in part by the Swedish Medical Research Council (grant 05958) and the Medical Faculty, Lund University, Sweden. Antisera raised to regulatory peptides at the Hammersmith Hospital, were produced in conjunction with Prof S.R. Bloom. We thank Mrs M.R. Alpiarça and A. Homem for excellent technical assistance.

References

Neuropeptides in Human Epicardial Coronal Arteries


Peptidergic innervation of human epicardial coronary arteries.
S Gulbenkian, O Saetrum Opgaard, R Ekman, N Costa Andrade, J Wharton, J M Polak, J Queiroz e Melo and L Edvinsson

Circ Res. 1993;73:579-588
doi: 10.1161/01.RES.73.3.579

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/73/3/579

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

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

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