Autonomic Modulation of Contractions to Endothelin-1 in Canine Coronary Arteries

Pertti Aarnio, Christopher G.A. McGregor, and Virginia Miller

Endothelin-1 contracts vascular smooth muscle and inhibits release of neurotransmitter from adrenergic and cholinergic neurons. Experiments were designed to investigate the interaction of these mechanisms in a blood vessel that receives both adrenergic and cholinergic innervation. Rings cut from canine left anterior descending coronary arteries were suspended in organ chambers for the measurement of isometric force. In some rings, the endothelium was removed. Endothelin-1 caused concentration-dependent increases in tension in all rings. During electrical stimulation (1 Hz, 9 V, 2 msec), the contractions to endothelin-1 were reduced significantly. In rings without endothelium, this decrease was greater in the presence of atropine (10^{-6} M) and was eliminated by a combination of phentolamine (10^{-5} M) and propranolol (5x10^{-7} M). Contractions to endothelin-1 during electrical stimulation in rings with endothelium were significantly less than those without endothelium. This difference was eliminated by atropine and L-N-monomethyl L-arginine (10^{-4} M). The presynaptic effects of endothelin-1 were studied by measurement of tritium-labeled norepinephrine. Phasic electrical stimulation induced release of norepinephrine; this was inhibited by endothelin-1 at high concentrations (4x10^{-7} M) in the presence of atropine. These results suggest that the major effect of endothelin-1 is postsynaptic in canine coronary arteries. However, contractions to endothelin-1 may be modulated by the level of sympathetic and parasympathetic tone. In situations in which innervation to the coronary arteries is altered, for example, in hearts used for transplantation, the contractile effects of endothelin-1 would prevail. (Hypertension 1993;21:680-686)

KEY WORDS • electric stimulation • norepinephrine • muscle, smooth, vascular • endothelins • coronary arteries • dog studies

Endothelin-1 is one of three 21-amino acid peptides named endothelin.1,2 Endothelin-1 not only contracts vascular smooth muscle,1,3 and stimulates release of vasoactive factors from endothelial cells but also inhibits presynaptic release of transmitter from adrenergic and cholinergic nerves.5,6 Postsynaptically, endothelin-1 enhances contractions initiated by activation of sympathetic nerves on mesenteric arterial smooth muscle.7 The interactions between the presynaptic and postsynaptic effects of endothelin-1 in a blood vessel receiving both adrenergic and cholinergic innervation are not known. Therefore, experiments were designed to determine the interactions between the presynaptic and postsynaptic effects of endothelin-1 in the canine coronary artery, a blood vessel receiving both sympathetic and parasympathetic innervation.

**Methods**

All animal care and use was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Random source crossbred hound dogs (male and female, 15–20 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and exsanguinated from the carotid arteries. The hearts were removed, and the left anterior descending coronary arteries were dissected free and placed in modified Krebs-Ringer bicarbonate solution (millimolar composition: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, calcium disodium edetate 0.26, and glucose 11.1; control solution).

**Organ Chamber Studies**

After removal of connective tissue, the arteries were cut into rings 4–5 mm long. The endothelium was removed by gently rubbing the luminal surface with the tip of a pair of watchmaker’s forceps.8 In some experiments, the endothelium was not removed deliberately. The rings were suspended between a fixed stirrup and force transducer for the measurement of isometric force in 25-mL organ chambers filled with the control solution at 37°C and bubbled with 95% oxygen and 5% carbon dioxide.

All rings were equilibrated at a passive tension of less than 2 g for 10 minutes. The rings were placed at the optimal point on length–tension curves by progressively stretching the rings and determining contractions to 20 mmol/L potassium chloride at each level of stretch. At
optimal length (basal tension), rings were incubated with either control solution alone, atropine \((1 \times 10^{-5} \text{ M})\), propranolol \((5 \times 10^{-6} \text{ M})\), phenolamine \((1 \times 10^{-6} \text{ M})\) plus propranolol, a combination of atropine and phenolamine plus propranolol, tetrodotoxin \((10^{-6} \text{ M})\), or \(N\text{O}^-\text{monomethyl L-arginine (L-NMMA, 10}^{-4}\text{ M})\) for at least 30 minutes before the next pharmaceutical agent was tested. Responses in the absence and presence of inhibitors were obtained in parallel on separate rings obtained from the same dog. Maximal contractions to KCl \((60 \text{ mmol/L})\) were measured in the absence and presence of inhibitors.

Transmural electrical field stimulation of arterial rings was accomplished with platinum electrodes placed parallel to the rings. Frequency–response curves to electrical stimulation \((0.2–16 \text{ Hz, 9 V, 2 msec})\) were obtained from optimal tension in the absence and presence of inhibitors.

After an equilibration period of 20 minutes, cumulative concentration–response curves were obtained to endothelin-1 \((1 \times 10^{-11} \text{ to } 10^{-7} \text{ M})\) in the presence and absence of electrical stimulation.

In separate experiments, concentration–response curves to endothelin-1 were obtained in the presence of isoproterenol \((1 \times 10^{-4} \text{ and } 1 \times 10^{-7} \text{ M})\).

### Superfusion Studies

In separate experiments, longitudinal strips of left anterior descending coronary arteries without endothelium were incubated 120 minutes in \([\text{H}]\text{norepinephrine (1} \times 10^{-7} \text{ M})\). After incubation, the strips were suspended at 2 g tension and superfused by means of a roller pump at 3 mL/min with aerated \((95\% \text{ oxygen, } 5\% \text{ carbon dioxide})\) control solution at \(37^\circ\text{C}\). For electrical stimulation, two platinum wires \((0.5 \text{ mm in diameter, } 10 \text{ cm long})\) were placed parallel to and in contact with the strips. Electrical impulses were 1 Hz, 10 V, and 2 msec.

After a 60-minute washout period, the superfusate was collected for 2-minute intervals by means of a fraction collector for measurement of the efflux of total radioactivity. One of two protocols was followed. The first protocol was designed to determine the effects of endothelin-1 on tonic release of autonomic transmitter. The strips were stimulated electrically for 14 minutes, and endothelin-1 \((1 \times 10^{-9}, 10^{-8}, \text{ or } 4 \times 10^{-7} \text{ M})\) was infused for an additional 14 minutes during the stimulation. The electrical stimulation continued for another 14 minutes after the cessation of the endothelin infusion. The second protocol was designed to determine the effects of endothelin-1 in phasic release of autonomic transmission. The strips were electrically stimulated for 6 minutes, after which they were superfused with endothelin-1 \((4 \times 10^{-7} \text{ M})\) for 24 minutes. The strips were again stimulated electrically at the end of endothelin-1 administration for 6 minutes. After a 32-minute washout period, the strips were electrically stimulated for another 6 minutes.

At the end of each experiment, the arteries were blotted dry and weighed. The strips were dissolved in Soluene 350 (Packard Instrument Co., Inc., Downer's Grove, Ill.) to determine the remaining tritium content in each tissue. Samples \((1 \text{ mL})\) of the perfusate were added to 10 mL Ultima Gold (Packard Instrument Co., Inc., Meriden, Conn.) and placed in a liquid scintillation counter (LS 9800, Beckman Instruments, Inc., Fullerton, Calif.) for measurement of radioactivity.

### Drugs and Chemicals

The following drugs were used: atropine (Sigma Chemical Co., St. Louis, Mo.), endothelin-1 (Peptides International, Inc., Louisville, Ky.), L-NMMA (Calbiochem Corp., La Jolla, Calif.), levo-[7]H(N)norepinephrine (specific activity, 2.7 Ci/mmol; New England Nuclear, Boston), phenolamine mesylate (CIBA-GEIGY, Somerset, N.J.), propranolol (Sigma), and tetrodotoxin (Sigma). Drugs were prepared daily in distilled water and kept chilled. Drugs were added to the organ chamber in volumes less than 0.5 mL.

Concentrations of drugs are expressed as final molar (moles per liter) concentrations \((M\text{ represents mol/L})\) in the organ bath or superfusion solution.

### Calculations and Statistical Analysis

All data are expressed as mean±SEM; \(n\) is the number of dogs from which tissue was taken. For organ chamber experiments, data are presented as grams tension. Responses to endothelin-1 during electrical stimulation were tested in parallel on rings with and without endothelium and in the absence and presence of the various inhibitors. Where appropriate, the effective concentrations causing 50\% of the maximal response \((ED_{50})\) were calculated for individual concentration–response curves, and the mean of these values is reported as the negative logarithm of the molar concentration. For superfusion experiments, the data are presented as fractional release of tritiated compounds calculated as the ratio of disintegrations per unit of time to the total tissue content. Statistical analysis was by one-way analysis of variance. When more than two means were compared, a significant \(F\) value was obtained, and post hoc Scheffe's test was used to identify differences among groups. A value of \(p<0.05\) was considered statistically significant.

### Results

#### Organ Chamber Experiments

In rings without endothelium, potassium chloride increased tension comparably; maximal contractions to potassium chloride \((60 \text{ mmol/L})\) were \(5.2±0.7\ g\) in control rings \((n=13)\). Comparable contractions to KCl were obtained in rings incubated with atropine or phenolamine plus propranolol. In the absence of electrical stimulation, endothelin-1 caused concentration-dependent contractions in all rings. Maximal tensions \((5.6±0.9\ g\text{ in control rings})\) to endothelin-1 were reached at a concentration of \(3 \times 10^{-5} \text{ M}\). These contractions were not significantly altered by atropine, phenolamine plus propranolol, or atropine and phenolamine plus propranolol (Figure 1).

Electrical stimulation caused frequency-dependent decreases from basal tension in all groups. The maximal decreases during electrical stimulation at 16 Hz ranged from \(0.52±0.11\ g\) in control rings to \(1.02±0.17\ g\) in rings incubated with atropine and phenolamine plus propranolol \((n=11)\). This difference was statistically significant \((p<0.05)\). Electrical stimulation at 1 Hz caused relaxation in all rings; however, these relaxations were not different statistically \((-0.4±0.1\ g, n=12\text{ in control})\).
During electrical stimulation (1 Hz, 9 V, 2 msec) the concentration–response curves of endothelin-1 in rings without endothelium were shifted significantly to the right compared with those obtained in the absence of electrical stimulation. The threshold for contraction was increased from $10^{-9}$ M to $10^{-8}$ M, and the maximal tensions were reduced significantly from $5.3 \pm 0.9$ (n=6) to $2.9 \pm 0.9$ g (n=10) in the absence of antagonists (Figures 1 and 2). During electrical stimulation, contractions to endothelin-1 were inhibited in the presence of atropine (Figure 2). Propranolol significantly increased contractions to endothelin-1 (10$^{-8}$ M) during electrical stimulation from $0.5 \pm 0.3$ (n=10) to $2.3 \pm 0.6$ g (n=7); maximal tensions (endothelin-1, 10$^{-7}$ M) were not significantly different in the absence (2.9±0.9 g, n=10) or presence (4.2±1.1 g, n=7) of propranolol. The $\text{ED}_{50}$ ($-\log M$) for contraction to endothelin-1 in the presence of propranolol was $7.8 \pm 0.1$ (n=7). In rings treated with phentolamine plus propranolol or atropine and phentolamine plus propranolol, contractions to endothelin-1 in the presence of electrical stimulation were not different from those obtained in the absence of electrical stimulation (Figures 1 and 2).

In four of six additional experiments, contractions to endothelin-1 (10$^{-8}$ M) during electrical stimulation were increased from $0.7 \pm 0.5$ to $2.6 \pm 1.4$ g (n=4) in the presence of tetrodotoxin ($10^{-6}$ M).

In rings with intact endothelium, in the absence of electrical stimulation, contractions to endothelin-1 were not different from those of rings without endothelium (data not shown, n=4). During electrical stimulation, contractions of rings with endothelium were not different statistically from those of rings without endothelium (Figure 3). However, in the presence of phentolamine plus propranolol during electrical stimulation, contractions of rings with endothelium were significantly less than contractions of rings without endothelium [ED$_{50}$].

**Figure 1.** Line graph shows contractions to endothelin-1 in canine left anterior descending coronary arteries without endothelium. Contractions were concentration dependent and not significantly altered by atropine, phentolamine plus propranolol, or atropine and phentolamine plus propranolol. Data are shown as gram increase in tension and expressed as mean±SEM.

**Figure 2.** Line graph shows contractions to endothelin-1 in canine left anterior descending coronary arteries without endothelium during electrical stimulation (1 Hz, 9 V, 2 msec). During electrical stimulation, concentration–response curves of endothelin-1 in rings incubated only in control solution (□) were inhibited compared with those obtained in the absence of electrical stimulation (see Figure 1). Atropine ($10^{-6}$ M) increased the threshold for contraction during electrical stimulation (□). In rings treated with phentolamine ($10^{-6}$ M) plus propranolol ($5 \times 10^{-6}$ M) or atropine and phentolamine plus propranolol, contractions to endothelin-1 were significantly shifted to the left (p<0.05, analysis of variance of concentration causing 50% of maximal response) and were not different from contractions to the peptide in the absence of electrical stimulation (see Figure 1). Data are shown as gram increase in tension and are expressed as mean±SEM.
In the presence of:

Phentolamine, 10^{-6} M

and propranolol, 5 \times 10^{-6} M

Atropine, 10^{-6} M plus phentolamine and propranolol

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Line graphs show contractions to endothelin-1 in canine left anterior descending coronary arteries with and without endothelium incubated in either control solution (left panel), phentolamine (10^{-6} M) and propranolol (5 \times 10^{-6} M) (middle panel), or atropine (10^{-6} M) plus phentolamine (10^{-6} M) and propranolol (5 \times 10^{-6} M) (right panel) during electrical stimulation (1 Hz, 9 V, 2 msec). Data are shown as gram increase in tension and are expressed as mean\pm SEM. Contractions of rings with endothelium were significantly less than rings without endothelium only in the presence of phentolamine (10^{-6} M) and propranolol (5 \times 10^{-6} M). The ED_{50} (–log M) for contraction was 7.9\pm0.1 and 8.3\pm0.1, n=4, in rings with and without endothelium, respectively (Student’s t test for paired observations, p<0.05).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Line graphs show contractions to endothelin-1 in canine left anterior descending coronary arteries with and without endothelium incubated with phentolamine (10^{-6} M) and propranolol (5 \times 10^{-6} M) in the absence (left panel) and presence (right panel) of \textsuperscript{L}-Nmethyl L-arginine (10^{-4} M) during electrical stimulation (1 Hz, 9 V, 2 msec). Data are shown as gram increase in tension and are expressed as mean\pm SEM. Significant difference as noted by asterisks (area under the curve, one-way analysis of variance, p<0.05) between contractions of rings with and without endothelium was eliminated by the arginine analogue.}
\end{figure}
In the presence of $10^{-8}$ M atropine, $9^{\text{o}}$ I o.s Endothelln, $10^{\text{s}}$ M Electrical stimulation, 1 Hz

**FIGURE 5.** Plots show endothelin-1 and release of $[^3\text{H}]$norepinephrine in canine left anterior descending coronary arteries without endothelium. When endothelin-1 ($1 \times 10^{-8}$ M) was administered during tonic electrical stimulation (1 Hz), there was no significant effect on tritium overflow in the presence (right panel) or absence (left panel) of atropine ($1 \times 10^{-6}$ M).

vation to that artery. The postsynaptic effect of endothelin-1 in the canine left anterior descending coronary artery in the absence of autonomic activation is contraction. This observation is consistent with the effects of endothelin-1 in canine left circumflex coronary arteries. Endothelin-1 probably does not interact with postsynaptic adrenergic receptors in this blood vessel, as the contractions to the peptide were not modified by either $\alpha$- or $\beta$-adrenergic antagonists in the absence of electrical stimulation. However, during electrical stimulation, the contractions to endothelin-1 are inhibited. This inhibition is probably neuronally mediated, as tetrodotoxin and adrenergic and cholinergic receptor antagonists modify the effects of electrical stimulation. In canine coronary arteries, electrical field stimulation releases transmitters from both adrenergic and cholinergic nerve endings. Norepinephrine release causes relaxation by activation of $\beta$-adrenergic receptors. It is likely that during electrical stimulation, stimulation of $\beta$-adrenergic receptors inhibits contractions to endothe-

**FIGURE 6.** Bar graphs show release of tritium in canine left anterior descending coronary arteries without endothelium in the absence (top panel) and presence (bottom panel) of endothelin-1 (ET-1; $4 \times 10^{-7}$ M). Phasic electrical stimulation (ES) significantly increased tritium overflow during two consecutive periods of ES (top panel). ES did not increase overflow of norepinephrine when basal overflow was high (bars 1 and 2, bottom panel). The amount of tritium overflow in the absence of ES was decreased significantly with infusion of ET-1 (bottom panel, bar 3). Each bar represents average (mean±SEM, $n=4-6$) tritium overflow during three consecutive 2-minute collection periods immediately before and during 6 minutes of ES. *Significance from overflow before ES under control conditions ($p<0.05$, one-way analysis of variance).
This conclusion is supported by the observations that during electrical stimulation, contractions to endothelin-1 are enhanced during adrenergic blockade and that the β-adrenergic agonist isoproterenol inhibits contractions to the peptide. Inhibition of contractions to endothelin-1 by β-adrenergic agonists in canine coronary arteries is consistent with what has been observed in porcine coronary arteries.

Potentiation of contractions to autonomic stimulation by endothelin-1 has been described in the mesenteric circulation. The results of the present study extend those observations from the mesenteric circulation to suggest that the combined postsynaptic effects of autonomic activation and endothelin-1 depend on the anatomic origin of the blood vessel and the net effect (stimulation or inhibition) of neuronal activation.

Acetylcholine released from parasympathetic nerve endings stimulates muscarinic receptors on sympathetic nerve endings inhibiting release of norepinephrine. When the muscarinic receptors were blocked by atropine in the present study, the threshold for contraction to endothelin-1 was increased during electrical stimulation. This is probably due to the increased release of sympathetic transmitter when the presynaptic effects of acetylcholine are blocked. Increased release of tritium during electrical stimulation of superfused arteries in the presence compared with the absence of atropine supports this conclusion. Alternatively, atropine could block cholinergic postsynaptic receptors that cause contraction of the smooth muscle.

The results of the present study also suggest that the contractions to endothelin-1 are modified by the endothelium during neuronal activation. In the presence of electrical stimulation, contractions to endothelin-1 were less in the rings with than without endothelium when tissue was incubated with phentolamine and propranolol. Acetylcholine released from nerve endings during electrical stimulation may cause release of inhibitory vasoactive factors from the endothelium. Alternatively, electrical stimulation per se may release such relaxing factors from the endothelium, which may be detected only when more potent relaxations mediated by sympathetic stimulation are blocked. Nitric oxide is probably the mediator of the inhibitory responses when endothelial cells are present during electrical stimulation, because the difference in contractions to endothelin-1 between rings with and without endothelium is reduced by an analogue of L-arginine.

Binding sites for porcine endothelin-1 have been detected in cultured rat vascular smooth muscle cells. In addition, binding sites of endothelin-1 have been found autoradiographically in organs other than vascular tissue. Endothelin has a neuromodulatory effect presynaptically, suggesting the existence of endothelin receptors on neuronal terminals. Wiklund et al showed that endothelin-1 inhibited the stimulation-induced fractional release of radiolabeled norepinephrine in the guinea pig femoral artery. Endothelin also inhibits the stimulated release of [3H]acetylcholine, whereas contractile responses to exogenous acetylcholine were enhanced in guinea pig ileum. The present study confirms the inhibition of norepinephrine release by endothelin-1 at the presynaptic level in canine coronary arteries. However, the inhibition was significant only at relatively
high concentrations of the peptide \( (4 \times 10^{-7} \text{ M}) \). It is not clear whether the presence or absence of the endothelium represents an important difference between the sensitivity of the presynaptic effects of endothelin-1 observed in the present study and those from others, as other endothelium-derived factors also inhibit adrenergic neurotransmission. Low sensitivity of the presynaptic receptor for endothelin-1 in the canine coronary artery suggests that this may not be of class A subtype. In the present study, presynaptic inhibition by endothelin-1 was observed with basal overflow of transmitter and with phasic electrical stimulation in the absence of atropine. Therefore, the endothelin receptors on the nerve terminals may be associated with intracellular events required before vesicular release of transmitter and share a common mechanism of action with muscarinic receptors.

In summary, the results suggest that in canine coronary arteries, contractions to endothelin-1 may be modulated by the level of sympathetic and parasympathetic tone. If these results can be generalized to human coronary arteries, then endothelin-1 may have more profound effects on vascular resistance in denervated hearts, for example, those used for transplantation.

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References

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