Effects of Endothelin on Coronary Flow, Mechanical Performance, Oxygen Uptake, and Formation of Purines and on Outflow of Prostacyclin in the Isolated Rabbit Heart

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Endothelin is a 21-residue peptide formed during incubation of isolated porcine endothelial cells. Due to its pronounced vasoconstrictor activity, endothelin has been proposed to play a role in the regulation of vascular tone. We studied the effects of synthetic endothelin (0.1–10 nM) on coronary flow, mechanical performance, myocardial oxygen uptake, and formation of purines, and outflow of 6-ketoprostaglandin F₁α (metabolite of prostacyclin) in rabbit hearts perfused with saline by the Langendorff method. Endothelin dose-dependently decreased the coronary flow, at 10 nM, by about 75%. Heart rate, ventricular contractility, myocardial oxygen uptake, and purine release were affected by endothelin no more than by a corresponding mechanical reduction of the coronary flow. In contrast, the diastolic relaxation appeared to be directly diminished by endothelin. The concentration of 6-ketoprostaglandin F₁α in the cardiac effluent was dose-dependently elevated by about 14 times by endothelin (10 nM) (p <0.001). A corresponding mechanical restriction of the coronary flow insignificantly affected the effluent concentration of 6-ketoprostaglandin F₁α. The calcium channel blocker nifedipine (1 μM) completely abolished the decrease in diastolic relaxation induced by endothelin and markedly counteracted the peptide-induced increase in effluent concentration of 6-ketoprostaglandin F₁α, but did not affect the vasoconstrictor activity. These data demonstrate that endothelin induces vasoconstriction and facilitates the outflow of prostacyclin in the rabbit heart. In addition, the peptide appears to affect diastolic relaxation in this organ. (Circulation Research 1990;66:46–54)

In the last few years it has become increasingly apparent that the vascular endothelium may modulate the tone of the blood vessels by producing powerful vasodilators (endothelium-derived relaxing factor and prostacyclin) or vasoconstrictor(s) (endothelium-derived constricting factor).1,2 In comparison to the extensive studies performed to characterize the actions and nature of endothelium-derived relaxing factor, relatively little is known about endothelium-derived constricting factor. Endothelium-dependent contractions have been demonstrated in response to different stimuli such as hypoxia,2 pressure changes,3 electrical stimulation,4 acetylcholine,5 and arachidonic acid.6 Also, a basal release of endothelium-derived constricting factor has been reported from cultured endothelium, suggesting a physiological role for the compound.7 The chemical identification of endothelium-derived constricting factor is still under investigation, and both peptide7 and prostanoïd5,8 structures of endothelium-derived constricting factor have been proposed.

Recently a novel vasoconstrictor agent was isolated from the supernatant of cultured porcine endothelial cells. It was identified as a 21-residue peptide and named endothelin.8 Endothelin contracts isolated vessels from several organs and species, with a reported EC₅₀ at least one order of magnitude lower than that of other known vasoconstrictors.8,9 Endothelin is a vasoconstrictor also in vivo, as shown in denervated rats.8 Besides, the peptide has been reported to stimulate the secretion of atrial natriuretic peptide from cultured rat atrial myocytes.10 The extent to which endothelin accounts for all endothelium-derived constricting factor reported earlier2–7 is still uncertain.

In the present study, the actions of endothelin on mechanical performance and coronary flow in the
isolated rabbit heart were investigated. In addition, the possible effect of endothelin on the formation of prostacyclin in the heart was studied.

Materials and Methods

Perfusion of Rabbit Hearts

A total of 52 New Zealand White rabbits of either sex, weighing 1.5–2.7 kg, were used for the study. After injection of heparin (1,000 IU/kg) into a marginal ear vein the animals were stunned by cervical dislocation and subsequently exsanguinated through the left carotid artery. A catheter was rapidly inserted into the aorta, allowing coronary perfusion to be quickly reestablished. The pulmonary artery was also cannulated. The heart was then dissected out and transferred to the perfusion apparatus, in which it was perfused according to Langendorff at a pressure of 5.6 kPa and a temperature of 38°C with a modified Tyrode’s solution of the following millimolar composition: NaCl 137, KCl 2.7, CaCl2 1.8, MgCl2 1.0, NaHCO3 11.9, Na2HPO4 0.4, EDTA 0.01, and glucose 5.6. The solution was aerated with 3% CO2 in O2, giving a final pH of 7.3 and PO2 of 90 kPa.

The apex of the heart was attached to a lever connected to an isotonic transducer (Harvard Apparatus, Millis, Massachusetts). The signal was recorded on a polygraph (model 7D, Grass Instruments, Quincy, Massachusetts) and calibrated in millimeters. Quantitative estimation of the systolic apical movement of the heart, as well as of the end-diastolic position of the apex, was performed from the chart. The coronary flow rate was continuously followed by timed collection of the effluent in preared beakers, which were weighed on an ordinary laboratory scale.

Protocol

After an initial stabilization period that lasted for about 30 minutes, the experiment was started by collection of basal effluent during 3 minutes. Then, a 5-minute infusion of endothelin (Peninsula Labs, Belmont, California) commenced, followed by a 30-minute perfusion without the drug. Endothelin was dissolved in saline and added directly to the perfusate, giving a final concentration of 0.1 nM (four hearts), 1 nM (eight hearts), 5 nM (five hearts), or 10 nM (eight hearts). Five hearts were given endothelin (10 nM) together with nifedipine (1–10 μM; Bayer AG). Another six hearts were given endothelin (5 nM) together with indomethacin (50 μM; Sigma Chemical, St. Louis, Missouri), and six more hearts were given endothelin (5 nM) together with aminophylline (0.1 mM; Teofyllamin ACO, Solna, Sweden). In a separate group of experiments, 10 hearts were subjected to a mechanical flow reduction by introduction of a roller pump into the perfusion system. In this series, the hearts were exposed in random order to each of four 5-minute periods of reduced coronary flow, amounting to 83%, 59%, 37%, or 25%, respectively, of their spontaneous coronary flow. The flow reduction periods were separated by 20-minute periods of perfusion at normal flow rate. The magnitude of the flow reductions were selected to give flow rates corresponding to those obtained in the series of hearts exposed to endothelin, which endothelin (0.1 nM) reduced the flow to 83% of control and endothelin (1, 5, and 10 nM) reduced the flow to 59%, 37%, and 25%, respectively, of control coronary flow.

Samples for analysis of purines and of 6-keto-prostaglandin F1α (6-keto-PGF1α) in the cardiac effluent were drawn during the basal perfusion period and after 3–5 minutes of exposure to endothelin or mechanical flow reduction. Samples for analysis of PO2 in the cardiac effluent were taken from the pulmonary artery catheter during the basal perfusion period and at the end of the endothelin exposure or mechanical flow reduction.

Analyses

All effluent samples were collected on ice. After volume determination, separate portions of the effluent for analysis of purines and of 6-keto-PGF1α were frozen and kept at −20°C until analysis.

Purines were analyzed in unextracted samples as adenosine, inosine, and hypoxanthine by use of liquid chromatography with adsorbance detection.11 Since the proportion of adenosine, inosine, and hypoxanthine varied between the samples, the sum of these three nucleotide metabolites is presented.

The prostacyclin metabolite 6-keto-PGF1α was analyzed with a stable isotope dilution technique using gas chromatography/mass spectrometry as follows. After addition of a tetradeuterated internal standard of 6-keto-PGF1α (1 ng/ml) a 2.5-ml portion of the sample was treated for 40 minutes with 30 mg methoxyamine hydrochloride dissolved in 0.75 ml acetate buffer (1.5 M, pH 4.8). After acidification, the reaction products were adsorbed on a disposable C18 cartridge, washed, and eluted with 2 ml ethyl acetate. After evaporation the residue was dissolved in 1 ml of 15% isopropanol in ethyl acetate (vol/vol) and applied to a disposable silica cartridge. After washing, the column was eluted with 2 ml of 40% isopropanol in ethyl acetate (vol/vol). After evaporation to dryness, the sample was converted to its pentafluorobenzyl ester, dried, and dissolved in 2 ml of 45% ethyl acetate in hexane (vol/vol). It was subsequently adsorbed to a dihydroxy column (2-OH; Analytichem), washed, and eluted with 2 ml of 60% ethyl acetate in hexane (vol/vol). After evaporation the residue was converted to its trimethylsilyl ether.

On analysis, the sample was dried and dissolved in 10 μl hexane. A 5-μl portion was injected in a Varian (Harbor City, California) 3400 gas chromatograph equipped with a 20-m medium polarity capillary column and operated at 275°C. The gas chromatograph was connected to a Finnigan Incos 50 mass spectrometer operated in the negative ion/chemical ionization mode with methane as reactant gas. Selective ion monitoring was performed at a mass-charge...
ratio (m/z) of 614 for the sample content of 6-keto-PGF$_{1a}$ and at m/z=618 for the tetradeutered internal standard.

Calculations

Heart rate was calculated from the recording chart. The systolic apical movement and the endothelin-induced change in diastolic apical position were also calculated from this chart and expressed in millimeters.

All data are presented as mean±SEM. Figures within brackets indicate number of experiments. Standard procedures have been used for calculation of statistical differences. A value of p≤0.05 has been considered as significant.

Results

The mean wet weight of the hearts was 5.8±0.1 g. After the stabilization period the basal coronary flow was 38±2 ml/min (n=24). The spontaneous beating rate was 163±6 beats/min (n=24). The systolic apical movement (2–3 mm) and the diastolic apical position were stable during the basal period. The fractional oxygen extraction was 0.51±0.04, and the myocardial oxygen consumption was 3.2±0.2 µmol/min/g heart weight. The pH of the effluent was 7.20±0.03.

Infusion of endothelin (0.1–10 nM) induced a dose-dependent decrease in coronary flow (Figure 1a). At the end of the 5-minute exposure to endothelin the coronary flow was reduced to 83±6% (0.1 nM, p<0.05), to 59±6% (1 nM, p<0.001), to 37±3% (5 nM, p<0.001), and to 25±6% (10 nM, p<0.001), respectively, of the basal coronary flow. The onset of the coronary vasoconstrictor action was rapid; after 1 minute of exposure to endothelin (10 nM), the flow was reduced to 44±7% (p<0.001, Figure 1b). After the end of the exposure the vasoconstrictor activity was slowly and incompletely reversed; after 30 minutes of washout, the coronary flow was still reduced to about 45% in comparison to basal flow (Figure 1b). Bolus injection of 0.1 mmol nitroprusside (Nipride, Roche Laboratories, Nutley, New Jersey) early during the washout period to hearts previously exposed to endothelin (5 nM) increased the coronary flow from 36±6% to 73±17% (n=4) of the basal (preendothelin) flow (see also Figure 2).

In parallel to the vasoconstriction, endothelin at 10 nM dose-dependently lowered the spontaneous heart rate by 29±7%. The decrease in heart rate during endothelin exposure did not differ from that elicited by a quantitatively similar mechanical flow reduction only (Figure 3).

The systolic apical movement was insignificantly affected by endothelin and did not differ from that induced by a quantitatively similar mechanical reduction in coronary flow (Figures 2 and 4).

The end-diastolic apical position was dose-dependently changed toward less efficient relaxation by endothelin. A quantitatively similar mechanical reduction in coronary flow elicited no more than a minor effect on the diastolic apical position (Figures 2 and 4).

The difference between endothelin and mechanical flow reduction in impairing the diastolic relaxation is highly significant (p<0.001, two-way analysis of variance).

Endothelin (10 nM) increased the oxygen extraction of the heart to 80±3% (p<0.02), decreased the oxygen uptake to 1.1±0.3 µmol/min/g wet wt (p<0.01), and decreased the pH of the effluent to 6.9±0.06 (p<0.025). Hence, despite a more efficient myocardial extraction of oxygen, the uptake of oxygen in the heart decreased during the endothelin-induced coronary vasoconstriction (Figure 3). The basal outflow of purines was 38.5±6.2 nmol/3 min, and the basal concentration of purines in the effluent was 0.34±0.06 µM. Infusion of endothelin (0.1 or 1 nM) did not affect the outflow or the effluent concentration of purines. Endothelin (5 and 10 nM) increased the outflow of purines about four times (p<0.05, Figure 3). The concentration of purines in the effluent was also elevated by endothelin, about
When endothelin (5 nM) was given together with aminophylline (0.1 mM) or indomethacin (50 μM), coronary flow, mechanical performance, or outflow of purines did not differ from the corresponding values observed after administration of endothelin only (Table 1).

The basal outflow of 6-keto-PGF$_{1\alpha}$ was 30.4±4.4 ng/3 min, and the basal concentration of 6-keto-PGF$_{1\alpha}$ in the cardiac effluent was 0.26±0.04 ng/ml. Exposure to endothelin (1–10 nM) elicited an increase in the outflow of 6-keto-PGF$_{1\alpha}$ from the heart ($p<0.001$, two-way analysis of variance), while a corresponding mechanical reduction of the flow had no such effect. Furthermore, the concentration of 6-keto-PGF$_{1\alpha}$ in the effluent was elevated more by endothelin than by a corresponding reduction in flow ($p=0.001$, two-way analysis of variance, Figure 7).

Nifedipine (1–10 μM) efficiently counteracted the increase in effluent concentration 6-keto-PGF$_{1\alpha}$ induced by endothelin (10 nM, Figure 8), and as a consequence, also the increased outflow of 6-keto-PGF$_{1\alpha}$ from the heart.

**Discussion**

In the present experiments, synthetic endothelin elicited a pronounced coronary vasoconstrictor effect in perfused rabbit hearts. Endothelin also affected the diastolic relaxation and stimulated the outflow of prostacyclin metabolite from the organ. These data demonstrate that the biological actions of endothelin are not restricted to contraction of vascular smooth muscle only.

The coronary vasoconstrictor action of endothelin was clearly dose dependent, with an estimated IC$_{50}$ of about 2 nM. This value refers to the concentration required to inhibit the absolute flow by 50% and is consequently not directly comparable to the EC$_{50}$ of 0.4 nM reported by Yanagisawa et al., which concerns the concentration at which the peptide was 50% effective. At 0.4 nM, the calculated coronary flow was about 67% of control in our experiments. Since 10 nM, the maximal concentration applied by us, inhibited the flow to about 25% of control, it appears that the IC$_{50}$ in the current preparation must be higher than that reported in vascular strips.

In accordance with earlier observations, the constrictor action of endothelin was long lasting and was only partially reversed after 30 minutes of washout. In this connection, it is of interest that bolus injection of nitroprusside during the washout period elicited considerable coronary vasodilation. Thus, the marked coronary vasoconstriction caused by the previous exposure to endothelin could partly be reversed by a nitrovasodilator.

Endothelin was originally proposed to exert vasoconstrictor activity by facilitating voltage-dependent Ca$^{2+}$ influx. Recent reports have questioned this hypothesis on two grounds: first, the peptide also acts as a vasoconstrictor in the absence of extracellular Ca$^{2+}$ and second, endothelin is not an agonist for the dihydropyridine-binding site that constitutes the
voltage-dependent Ca\textsuperscript{2+} receptor.\textsuperscript{15–17} In our experiments the Ca\textsuperscript{2+} channel blocker nifedipine, at a concentration of 1 \muM, was unable to counteract the peptide-induced vasoconstriction. At 10 \muM, nifedipine partly elevated the flow in coronary vasculature constricted by endothelin (10 nM). To some extent, this effect may have been due to the ceased ventricular activity at this concentration of nifedipine, which in itself might promote coronary flow for mechanical reasons. Hence, the present experiments do not allow any conclusions concerning the type of receptor activated by endothelin.

The mechanical performance of the heart was also affected by endothelin. The moderate, dose-dependent bradycardia we observed appeared related to the vasoconstrictor action of the peptide, since mechanical reduction of the coronary flow had a similar negative chronotropic effect. In this connection, it may be noted that endothelin had a positive chronotropic action in isolated, spontaneously beating guinea pig atria.\textsuperscript{18} Whether a similar direct effect of the peptide on pacemaker activity was absent in the present experiments or was masked by the vasoconstrictor action remains to be studied.

Endothelin also changed the apical position of the heart during diastole toward less efficient diastolic relaxation. This action of endothelin did not appear to be based on the flow reduction only, since mechan-
tical restriction of flow had no more than a minor effect in this respect. Endothelin has been reported to have positive inotropic action in guinea pig and rat atria\(^{19,20}\), such an action was not observed in the present experiments. Hence, also the effects of endothelin on myocardial contractility and relaxation may be tissue and species dependent. In this connection it is notable that the decrease in diastolic relaxation induced by endothelin was counteracted by nifedipine (1 \(\mu\)M).

Parallel to the progressive coronary vasoconstriction, endothelin induced a dose-dependent decrease in myocardial oxygen consumption, despite an increase in myocardial oxygen extraction, and a lowered pH in the effluent. These actions of endothelin were clearly

**Figure 5.** Typical experiment demonstrating the effect of parallel infusion of endothelin (final concentration, 10 nM) and nifedipine (final concentration, 1 \(\mu\)M) in isolated Langendorff-perfused rabbit hearts. Infusion time indicated by the box. Upper diagram displays coronary flow and heart rate, and the lower tracing represents the apical movement of the heart. Note that the baseline of the apical tracing is stable during the infusion, in contrast to the corresponding baseline demonstrated in Figure 2. Nifedipine impaired the systolic apical movement slightly, indicating a negative inotropic effect by the drug.

**Figure 6.** Coronary flow, heart rate, myocardial oxygen uptake (MVO2), and outflow of purines from perfused rabbit hearts in the basal state and during exposure to endothelin (10 nM), alone or in combination with nifedipine (1 or 10 \(\mu\)M). All variables expressed in relation to basal (=100\%, value before drug). Columns and bars represent mean \(\pm\) SEM. Nifedipine (1 \(\mu\)M) did not reverse any of the effects of endothelin (10 nM), while nifedipine (10 \(\mu\)M) counteracted the increase in purine outflow induced by the peptide.
TABLE 1. Effect of Aminophylline or Indomethacin on Endothelin-Induced Changes in Coronary Flow, Heart Rate, Change in Diastolic Apical Position, Change in Systolic Apical Movement, and Outflow of Purines and Prostacyclin Metabolite in Isolated Langendorff-Perfused Rabbit Hearts

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<td>Coronary flow (ml/min)</td>
<td>Heart rate (beats/min)</td>
<td>Change in diastolic apical position (mm)</td>
<td>Change in systolic apical movement (%)</td>
<td>Purine outflow (nmol/3 min)</td>
<td>6-ketoprostaglandin F1α outflow (ng/3 min)</td>
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<td>Endothelin (5 nM; n=5) 43±2</td>
<td>16±1*</td>
<td>146±19†</td>
<td>2.2±0.5*</td>
<td>10±10NS</td>
<td>26±5</td>
<td>109±20†</td>
<td>26±8</td>
<td>92±22†</td>
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<td>Endothelin (5 nM)+ aminophylline (0.1 mM; n=6) 43±2</td>
<td>18±4‡</td>
<td>169±11</td>
<td>155±18NS</td>
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<td>151±14NS</td>
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<td>7.6±0.5‡</td>
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Data presented as mean±SEM. n, number of experiments. "Basal" represents values obtained before exposure to endothelin. "Drug(s)" indicates values obtained during 5 minutes of exposure to the drug(s). The changes in diastolic apical position and in systolic apical movement are expressed as the arithmetic or geometric differences between the basal values and the values obtained during drug exposure. * and † and ‡ indicate that the data differ significantly (by p<0.001, p<0.05, and p<0.01, respectively) from the corresponding basal values.

related to the vasoconstriction since mechanical flow reduction elicited similar effects. The outflow of purines was also increased by endothelin (5–10 nM). An increased cardiac outflow of adenosine and its degradation products, inosine and hypoxanthine, usually reflects impairment of oxygen supply.21,22 Also, this effect of endothelin appeared to be flow dependent, inasmuch as mechanical flow reduction caused an increase in purine outflow that did not differ from that caused by endothelin. Hence, it appears that at least a major part of the change in myocardial metabolism in response to endothelin exposure in the present study was caused by the coronary vasoconstriction and subsequent oxygen deprivation.

Ventricular performance was monitored by recording of apical movement during the cardiac cycle. The upward apical movement, representing the systolic shortening of the heart, was not affected either by endothelin (0.1–1 nM) or by a corresponding mechanical reduction in flow. This may seem surprising in light of the decreased myocardial oxygen uptake and increased purine outflow. However, the Langendorff-perfused rabbit heart has considerable glycolytic capacity, as revealed by a marked increase in lactate release during oxygen deprivation.23 Obviously, the glycolytic capacity of the present hearts was sufficient to meet the reduction in oxygen uptake and thereby to allow mechanical performance to be maintained.

Nifedipine (1 μM) did not significantly counteract the endothelin-induced release of purines from the heart. This agrees with the lack of effect of this concentration of nifedipine on endothelin-induced vasoconstriction. In contrast, nifedipine (10 μM) completely reversed endothelin-induced outflow of purines, despite no more than a partial reversal of the vasoconstriction. We interpret this lowered outflow of purines in the presence of nifedipine (10 μM) as secondary to a diminished myocardial demand of oxygen; as stated above, nifedipine (10 μM) completely abolished the mechanical activity of the ventricles.

FIGURE 7. Outflow of prostacyclin metabolite (left diagram) and effluent concentration of prostacyclin metabolite (right diagram) in perfused rabbit hearts during infusion of endothelin (1–10 nM) or during mechanical reduction of flow to levels corresponding to the vasoconstriction elicited by the different concentrations of endothelin (for further details see text to Figure 3). Columns and bars indicate mean±SEM. Endothelin, but not mechanical flow reduction, elevated the outflow of the prostacyclin metabolite 6-ketoprostaglandin F1α (p<0.001, two-way analysis of variance). Mechanical flow reduction elevated the concentration of 6-ketoprostaglandin F1α in the effluent (p<0.01), but endothelin was more efficient in this respect (p=0.001, two-way analysis of variance).
facilitated the vasoconstrictor action of endothelin. The lack of effect indicates that the amounts of adenosine and prostacyclin released under the prevailing conditions were unable to elicit any vasodilator activity in the coronary vessels.

In summary, the current study demonstrates that endothelin elicits significant effects on several aspects of cardiac function. The cardiac actions of endothelin, involving interference with vascular tone and formation of prostacyclin and possibly diastolic relaxation, renders endothelin a compound of considerable importance also in relation to cardiac physiology under normal and pathological conditions.

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References


KEY WORDS • adenosine • diastolic relaxation • coronary flow • endothelin • isolated heart • prostacyclin
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