Effects of Endothelin on the Coronary Vascular Bed in Open-Chest Dogs

Jean-Paul Clozel and Martine Clozel

The goal of the present study was to evaluate the effects of endothelin, a newly discovered very potent vasoconstrictor secreted by endothelial cells, on the coronary vascular bed. For this purpose, the effects of endothelin injected intracoronarily were tested in open-chest anesthetized dogs with the circumflex coronary artery cannulated and perfused at a constant pressure of 100 mm Hg. Circumflex blood flow, transmural distribution of coronary blood flow (radioactive microspheres), circumflex coronary artery diameter (piezoelectric crystals), and circumflex luminal surface area were measured. Endothelin decreased coronary blood flow by 30% and 61% with doses of 1 and 3 ng, respectively. A dose of 10 µg was lethal. The decrease of coronary blood flow was larger in the subepicardium than in the subendocardium, which explains that the endocardial-epicardial blood flow ratio increased from 1.27±0.05 to 1.98±0.23 (p<0.001) with a dose of 3 µg endothelin. Circumflex surface area decreased by 7% (p=NS) and 20% (p<0.01) with doses of 1 and 3 µg endothelin, respectively. The action of endothelin was not modified by the concomitant ß-adrenergic blockade, serotonergic blockade, angiotensin converting enzyme inhibition, or cyclooxygenase inhibition. We conclude that endothelin is a potent coronary vasoconstrictor with a selective effect on the subepicardium. At least part of the increase of coronary vascular resistance is due to a constriction of the large coronary arteries. Further studies are required to determine the physiopathological role of endothelin, especially in coronary vasospasm. (Circulation Research 1989;65:1193-1200)

The effects of endothelin on coronary arteries have been assessed in vitro in isolated perfused hearts or isolated rings. Recently, endothelin, a 21-amino acid peptide and the most potent vasoconstrictor known so far, was isolated from medium of porcine endothelial cells in culture. The complementary DNA coding for the same peptide is also present in human placenta. Endothelin can constrict not only coronary arteries but also strips or rings of rat aorta, cat basilar artery, rabbit and dog mesenteric arteries, and human mesenteric and pulmonary artery branches.

The effects of endothelin on coronary arteries have been assessed in vitro in isolated perfused hearts or isolated rings. Recently, endothelin was shown to induce coronary vasoconstriction in conscious dogs after systemic administration. However, in this model endothelin also induced systemic changes, and the direct effects of endothelin on the coronary circulation were difficult to assess. Therefore, the goal of the present study was to evaluate in vivo the effects of endothelin injected intracoronarily on the coronary vascular bed. Moreover, since it is known that not only coronary flow but also its distribution through the myocardial wall are important characteristics of the coronary circulation, we have measured the effects of endothelin not only on total coronary blood flow but also on its transmural distribution. The effects of endothelin on large coronary arteries were also assessed since it has been shown recently that large coronary arteries contribute significantly to the global coronary vascular resistance. Finally, the mechanism of action of endothelin was explored by evaluating the effect of ß-adrenergic blockade, serotonergic blockade, angiotensin converting enzyme inhibition, and cyclooxygenase inhibition.

Materials and Methods

General

Twenty-six dogs (20-30 kg) were given 20 mg/kg i.v. thiobarbiturate (Surital, Parke Davis, München, FRG). Then, the trachea was intubated, and the dogs were anesthetized with halothane (0.5-1%). The lungs were ventilated with 50% O₂ by a ventilator (model 607, Harvard Apparatus, South Natick, Massachusetts), which operated with a positive expiratory pressure of 3-5 cm H₂O; tidal volume...
was 15 ml/kg, and ventilator frequency was 10-15 cycles/min. Arterial blood gases and pH were measured periodically, and metabolic acidosis was corrected by giving sodium bicarbonate intravenously.

**Measurement of General Hemodynamics and Regional Myocardial Shortening**

Blood pressure in the thoracic aorta was measured with a high fidelity micromanometer (Millar Instruments, Houston, Texas) introduced via a femoral artery. After opening the chest in the fourth left intercostal space, a high-fidelity micromanometer was introduced into the left ventricle via a stab wound made through the apex to measure left ventricular pressure. Left ventricular first derivative (LVdp/dt) was derived from the left ventricular pressure signal. LVdp/dt max+ was defined as the positive maximum of LVdp/dt. Heart rate was recorded by a cardiotachometer triggered by the aortic pressure. Regional myocardial segmental shortening was measured with two pairs of piezoelectric crystals implanted into the subendocardium as described before and connected to an ultrasonic transit time dimension gauge (Sonomicrometer 120, Triton Technology, San Diego, California). One pair was implanted into the region perfused by the circumflex coronary artery. The other pair was implanted into the region perfused by the left anterior descending coronary artery. The measurements of end-diastolic and end-systolic segmental lengths as well as segmental shortening were made as described. The signals representing blood pressure, heart rate, and other variables described below (coronary blood flow and coronary perfusion pressure) were recorded on a 12-channel physiological recorder (Linearcorder, model WR3/01, Graphtec, Tokyo, Japan).

**Measurement of Coronary Blood Flow**

As shown in Figure 1, the circumflex coronary artery was cannulated via the right common carotid artery and perfused from a constant-pressure reservoir; flow into the circumflex artery was measured by a flowmeter. The right common carotid artery was separated from the vagosympathetic trunk. Sodium heparin (75 units/kg) was injected intravenously. The external circuit was primed with 6% dextran, which was pumped into the systemic circulation and replaced by blood from the left femoral artery before the coronary artery was cannulated. The total volume of the perfusion circuit was about 200 ml, and the reservoir usually contained about 50 ml.

A stainless steel cannula that was connected at its upstream end to the reservoir was inserted via the right carotid artery, aorta, and left coronary ostium, and its tip was wedged in the circumflex coronary artery. Blood from the left femoral artery was delivered to the perfusion reservoir by a roller pump, whose speed was controlled by a feedback circuit to maintain a constant level of blood in the reservoir. The temperature of the external perfusion circuit was maintained at 37°C by a thermostatically controlled water jacket. Because resistance along the cannula varied with coronary flow, we monitored perfusion pressure at the tip of the cannula in the circumflex coronary artery with a Statham strain gauge (model P23Gb, Gould Instruments, Cleveland, Ohio) and, as coronary flow varied, kept cannula-tip pressure constant by regulating the pressure in the reservoir. Reservoir pressure was in turn regulated by a variable flow of compressed air, controlled by a solenoid (model 60, Fairchild, Winston-Salem, North Carolina) connected by a servoloop to the cannula-tip pressure transducer. During the whole study, perfusion pressure was kept at 100 mm Hg.

Blood flow to the circumflex artery was measured by a cannulating electromagnetic transducer (type E, lumen 3 mm, Hellige, Freiburg, FRG), located in the perfusion circuit between reservoir and cannula and connected to a flowmeter (Recomed, Hellige). The flowmeter was calibrated with the dog’s blood at the end of the experiment.

We tested the effectiveness of the seal between the wedge-shaped tip of the cannula and the circumflex artery by briefly obstructing the inflow to the cannula. A rapid decrease in cannula-tip pressure to below 25 mm Hg during the 10-second period of occlusion confirmed that the seal was effective. We also tested the seal at the end of the experiment by injecting methylene blue into the cannula.

**Measurements of Transmural Distribution of Coronary Blood Flow**

To determine whether the coronary vasoconstriction induced by endothelin was accompanied by changes in the transmural distribution of coronary blood flow, radioactive microspheres were injected into the circumflex coronary artery. After being
FIGURE 2. Recordings showing hemodynamic effects of the intracoronary (i. coronary) injection of endothelin (1 and 3 μg) or of its vehicle in an open-chest anesthetized dog. LAD, left anterior descending coronary artery.
mixed vigorously, about $10^5$ microspheres (15 $\mu$m in diameter) contained in 0.5 ml saline were injected into the perfusion circuit through a mixing chamber located 10 cm upstream to the cannula. Preliminary experiments confirmed that this method of injection resulted in a good mixing of the microspheres. Each of the four injections was labeled with one of eight different isotopes ($^{141}$Ce, $^{114}$In, $^{51}$Cr, $^{113}$Sn, $^{103}$Ru, $^{85}$Sr, $^{95}$Nb, and $^{46}$Sc).

At the end of the experiment, while the heart was still beating, methylene blue was injected into the cannula to delineate the vascular territory of the circumflex coronary artery. Immediately after this injection, the heart was arrested by injecting potassium chloride intravenously; it was then removed and fixed in 10% formalin for 3 days. A specimen (around 30 g) of the left ventricular wall was taken from the center of the region of myocardium perfused by the circumflex coronary artery and was cut into three slices of equal thickness. Each slice was cut into three sections, and each section was cut into three portions from endocardium to epicardium. Thus, a total of 27 specimens were counted in a well-type scintillation counter by use of a germanium crystal (Enertec, Strasbourg, France).

Coronary flow per gram of tissue was calculated by multiplying radioactivity per gram of tissue by the ratio of circumflex coronary flow (measured by the flowmeter) to the total radioactivity of the heart. The flows of all the samples in each layer (endocardium, mesocardium, and epicardium) were averaged.

**Measurement of Coronary Diameter**

The diameter of the circumflex artery was measured continuously with an ultrasonic dimension gauge (Sonomicrometer 120, Triton Technology). Two 5-MHz piezoelectric crystals were attached to opposing sides of a stainless steel clip to align the crystals throughout the experiment. The two crystals were positioned on each side of the coronary artery. With this implantation technique, we could avoid sewing the crystals on the adventitia of the artery.

The cross-sectional area of the coronary artery was also calculated by taking into account the external diameter and the wall volume. The wall volume was calculated as the quotient of mass and density ($d=1.06$ g/cm$^3$) of the coronary wall. After the experiment, the wall mass was measured by weighing a known length of the coronary artery excised in the region where the crystals were implanted.
TABLE 1. Hemodynamic Effects of Endothelin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Arterial blood pressure (mm Hg)</th>
<th>CBF (ml/min)</th>
<th>LVdp/dt max (+) (mm Hg/sec)</th>
<th>LVEDP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Segmental shortening (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>116±5</td>
<td>64±12</td>
<td>1,943±102</td>
<td>4.6±0.4</td>
<td>101±2</td>
<td>15.1±2.5</td>
</tr>
<tr>
<td>Solvent</td>
<td>114±5</td>
<td>67±13</td>
<td>1,957±104</td>
<td>4.6±0.4</td>
<td>101±2</td>
<td>15.7±2.9</td>
</tr>
<tr>
<td>Endothelin</td>
<td>1 µg i.c.</td>
<td>106±5*</td>
<td>90±5</td>
<td>1.16±0.11</td>
<td>4.1±0.4</td>
<td>17.9±3.2</td>
</tr>
<tr>
<td></td>
<td>3 µg i.c.</td>
<td>80±3†</td>
<td>54±4†</td>
<td>1.25±0.05</td>
<td>4.7±0.6</td>
<td>21.7±2.6†</td>
</tr>
</tbody>
</table>

All variables were measured at baseline and during the peak effect of endothelin. Values are mean±SEM.

CBF, coronary blood flow; LVdp/dt max(+), left ventricular dp/dt max(+); LVEDP, left ventricular end-diastolic pressure; LAD, left anterior descending artery; CIRC, circumflex artery.

*p<0.05 vs. baseline.

Study Design

The study was performed in four phases. First, a dose-response curve was determined in six dogs given doses of endothelin intracoronarily that increased from 0.1 to 10 µg. Then two of these doses (1 and 3 µg) were used in another group of 10 dogs in which coronary blood flow distribution, in addition to hemodynamics, was measured with radioactive microspheres. In a third group of six dogs, the effects of increasing doses of endothelin (from 0.3 to 3 µg) on the circumflex coronary artery diameter were evaluated. In the last group of four dogs, the effects of 3 µg endothelin were evaluated before and 10 minutes after the simultaneous and intravenous administration of cilazapril (1 mg/kg), indomethacin (5 mg/kg), phen tolamine (2 mg/kg), methylergide (0.5 mg/kg), ketanserin (0.2 mg/kg) to induce angiotensin converting enzyme and cyclooxygenase inhibition and adrenergic and serotoninergic blockade (5-HT1 and 5-HT2), respectively. For the four types of experiments, the effects of the endothelin vehicle were also checked.

Injection of endothelin. Human endothelin (Peninsula Laboratories, Belmont, California) was dissolved in methanol:water (1:1) at a concentration of 100 µg/ml and further diluted in normal saline. A constant volume of 300 µl was injected into the perfusion circuit.

Statistical analysis. The effects of endothelin were evaluated by ANOVA. When multiple comparisons were performed within a group, a Bonferroni correction was made to adjust for multiple comparisons. All data are given as mean±SEM. A value of p<0.05 was considered significant.

Results

Effects of Endothelin on Coronary Blood Flow and Hemodynamics

The endothelin vehicle produced either no change of coronary flow (Figure 2) or a transient increase of coronary flow (Figure 3) that lasted less than 1 minute. Thus, the effects of endothelin were always measured at least 1 minute after injection of the solution. Endothelin did not produce a significant change in coronary blood flow with doses of 0.1 and 0.3 µg (Figure 4). Endothelin began to decrease coronary blood flow (30±5%, p<0.01) with a dose of 1 µg. With a dose of 3 µg, endothelin reduced coronary blood flow by 61±10% (p<0.001). With a dose of 10 µg, endothelin produced a coronary vasodilator abolishing coronary blood flow. This was associated in each animal with a ventricular fibrillation occurring after 2–3 minutes.

For each dose, the effect of endothelin was sustained. With a dose of 3 µg, after 30 minutes coronary blood flow was still decreased by at least 50%.

The decrease of coronary blood flow was associated with a decrease of segmental shortening in the myocardium perfused by the circumflex coronary artery (Table 1 and Figure 2). In contrast, segmental shortening increased in the myocardium perfused by the left anterior descending artery, which did not receive endothelin. Left ventricular end-diastolic pressure increased and LVdp/dt max+ decreased (Table 1). After 3 µg endothelin, arterial pressure decreased and recovered in parallel with coronary blood flow.

TABLE 2. Effects of Endothelin on Transmural Distribution of Coronary Blood Flow

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Coronary blood flow (ml/min)</th>
<th>Endo/epi blood flow ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.17±0.11</td>
<td>1.07±0.09†</td>
</tr>
<tr>
<td>Solvent</td>
<td>1.20±0.10</td>
<td>1.08±0.09*</td>
</tr>
<tr>
<td>Endothelin</td>
<td>1 µg i.c.</td>
<td>0.97±0.09*</td>
</tr>
<tr>
<td></td>
<td>3 µg i.c.</td>
<td>0.97±0.09†</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Regional blood flow was measured with radioactive microspheres in 10 dogs. Endo, endocardial; meso, mesocardial; epi, epicardial.

*p<0.01 vs. baseline.

fp<0.001 vs. baseline.
Effects of Endothelin on Coronary Blood Flow Distribution

The radioactive microspheres were always injected 5 minutes after endothelin to avoid the transient coronary blood flow increase induced by the vehicle. After this delay, the endothelin vehicle did not change coronary blood flow or its distribution (Table 2). The effect of endothelin was larger in the epicardium than in the endocardium (Table 2) and the endocardial/epicardial blood flow ratio increased from 1.27±0.05 to 1.98±0.23 (p<0.001).

Effects of Endothelin on Coronary Artery Diameter and Cross-sectional Surface Area

Endothelin decreased in a dose-dependent manner the circumflex artery diameter (Figure 5). This effect appeared with a dose of 1 μg, which produced a decrease of 3.1±1.3% (p=NS) of the coronary artery diameter, and was maximal with a dose of 3 μg, which reduced mean coronary diameter by 9.6±3.1% (p<0.001). The corresponding reductions of coronary surface area were 6.7±2.7% (p=NS) and 19.7±2.7% (p<0.01).

Effects of Inhibitors on the Coronary Vasoconstriction

Injection of the various inhibitors did not significantly modify the coronary vasoconstriction induced by endothelin (Figure 6). The decrease of coronary blood flow was 60±8% and 77±3% before and after injection of inhibitors, respectively (p=NS).

Discussion

The present results show that in anesthetized dogs endothelin produces a dose-related coronary vasoconstriction larger in the subepicardium than in the subendocardium and a vasoconstriction of the large coronary arteries.

In all experiments, the circumflex coronary artery was cannulated and perfused at constant pressure. Thus, the changes of coronary flow were directly related to the changes of coronary vascular resistance. Moreover, changes of coronary vascular resistances due to autoregulation of the coronary vascular bed were avoided since coronary perfusion pressure was kept constant. The direct effect of endothelin on the transmural myocardial blood flow...
Coronary Constriction Evoked by Endothelin

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could also be evaluated without the interference of changes of coronary perfusion pressure. However, the experiments were performed during anesthesia, which is known to modify the physiological responses of the animals. Thush, in conscious animals coronary responses to endothelin might be different than in anesthetized animals, due to stimulation of reflex pathways.

The effect of endothelin was dose-related and began to be significant with a dose of 3 μg. With a dose of 10 μg, endothelin produced a dramatic decrease of coronary blood flow associated with ventricular fibrillation. Therefore, the data with 10 μg endothelin have not been analyzed. For the experiments in which coronary blood flow distribution was measured with radioactive microspheres, we have chosen doses of 1 and 3 μg, which decreased coronary blood flow by 26% and 61%, respectively. With these two doses, endothelin significantly decreased segmental shortening in the myocardium perfused by the circumflex artery. In contrast, the segmental shortening of the myocardium perfused by the left anterior descending artery increased, most likely to compensate for the decrease of the function of the circumflex zone. This decrease of shortening of the circumflex zone was also associated with a decrease of the global left ventricular function since LVdp/dt max+ decreased and left ventricular end-diastolic pressure increased.

To assess whether this depressive effect on the myocardium was due to a decrease of coronary blood flow or to a direct effect of endothelin on the myocardium, we increased coronary perfusion pressure in two dogs after endothelin injection until coronary blood flow was back to baseline. The normalization of coronary flow was associated with a nearly complete normalization of segmental shortening. Therefore, the depressive effect of endothelin on the myocardium is most likely secondary to the dramatic decrease of coronary blood flow. Moreover, endothelin, in vitro, has a direct positive inotropic effect and not a negative one.

The experiments with the radioactive microspheres showed that the vasoconstriction induced by endothelin was more pronounced in the subepicardial arteries than in the subendocardium. This is not likely to be secondary to general hemodynamic changes, which were moderate. The selective decrease of subepicardial blood flow would rather indicate that endothelin is a selective vasoconstrictor of subepicardial arteries. It might also indicate that vasodilator mechanisms can partly antagonize the vasoconstrictor effect of endothelin in the subendocardium.

To assess whether endothelin was acting only on the small resistance vessels or also on the large capacitance arteries, we measured the effects of endothelin on the circumflex coronary artery diameter and calculated coronary surface area. The decrease of coronary surface area induced by endothelin was 19% for a dose of 3 μg. Since resistance is a function of the square of the coronary surface area (Poiseuille's law), it is possible to estimate that endothelin increased by 34% the vascular resistance in the circumflex coronary artery. Moreover, recently it has been shown that 50% of resting coronary vascular resistance can be attributed to vessels larger than 100 μm. Therefore, the constriction of the circumflex coronary artery is responsible for a significant part of the decrease of the total coronary blood flow that was observed in this study.

The physiological and pharmacological mechanisms responsible for the vasoconstriction of the large epicardial coronary arteries have been recently reviewed. Large coronary arteries can be constricted by the stimulation of α-adrenoceptors, injection of serotonin, ergonovine, thromboxane, or aggregated platelets. Moreover, these mechanisms can be markedly enhanced when atherosclerosis is present or during acute hypertension. Endothelin is known to be able in vitro to constrict arteries with a mechanism independent of the factors mentioned above. We confirmed this finding by showing that the effects of endothelin on coronary blood flow were not decreased by pharmacological blockade of α-adrenoceptors (phentolamine) or 5-HT 1, and 5-HT 2 receptors (methysergide and ketanserin) or by angiotensin converting enzyme (cilazapril) or cyclooxygenase inhibition (indomethacin).

The physiopathological significance of the findings of the present study is difficult to assess since we have injected intracoronarily pharmacological doses of endothelin leading to regional concentrations that perhaps are never reached under physiological or pathological conditions. Moreover, when injected intravenously, endothelin seemed to be a more potent vasoconstrictor on the renal than on the coronary vascular bed. Endothelin is secreted by endothelial cells in culture although its secretion in vivo has not been proven. Endothelin might act only locally on the smooth muscle cells.

Coronary vasospasm is a very frequent feature that can occur in patients with Prinzmetal's angina as well as in patients with unstable angina or myocardial infarction. In most of these clinical conditions, injury of the arterial wall due to the presence of arterial thrombus or plaque rupture has been described. It is tempting to believe that endothelin secretion could be induced in these conditions and could be responsible for coronary vasospasm.

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References


KEY WORDS: coronary blood flow • vasospasm • transmural distribution • coronary diameter • endothelin • dogs
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