Vascular Responses to Endothelin-1 in Atherosclerotic Primates

J. Antonio G. Lopez, Mark L. Armstrong, Donald J. Piegors, and Donald D. Heistad

Endothelin-1 (ET-1) is a vasoactive peptide that is released by endothelial cells. This study was performed to determine whether vascular responses to ET-1 are altered by atherosclerosis. ET-1 (1 or 10 nmol) was injected intra-arterially into the perfused hind limb of normal cynomolgus monkeys and monkeys fed an atherogenic diet for 19 months. We calculated the resistance of the total limb and large arteries and estimated the resistance of the small vessels. The major finding was that ET-1 had minimal effects on large arteries in normal monkeys but produced pronounced constriction of large arteries in atherosclerotic monkeys. In both groups, ET-1 produced dilatation of small vessels at 1 nmol and constriction at 10 nmol. Indomethacin (6 mg/kg intravenously) did not affect the responses to ET-1 in normal or atherosclerotic monkeys. In summary, the major finding is that the constrictor responses of large arteries to ET-1 are potentiated by atherosclerosis. (Arteriosclerosis 10:1113–1118, November/December 1990)

Vasospasm or increased vasoconstriction is an important complication of atherosclerosis.1 Vasoconstrictor responses to several agonists are potentiated by atherosclerosis,2–7 probably due in part to impaired endothelium-dependent relaxation.5,6,8–12 The mechanisms by which atherosclerosis predisposes to vasospasm are not clear.

Endothelium releases two types of relaxing factors, prostacyclin13 and endothelium-derived relaxing factor (EDRF),14 which modulate vascular responses to many vasoactive agents. In addition, the supernatants of endothelial cells in culture release a vasoconstrictor peptide, endothelin-1 (ET-1).15–19 ET-1 is a potent mitogen in fibroblasts20 and rat aortic smooth muscle cells,21 which raises the possibility that ET-1 may play a role in the pathogenesis of atherosclerosis.22 It is not known whether vascular responses to ET-1 are altered by atherosclerosis.

In this study we tested the hypothesis that vasoconstrictor responses to ET-1 are altered by atherosclerosis. We also examined responses to ET-1 after administration of indomethacin to inhibit synthesis of prostacyclin and other vasodilator prostaglandins.

Methods

Animals

Two groups of adult male Malaysian monkeys were studied. Nineteen normal monkeys were fed commercial laboratory chow (Purina Monkey Chow, Ralston Purina, Richmond, IN). Fourteen monkeys were fed the atherogenic diet, which contained cholesterol (1 mg/calorie) and fat (43% of total calories) for 19 ± 0.5 (mean ± SEM) months. The normal monkeys weighed 6.3 ± 0.2 kg and the atherosclerotic monkeys weighed 5.6 ± 0.3 kg. At intervals of 3 to 4 months, the monkeys were sedated with ketamine HCl (10 mg/kg intramuscularly), and a venous blood sample was obtained. Total cholesterol and triglyceride levels were determined with the method used by the Lipid Research Clinics Protocols for the AutoAnalyzer II (Technicon Instruments, Tarrytown, NY).

Hemodynamic Studies

At the time of study, the monkeys were sedated with ketamine (15 mg/kg intramuscularly) and were anesthetized with chloralose (100 mg/kg intravenously). A tracheotomy was performed, and the monkeys were intubated and ventilated with room air and supplemental oxygen. Gallamine triethiodide (5 mg/kg intravenously) was given for paralysis of skeletal muscles, and heparin sodium (500 U/kg intravenously) was given for anticoagulation. Arterial blood gases and pH were monitored during each study and were maintained at normal levels by adjustment of the ventilatory rate and tidal volume or by intravenous injection of small amounts of sodium bicarbonate. Rectal temperature was maintained at 37° to 38°C with a heating pad.

A polyethylene catheter was inserted into the right brachial artery for measurement of aortic pressure and obtaining blood samples. Catheters were also inserted into the right and left brachial veins for injection of fluids and drugs.

Through a laparotomy, the bifurcation of the abdominal aorta and the proximal left iliac artery were exposed, isolated, and cannulated with perfusion tubing. The left dorsal pedal artery also was exposed, and a PE-50
A calibrated Harvard Model 1210 pulsatile perfusion catheter was inserted retrogradely to measure pressure. A calibrated Harvard Model 1210 pulsatile perfusion pump (Harvard Apparatus, South Natick, MA) was used to perfuse the left iliac artery at a constant flow with blood from the abdominal aorta, and the iliac perfusion pressure was measured continuously. When the pump was stopped, perfusion pressure decreased rapidly to 10 to 15 mm Hg, which indicated that vascular isolation was adequate. The baseline perfusion pressure of the hind limb was established by adjusting blood flow so that the perfusion pressure was similar to the animal’s mean systemic arterial pressure.

Total limb resistance (TLR) was calculated from the equation:

\[ \text{TLR} = \frac{\text{iliac perfusion pressure (mm Hg)}}{\text{hind limb blood flow (ml/min)}} \]

This included the resistance of large and small vessels. The difference between the iliac perfusion pressure and the dorsal pedal pressure at constant flow indicates a resistance of the large arteries in the limb. The resistance of the small vessels was estimated from the total limb resistance—the large artery resistance. Our estimate of the resistance of small vessels does not distinguish between the resistance of small arteries, arterioles, and venules. We have described this method in detail previously.3

**Experimental Protocols**

We studied the effects of phenylephrine (Sigma Chemical, St. Louis, MO) and ET-1 (Peninsula Laboratories, Belmont, CA). Phenylephrine (5 x 10⁻⁹ and 5 x 10⁻⁸ mol) and ET-1 (10⁻¹⁰ and 10⁻⁹ mol) were injected as a 0.1 ml bolus into the iliac perfusion tubing. The injection of the vehicle (0.1 ml) produced minimal hemodynamic effects. Because the pressor response to ET-1 was prolonged,18,24 the responses to phenylephrine were examined first. Hemodynamic measurements were taken before the systemic effects.

To investigate the possibility that ET-1 may release prostacyclin or other vasodilator prostaglandins, we studied the responses to ET-1 in eight normal and six atherosclerotic monkeys at 30 minutes after indomethacin injection (6 mg/kg intravenously, Sigma Chemical). The dose of indomethacin used has been shown to abolish the responses to arachidonic acid in vivo.23 The responses to ET-1 were not evaluated before and after indomethacin in the same animal because the constrictor response to ET-1 was prolonged.18,24 The maximal changes in the perfusion pressures of the iliac and the dorsal pedal artery were measured after injections.

At the end of the experiment, each anesthetized monkey was killed with an intravenous injection of KCl. The leg was severed through the hip joint and weighed, so that limb blood flow and vascular resistance could be expressed per 100 grams of limb.

**Morphologic Studies**

After termination of the experiment, the iliac artery was removed, was examined for gross lesions, and was fixed by immersion in 10% buffered formalin. Specimens were taken at standardized sites from the proximal segment of the iliac artery, as described previously.3,6,25 The morphologic study was carried out on paraffin-embedded sections. Sections were stained with hematoxylin-eosin and Verhoff-Van Gieson stains. Morphometric determinations of the sizes of the intima and media were performed with an image analyzer, as described previously.3,6,25

**Statistical Analysis**

The mean values were analyzed with an analysis of variance with a repeated-measure procedure from statistical analysis systems (SAS Institute, Cary, NC). This model was used to determine which pairs of means were significantly different.26 A statistical significance was considered to be \( p < 0.05 \).

**Plasma Lipids**

Plasma total cholesterol was 129±6 mg/dl in normal monkeys and 618±34 mg/dl in atherosclerotic monkeys. Plasma triglycerides were <40 mg/dl in both groups.

**Morphologic Changes**

In atherosclerotic monkeys, the morphologic changes were similar to those described previously.3,6,25 There was dense fibrofatty intimal thickening, with intimal necrosis of the proximal segment of the iliac artery.

Morphometry demonstrated increases in the intimal area in the iliac artery of atherosclerotic monkeys. The intimal area was 0.2±0.1 mm² in normal monkeys and 2.7±0.4 mm² in atherosclerotic monkeys (\( p < 0.05 \) vs. normal monkeys). The medial area was not significantly different between groups.

**Hemodynamic Studies**

**Baseline Values**

Total limb vascular resistance tended to be greater and large artery resistance was significantly greater in the atherosclerotic monkeys than in the normal monkeys (Table 1).

**Responses to Endothelin-1 and Phenylephrine**

The low dose (10⁻¹⁰ mol) of ET-1 produced vasodilation in the limbs of both normal and atherosclerotic

<table>
<thead>
<tr>
<th>Table 1. Baseline Hemodynamic Values</th>
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<tr>
<td>Measurement site/variable</td>
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<td>---------------------------</td>
</tr>
<tr>
<td>Blood flow (ml/min/100 g)</td>
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<tr>
<td>Iliac perfusion pressure (mm Hg)</td>
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<tr>
<td>Hind limb vascular resistance (mm Hg/ml/min/100 g)</td>
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</table>

Values are means±SEM in 19 normal and 14 atherosclerotic monkeys.

\*\( p < 0.05 \) vs. normal.
monkeys (Figure 1). The dilator response to ET-1 tended to be less in atherosclerotic monkeys ($p>0.05$ vs. normal monkeys). The higher dose of ET-$1$ ($10^{-9}$ mol) produced vasoconstriction.

ET-1 produced minimal constriction of the large artery segment in the limbs of normal monkeys (Figure 2). There was potentiation of constrictor responses of the large artery segment to ET-$1$ ($10^{-9}$ mol) in atherosclerotic monkeys ($p<0.05$ vs. normal monkeys, Figure 2).

The response of ET-1 in the limb was rapid (<20 seconds), and a delayed systemic response occurred about 50 seconds after local administration. Hemodynamic measurements were obtained before the systemic effects.

Phenylephrine produced vasoconstriction in the limbs of both normal and atherosclerotic monkeys (Figure 3). The constrictor response tended to be less in atherosclerotic monkeys ($p>0.05$ vs. normal monkeys).

Phenylephrine produced minimal constriction of the large arteries in the limbs of normal monkeys. The response was potentiated in atherosclerotic monkeys ($p<0.05$ vs. normal monkeys, Figure 4).

Baseline Values after Indomethacin

Intravenous administration of indomethacin increased baseline hind limb vascular resistance similarly in eight normal and six atherosclerotic monkeys (0.9±0.4 in normal and 1.0±0.8 mm Hg/ml/min per 100 g in atherosclerotic monkeys). There was no significant effect of indomethacin on the change in large artery resistance between groups ($-0.1±0.2$ in normal and $+0.4±0.5$ mm Hg/ml/min per 100 g in atherosclerotic monkeys).

Responses to Endothelin-1 and Phenylephrine after Indomethacin

After treatment with indomethacin, the low dose of ET-$1$ ($10^{-10}$ mol) produced vasodilatation in the limbs of both normal and atherosclerotic monkeys (Figure 5). The dilator response to endothelin-1 was similar in both groups. The higher dose of ET-$1$ ($10^{-9}$ mol) produced vasoconstriction in the limbs of normal and atherosclerotic monkeys.

ET-1 produced minimal constriction of the large artery segments in the limbs of normal monkeys after indomethacin (Figure 6). After indomethacin, the constrictor responses of the large artery segment to ET-1 were greater in atherosclerotic monkeys (Figure 6) than in normal monkeys. Indomethacin did not alter the constrictor responses to ET-$1$ ($10^{-8}$ mol) in the large arteries of normal or atherosclerotic monkeys (Figure 2 vs. Figure 6).
Normal Atherosclerotic

Limb Resistance
\[ \text{mmHg/ml/min per 100g} \]

Endothelin (moles)

Figure 5. The changes in total limb resistance in response to an intra-arterial injection of endothelin-1 after indomethacin. The values are the means±SEM in eight normal and six atherosclerotic monkeys.

Large Artery Resistance
\[ \text{mmHg/ml/min per 100g} \]

Endothelin (moles)

Figure 6. The changes in large artery resistance in response to an intra-arterial injection of endothelin-1 after indomethacin. Constrictor responses to endothelin-1 \((10^{-9} \text{ mol})\) were augmented in atherosclerotic monkeys \((p<0.05)\). The values are the means±SEM in eight normal and six atherosclerotic monkeys. The constrictor responses to endothelin-1 were greater in atherosclerotic than in normal monkeys \((p<0.05)\).

The constrictor responses of large arteries to ET-1 were augmented by atherosclerosis. It should be noted, however, that potentiation of responses to ET-1 was less than we have observed previously with serotonin and vasoactive products that are released by leukocytes.

There are several mechanisms by which atherosclerosis may alter vascular responses to ET-1. Fourth, there may be an impairment of ET-1 clearance by atherosclerosis. Fifth, changes in membrane lipids of endothelial and smooth muscle cells may change receptor number or affinity.

Endothelin-1 acts at a site closely coupled to the calcium channel to produce vasodilatation at low doses and vasoconstriction at high doses. Vasodilator responses to ET-1 may be due to the release of prostacyclin or to endothelin-derived relaxing factor. Our finding in the hind limbs of normal monkeys, in which ET-1 produced dilatation of small vessels at low doses and constriction at higher doses, is similar to previous reports.

**Effects of Atherosclerosis on Vascular Responses**

The constrictor responses of large arteries to ET-1 were augmented by atherosclerosis. It should be noted, however, that potentiation of responses to ET-1 was less than we have observed previously with serotonin and vasoactive products that are released by leukocytes.

**Discussion**

This study demonstrates that atherosclerosis potentiates the constrictor responses of large arteries to ET-1. The constrictor responses of the large arteries to ET-1 were not augmented by indomethacin, which suggests that ET-1 does not release vasodilator prostaglandins in large arteries. At a low dose, ET-1 produced dilatation of the small vessels, which was not altered by indomethacin.

**Previous Studies in Normal Animals**

ET-1 is a potent constrictor of large arteries. The major site of vasoconstriction in response to endothelin, however, is small vessels. ET-1 has been reported to produce vasodilatation at low doses and vasoconstriction at high doses. Vasodilator responses to ET-1 may be due to the release of prostacyclin or to endothelin-derived relaxing factor. Our finding in the hind limbs of normal monkeys, in which ET-1 produced dilatation of small vessels at low doses and constriction at higher doses, is similar to previous reports.
agonists were selected to avoid significant systemic
effects after their local administration. Due to the long
half-life and marked pressor effects of ET-1, it was nec-
essary to administer this agonist last.

The constrictor responses of large arteries to phenyl-
ephrine were modestly potentiated in the atherosclerotic
monkeys. The responses to phenylephrine are poten-
tiated in the colon of atherosclerotic monkeys,47 and
constrictor responses to sympathetic stimulation are aug-
mented in rabbit carotid48 and in human coronary arter-
ies.49,50 In previous studies, we did not observe any
potentiation of responses to norepinephrine or phenyl-
ephrine in the limbs of atherosclerotic monkeys.6,45,46
The finding that responses to phenylephrine are mod-
estly potentiated by atherosclerosis suggests that the
potentiation of responses to ET-1 may be due in part to
nonspecific potentiation of vasoconstrictor responses.

Phenylephrine produced vasoconstriction in the entire
limbs of both normal and atherosclerotic monkeys. The
constrictor response tended to be less in atherosclerotic
monkeys, but this effect did not achieve statistical sig-
ficance. The constrictor responses of the entire limbs
tended to be reduced in atherosclerotic monkeys despite
the increase in the response of large arteries. Thus,
the constrictor responses of the small vessels to phenyl-
ephrine must have been reduced in atherosclerotic monkeys.
In previous studies, we observed that responses to phene-
lyphrine were either similar45,46 or tended to be less6
in atherosclerotic monkeys than in normal monkeys. Thus,
the effects of atherosclerosis on the responsiveness of
both large and small vessels are relatively modest and
inconsistent. The mechanisms that contribute to alteration
of responses to phenylephrine are not clear.

Responses after Indomethacin

ET-1 releases prostacyclin from the pial arterioles of
piglets50 and the isolated lungs of guinea pigs and rats.36
The constrictor responses to ET-1 in mesenteric arteries
are potentiated by indomethacin, suggesting a release of
prostacyclin.51 Atherosclerosis may increase52 or de-
crease53 the production of prostacyclin. Our finding,
that indomethacin did not augment the constrictor effects
of ET-1 on large arteries in normal and atherosclerotic
monkeys, suggests that vasodilator prostaglandins are
not released in response to ET-1 in the cynomolgus
monkey.

ET-1 produces a dilatation of small vessels at low
doses. The potential mechanisms of vasodilatation include
a release of prostacyclin,7,29,36 EDRF,35,36 or prostaglan-
din E2.7 Our experiments with the inhibition of cyclooxy-
genase with indomethacin suggest that vasodilatation
may be secondary to a direct effect of ET-1 on vascular
smooth muscle or to a concomitant release of EDRF.

Implications

Vasospasm is an important complication of athero-
scerosis. The expression of the ET-1 gene in endothelial cells
is stimulated by thrombin18 and transforming growth
factor-beta,54 which is released by platelets and lympho-
cytes. We speculate that the aggregation of platelets, the
adherence of leukocytes, or thrombosis at atherosclerotic
lesions may stimulate the production of ET-1 by endothe-
lium cells. Thus, release of ET-1, coupled with augmented
vasoconstrictor responses to ET-1 in atherosclerotic ar-
teries, may play a role in the pathogenesis of vasospasm.

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Index Terms: endothelin • peripheral circulation • cynomolgus monkeys


Vascular responses to endothelin-1 in atherosclerotic primates.

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