We determined the effects of mono-L-arginine–containing compounds on pial arterioles of anesthetized piglets. A closed cranial window was implanted, and the diameter of one pial arteriole was determined by intravital microscopy. Diameter was determined during application of artificial cerebrospinal fluid containing no drugs and during application of 10^-5, 10^-4, 10^-3, and 10^-2 M L-arginine (ARG), L-arginine ethyl ester (AEE), Nω-benzoyl-L-arginine (NBA), Nω-benzoyl-L-arginine ester ethyl (BAEE), and L-citrulline (CIT). Initial diameters were 100–200 μm. All of these compounds dilated arterioles, but the threshold concentration needed to elicit dilation varied: 10^-5 M for NBA (n=5), 10^-3 M for AEE (n=9) and BAEE (n=6), and 10^-2 M for ARG (n=6) and CIT (n=4). Maximal responses were 15±2% for CIT, 17±4% for ARG, 19±8% for BAEE, 28±5% for NBA, and 27±6% for AEE. Indomethacin pretreatment (5 mg/kg i.v.) did not change arteriolar responses to AEE, NBA, and BAEE. However, coadministration of methylene blue (0.5×10^-4 M or 0.5×10^-3 M) abolished dilation to 10^-3 M AEE or BAEE and attenuated dilation to 10^-2 M NBA. In addition, coadministration of hemoglobin (0.4×10^-4 M) abolished dilation to AEE, BAEE, or NBA. Last, intravenous (5 mg/kg) and coadministration (10^-3 M) of N^ω-methyl-L-arginine blocked dilation to NBA or AEE. We conclude that mono-L-arginine–containing compounds produce pial arteriolar dilation in piglets, possibly involving an endothelium-derived relaxing factor. (Circulation Research 1990;67:1374–1380)

The nonprostanoid endothelium-derived relaxing factor (EDRF) originally described by Furchgott and Zawadzki is a labile substance that is released in response to activation of muscarinic receptors and dilates blood vessels via a mechanism that involves cyclic GMP. The exact nature of EDRF is not known with certainty, but it may be nitric oxide or a related compound. Dilation due to EDRF is blocked or attenuated by several substances, including methylene blue, hemoglobin, and inhibitors of nitric oxide synthesis. Recent studies indicate that EDRF is an important control mechanism in many vascular beds. However, in several regional circulations, such as the cerebral circulation of the pig, EDRF-dependent responses appear to be absent or insignificant. For example, acetylcholine in piglets and juvenile pigs may cause modest dilation at low doses and causes potent constriction at high doses. Indomethacin pretreatment eliminates the dilation and abolishes the constriction without revealing relaxation in response to acetylcholine in piglets. In addition, in juvenile pigs, removal of the endothelium does not abolish either acetylcholine-induced dilation or constriction. Results from experiments involving topical application of other substances thought to work through EDRF—such as serotonin, substance P, and the calcium ionophore A23187—do not indicate that EDRF plays a significant role in responses to these substances in pial cerebral arterioles (unpublished observations, 1988). Specifically, application of A23187 constricts pial arterioles, and serotonin and substance P have little effect on diameter. However, bradykinin dilates pial cerebral arteries via another, yet unidentified, relaxing factor derived from the endothelium. It is unknown whether the failure to observe EDRF-related responses in piglets is due...
to lack of EDRF production or lack of responsiveness to EDRF once it is produced. Application of L-arginine (ARG) or other mono-L-arginine–containing compounds has been reported to cause relaxation of blood vessels and to produce nitric oxide.\(^8\)\(^{15-18}\) Whether this occurs in the cerebral circulation of the piglet or any other species is unclear.

The purpose of this study was to examine the influence of mono-L-arginine–containing compounds on pial arterioles of piglets. We tested the hypotheses that administration of these compounds would dilate pial arterioles and that this dilation would involve an EDRF mechanism.

**Materials and Methods**

Piglets initially were anesthetized with ketamine hydrochloride and acepromazine (33 and 3.3 mg/kg i.m., respectively), followed by alpha-chloralose (50 mg/kg i.v., initially, plus 5–10 mg/kg/hr). Catheters were placed into a femoral artery to record blood pressure and to sample for gases and pH and into a femoral vein for injection of drugs and fluids. The animals were intubated and ventilated with air. Body temperature was maintained at 37–38°C using a water-circulating rubber heating pad. After the scalp was removed, a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura and arachnoid membranes were cut without touching the brain, and all cut edges were reflected over the bone. A stainless steel and glass cranial window was placed in the hole and cemented to the skull with dental acrylic. The space under the window was filled, through needles incorporated into the sides of the windows, with artificial cerebrospinal fluid (aCSF) of the following composition (mM): KCl 2.9, MgCl\(_2\) 1.4, CaCl\(_2\) 1.2, NaCl 132, NaHCO\(_3\) 24.6, urea 6.7, and glucose 3.7. The aCSF was warmed to 39°C and equilibrated with 6.5% CO\(_2\)-6% O\(_2\) (balance N\(_2\)). The pH and gas values were pH 7.33; Pco\(_2\) 46 mm Hg; and Pao\(_2\) 43 mm Hg. The volume of fluid directly under the window was 500 μl and was contiguous with the subarachnoid space. Pial arterioles were observed with a trinocular stereomicroscope (Wild Leitz USA, Inc., Rockleigh, N.J.). Pial arteriolar diameter was measured with a television camera mounted on the microscope, a video monitor, and a video microscaler (model VPA-1000, For-A Corp., Newton, Mass.).

**Experimental Design**

The cranial window was flushed with aCSF several times. The diameter of one pial arteriole was measured in each animal. Maximal responses to each concentration were recorded. After a corresponding control period in which aCSF containing no drug was infused under the window and blood gases and arterial pressure were within normal limits, the arterioles were exposed to increasing concentrations (10\(^{-5}\), 10\(^{-4}\), 10\(^{-3}\), 10\(^{-2}\) M) of either ARG HCl, ARG ethyl ester (AEE), Na-benzoyl-L-arginine (NBA), Na-benzoyl-L-arginine ethyl ester (BAEE), or L-citrulline (CIT) for up to 5 minutes (all from Sigma Chemical Co., St. Louis). AEE, BAEE, ARG, and CIT were dissolved in aCSF. NBA required prolonged sonication to be dissolved in aCSF or distilled water. Aliquots of the NBA solution were further diluted in aCSF. In addition, responses to AEE, NBA, or BAEE were examined before and after intravenous administration of indomethacin trihydrate (5 mg/kg) (a gift from Merck Sharp & Dohme, West Point, Pa.). We have shown previously that this dose of indomethacin is able to reduce prostanoid levels in CSF to nondetectable levels and to substantially reduce pial arteriolar responses to prostanoid-dependent stimuli.\(^19\)\(^\)\(^20\)

In additional experiments to determine whether dilation was due to the release of an EDRF-like substance, we measured diameter during topical application of methylene blue (Sigma) or porcine hemoglobin (Sigma) in aCSF alone or with AEE, BAEE, or NBA. We also examined whether N\(^\cdot\)methyl-L-arginine (NMA) (Sigma), an inhibitor of EDRF production, was able to reduce pial arteriolar dilation to NBA and AEE. We applied 0.5 × 10\(^{-4}\) M methylene blue with AEE (10\(^{-3}\) M), BAEE (10\(^{-3}\) M), or NBA (10\(^{-5}\), 10\(^{-4}\) M) and 0.5 × 10\(^{-3}\) M methylene blue with NBA (10\(^{-3}\), 10\(^{-4}\) M). To test dilator ability in the presence of methylene blue, we measured diameter when the animals were breathing room air and during inspiration of 10% CO\(_2\) in air. We applied 0.4 × 10\(^{-4}\) M hemoglobin with AEE (10\(^{-3}\) M), BAEE (10\(^{-3}\) M), or NBA (10\(^{-4}\) M). To assess dilator ability in the presence of hemoglobin, we determined arteriolar diameter during coapplication of isoproterenol (10\(^{-4}\) M). Hemoglobin was reduced to the ferrous form before use by the method of Martin et al.\(^17\) Briefly, a 10\(^{-3}\) M hemoglobin solution was combined with a 10-fold molar excess of sodium dithionite, and the dithionite was removed by dialyses against a 100:1 volume of distilled water. Final hemoglobin concentration was measured by spectrophotometry (IL co-oximeter, model 282, Lexington, Mass.) and was found to be 0.8 × 10\(^{-3}\) M. Aliquots were frozen at −20°C until use. We gave NMA intravenously (5 mg/kg) and applied 10\(^{-3}\) M NMA with NBA (10\(^{-3}\), 10\(^{-4}\) M) and AEE (10\(^{-3}\) M). To assess dilator capacity in the presence of NMA, we determined arteriolar diameter during inspiration of 10% CO\(_2\) in air.

In two piglets, arteriolar diameter was measured in the presence of aCSF alone and aCSF containing 10\(^{-4}\) M 5-nitros o-N-acetyl penicillamine (SNAP) (a gift from Dr. T. Patel, University of Tennessee, Memphis), which forms nitric oxide in solution.

Arterial blood gas values under control conditions pooled for the animals were pH 7.52 ± 0.03; Pco\(_2\), 36 ± 1 mm Hg; and Pao\(_2\), 74 ± 2 mm Hg (n = 34).

**Statistical Analysis**

Values are presented as mean ± SEM. Because of missing data, data points for diameter and blood
pressure for BAEE (10⁻⁵ M dose) and NBA (10⁻² M dose) were estimated before further analysis.²¹ Statistical analyses comparing baseline values to treatment values were conducted using paired $t$ tests or repeated measures analysis of variance. If the $F$ value was significant, pairwise comparisons were made using the Student-Newman-Keuls test. For comparison of percent change data from different animals, unpaired $t$ tests were used. For all percentage data, an arcsine transformation was done to normalize values before additional statistical treatment.

## Results

Topical application of mono-L-arginine compounds diluted piglet pial arterioles (Table 1 and Figure 1). The dose needed to elicit a statistically significant dilation was 10⁻² M for NBA, 10⁻³ M for AEE and BAEE, and 10⁻² M for ARG and CIT. In some cases, dilation was not reversible by flushing out the arginine compounds with aCSF. In 13 of 30 cases, the dilation seen with the arginine compounds persisted in spite of repeated flushing of the window with aCSF containing no arginine compounds. When this situation occurred and it was not the intended end of the experiment, the window was flushed with aCSF containing 10⁻³ M L-norepinephrine, which constricted the arterioles. With subsequent flushing with aCSF, arterioles returned to baseline levels. After this occurred, arterioles still dilated normally in response to other arginine compounds. For the specific compounds, dilation was sustained in seven of nine cases for AEE, three of five for NBA, two of six for BAEE, one of six for ARG, and zero of four for CIT.

Indomethacin pretreatment did not prevent dilation in response to AEE, BAEE, or NBA (Table 2). Prolonged dilation occurred in six of 11 trials (AEE, BAEE, and NBA combined).

Administration of methylene blue (0.5×10⁻⁴ M or 0.5×10⁻³ M) did not change baseline arteriolar diameter. In the presence of aCSF alone, diameter was 131±11 μm; in the presence of aCSF plus methylene blue, diameter was 127±11 μm (NS; $n=12$). Methylene blue at 0.5×10⁻⁴ M blocked dilation to AEE (10⁻³ M) (initial diameter, 108±12 μm; $n=3$) or BAEE (10⁻³ M) (initial diameter, 124±5 μm; $n=4$) but not to NBA (10⁻³, 10⁻⁴ M) (initial diameter, 122±32 μm; $n=3$) (Figure 2). In the presence of methylene blue (0.5×10⁻⁴ M), arterioles still dilated in response to arterial hypercapnia (initial diameter, 115±11 μm; $n=4$) (Figure 2, upper panel). However,
TABLE 2. Effects on Pial Arterioles of L-Arginine Ethyl Ester or Na-Benzoyl-L-arginine Ethyl Ester in Indomethacin-Pretreated Piglets

<table>
<thead>
<tr>
<th>Compound</th>
<th>Baseline</th>
<th>10^{-4} M</th>
<th>10^{-3} M</th>
<th>10^{-2} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine ethyl ester (n=3)</td>
<td>Diameter (μm)</td>
<td>109±8</td>
<td>112±8</td>
<td>122±6</td>
</tr>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>74±7</td>
<td>72±9</td>
<td>71±8</td>
</tr>
<tr>
<td>Na-Benzoyl-L-arginine ethyl ester (n=5)</td>
<td>Diameter (μm)</td>
<td>102±7</td>
<td>104±6</td>
<td>111±8</td>
</tr>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>73±76</td>
<td>72±6</td>
<td>68±6</td>
</tr>
<tr>
<td>Na-Benzoyl-L-arginine (n=3)</td>
<td>Diameter (μm)</td>
<td>132±25</td>
<td>169±33*</td>
<td>170±36*</td>
</tr>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>55±5</td>
<td>53±4</td>
<td>53±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial blood pressure.

* p<0.05 compared with baseline.
† p<0.05 compared with 10^{-3} M.
‡ p<0.05 compared with 10^{-4} M.
§ p<0.05 compared with 10^{-3} M.

FIGURE 2. Upper panel: Percent change from baseline for arteriolar diameter during coadministration of methylene blue (0.5x10^{-4} M) with L-arginine ethyl ester (AEE) or Na-benzoyl-L-arginine ethyl ester (BAEE) and for methylene blue plus arterial hypercapnia. Values are mean±SEM. * p<0.05 compared with baseline. Lower panel: Percent change from baseline for arteriolar diameter for Na-benzoyl-L-arginine (10^{-5}, 10^{-4} M) in the absence of methylene blue (No MB), 0.5x10^{-3} M methylene blue (Low MB), and 0.5x10^{-2} M methylene blue (High MB). Values are mean±SEM. * p<0.05 compared with baseline; † p<0.05 compared with corresponding value during No MB.

FIGURE 3. Percent change from baseline for arteriolar diameter for L-arginine ethyl ester (AEE) (10^{-3} M), Na-benzoyl-L-arginine ethyl ester (BAEE) (10^{-3} M), Na-benzoyl-L-arginine (NBA) (10^{-4} M), and isoproterenol (ISO) (10^{-4} M) in the presence of 0.4x10^{-4} M hemoglobin. Values are mean±SEM. * p<0.05 compared with baseline.

a higher dose of methylene blue (0.5x10^{-3} M) attenuated the dilation to 10^{-3} M NBA (initial diameter, 185±24 μm; n=3) (Figure 2, lower panel). In the presence of methylene blue (0.5x10^{-3} M), pial arterioles dilated from a baseline value of 127±31 to 149±26 μm (n=5) during inhalation of 10% CO_2 (percent change, 28±12; p<0.05).

Administration of hemoglobin (0.4x10^{-4} M) did not change baseline arteriolar diameter. In the presence of aCSF, diameter was 180±12 μm; in the presence of aCSF plus hemoglobin, diameter was 184±12 μm (NS; n=11). Hemoglobin blocked dilation to AEE (10^{-3} M) (initial diameter, 155±10 μm; n=3), BAEE (10^{-3} M) (initial diameter, 198±22 μm; n=5), or NBA (10^{-4} M) (initial diameter, 188±22 μm; n=3), whereas dilation to 10^{-4} M isoproterenol was preserved (initial diameter, 104±13 μm; n=8) (Figure 3).
Administration of NMA (5 mg/kg i.v., 10⁻³ M topical) had no effect on arteriolar diameter. In the presence of aCSF, diameter was 114±16 μm; in the presence of NMA, diameter was 110±17 μm (n=4). NMA treatment abolished the dilator response to NBA (initial diameter, 110±17 μm; n=4) and AEE (initial diameter, 100±16 μm; n=3) but did not prevent dilation to CO₂ inspiration (initial diameter, 102±μm; n=3) (Figure 4).

In the two animals studied, 10⁻⁴ M SNAP caused substantial dilation. In one, arteriolar diameter increased from 256 μm (mean arterial blood pressure, 60 mm Hg) during the control period to 353 μm (mean arterial blood pressure, 60 mm Hg) during application of 10⁻⁴ M SNAP. In the other animal, SNAP increased diameter from 175 μm (mean arterial blood pressure, 68 mm Hg) to 300 μm (mean arterial blood pressure, 63 mm Hg).

**Discussion**

We found that mono-L-arginine-containing compounds produce dilation in the piglet cerebral circulation. In addition, these substances do not cause dilation via a prostanooid mechanism but operate through the release of an EDRF-like substance. Thus, under appropriate conditions, piglet pial arterioles can be induced to generate or respond to a substance that may be the classical EDRF.

Previous results from our laboratories indicate that the piglet cerebral circulation does not normally make EDRF. Acetylcholine, the generally recognized “gold standard” for eliciting EDRF responses, operates via a prostanooid rather than an EDRF mechanism in pial arterioles of piglets and juvenile pigs.¹⁻¹³ Thus, acetylcholine may dilate or constrict arterioles at low doses and only constrict them at higher doses, but all responses are due to production and/or presence of prostanooids and are blocked by indomethacin. Furthermore, even at high doses of acetylcholine, indomethacin pretreatment does not unmask a dilator response. In juvenile pigs, removal of endothelium does not affect responsiveness of cerebral arteries to acetylcholine.¹² In addition, application of other substances—such as substance P, the calcium ionophore A23187, and serotonin—indicates that these substances do not elicit arteriolar changes via an EDRF mechanism in newborn pigs (unpublished results, 1988). Cerebral vessels may dilate in response to bradykinin via another type of dilator substance released from the endothelium.¹⁴ Ours is the first study to show that an EDRF-like substance can be produced by L-arginine-containing compounds in a circulation that does not normally make EDRF. The reason that the piglet cerebral circulation does not dilate via an EDRF mechanism in response to acetylcholine is unclear but probably does not represent a response unique to the neonate, because we find that lamb pial arterioles dilate in response to acetylcholine (unpublished observations, 1989). However, many studies indicate that normal coronary²²,²³ and pulmonary²⁴ arterioles from several species, dog saphenous vein,²⁵ and dog basilar artery¹⁵ do not dilate but rather constrict in response to acetylcholine, and this phenomenon may mean that many vascular beds do not rely heavily on the classical EDRF mechanism. Rather, other mechanisms, such as the prostanooid system, may be more important in mediating vascular responses in those circulations.

To our knowledge, there have been no previously published studies examining the effects of L-arginine and derivative compounds on cerebral vessels. However, there are several studies in which these compounds have been applied to other circulations. Thomas and Ramwell²⁶ have reported that BAEE, NBA, and AEE, but not ARG, were dilator stimuli in rat aortic rings. Furthermore, dilator responses, particularly to NBA, were reduced after endothelium removal. In the rabbit aorta, ARG induced a modest relaxation.¹⁸ In addition, Thomas et al²⁷ reported that porcine internal mammary artery dilates in response to BAEE, NBA, and AEE, but not to ARG. Also, Thomas and coworkers²⁸ showed that AEE and BAEE dilate mesenteric arteries of rats. Basic polyamino acids, rich in arginine, also cause relaxation in the rat aorta and bovine intrapulmonary artery and vein.²⁹,³⁰ Our results extend these findings to the piglet pial circulation and show that NBA, BAEE, AEE, CIT, and ARG all are able to dilate the piglet pial arterioles. On a molar basis, NBA caused dilation at the lowest dose (10⁻⁵ M), followed by AEE and BAEE (10⁻⁴ M). However, ARG and CIT did not cause significant dilation until 10⁻² M. It is possible that some of the dilation at 10⁻² was due to a slight acidification of CSF by HCl in these compounds. Normal pH for our gassed aCSF was approximately 7.33. At 10⁻² M, CSF pH was approximately 0.08 lower. At 10⁻⁵–10⁻³ M, no detectable change in CSF pH was observed.
Based on two criteria, NBA and AEE were the most potent of the compounds examined. First, they produced the largest dilations. Second, persistence of dilation was greatest for AEE and NBA (occurring in 78% and 60% of the trials, respectively). For BAEE, sustained dilation occurred in 33% of the animals. For ARG and CIT combined, sustained dilation occurred only once out of 10 times. Thus, it appears that addition of a benzoyl and/or ethyl ester group is necessary for L-arginine to be readily metabolized to an EDRF-like substance. Greater potency for NBA compared with AEE and BAEE may be due to decreased lipid solubility due to the ethyl ester moiety, which could reduce incorporation of AEE and BAEE into cells.

The mechanism for the dilator response to these compounds is not known with certainty but appears to involve synthesis of an EDRF-like substance. The evidence for this view is provided from experiments in which three different pharmacological probes working through distinct mechanisms were able to block dilator responses to mono-L-arginine-containing compounds. First, methylene blue blocked responses to AEE, BAEE, and NBA but not dilation in response to arterial hypercapnia. Methylene blue has been shown to be a potent inhibitor of vascular responsiveness to EDRF,6,7 apparently acting indirectly by inhibiting production of cyclic GMP through blockade of guanylate cyclase.6,7 Second, the presence of hemoglobin prevented dilator responses to AEE, BAEE, and NBA. Hemoglobin has been shown to effectively inhibit the actions of EDRF, probably by binding with EDRF. Consistent with this view, it has been reported that experimental subarachnoid hemorrhage selectively blocks EDRF responses in the rabbit basilar artery.31 Third, NBA, which prevents conversion of mono-L-arginine-containing compounds into nitric oxide,16 blocked arteriolar dilation to NBA and AEE. Because indomethacin did not blunt the dilator response to the arginine-containing compounds, it appears that prostanooids are not involved.

Recent evidence indicates that one EDRF may be nitric oxide or a related compound, and arginine compounds may be precursors to nitric oxide.4,5,18,26 For example, ARG and CIT are able to induce nitric oxide production in porcine or rabbit aortic endothelial cells.16 Nitric oxide appears to be derived from the terminal guanido nitrogen atom(s) of arginine.9,16 Because conversion of CIT to nitric oxide may involve an additional step, in that CIT needs to be converted first into arginine,16 it seems unlikely that this conversion occurred during the short time of our experiments. As mentioned before, slight acidosi at $10^{-2}$ M is more likely to contribute to dilation. We believe that our results support the concept that mono-L-arginine-containing compounds can be precursors for EDRF in the piglet pial circulation.

On a molar basis, application of SNAP produced much more dilation than even the highest dose of the L-arginine-containing compounds. The reason for the difference probably is that only a portion of the L-arginine molecules are metabolized into nitric oxide, whereas the amount of nitric oxide produced from SNAP is likely to be much higher.

The reason for prolonged dilation, especially after application of AEE or NBA, is unclear. The persistent dilation is not attributable to damage to the vessels. After application of norepinephrine and the resulting constriction, flushing CSF over the vessels restores initial diameter. At that time, the vessels once again can dilate in response to other arginine-containing compounds. We reasoned that generation of activated oxygen species could produce strong, irreversible dilation. However, free radicals would have to be generated via a process other than the prostanooid pathway. We have found that generation of superoxide anion is largely cyclooxygenas dependent in the piglet pial circulation during ischemia and reperfusion,32 asphyxia and reventilation,33 and seizures.34 In the present study, dilation in response to AEE, BAEE, and NBA still occurred after pretreatment with indomethacin, and prolonged dilation still occurred over 50% of the time. It is possible that the prolonged response is due to the slow conversion of AEE, BAEE, and NBA into derivative products after loading of tissues, as Palmer et al16 suggest occurs for CIT. Simultaneous application of norepinephrine will counteract the dilation with a constrictor influence and may stop conversion of arginine compounds to their dilator product via an as yet unknown mechanism.

In summary, mono-L-arginine-containing compounds dilate piglet pial arterioles via a nonprostanooid mechanism, which appears to involve synthesis of an EDRF-like substance. Thus, induction of the EDRF-related mechanism for dilation can occur in a circulation that appears not to rely heavily on EDRF for regulation of vascular tone.

Acknowledgments

We thank Diane Loria, Ronnie Sabbah, Alex Fedinec, Joel Giddens, and John Pirani for technical assistance and Jim Emerson-Cobb and Collean Payne for typing the manuscript.

References


**KEY WORDS** • neonate • endothelium-derived relaxing factor • cerebral circulation
Mono-L-arginine-containing compounds dilate piglet pial arterioles via an endothelium-derived relaxing factor-like substance.

D W Busija, C W Leffler and L C Wagerle

*Circ Res.* 1990;67:1374-1380
doi: 10.1161/01.RES.67.6.1374

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 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/67/6/1374

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/