Vasodilator mechanisms in the coronary circulation of endothelial nitric oxide synthase-deficient mice

KATHRYN G. LAMPING, DANIEL W. NUNO, EDWARD G. SHESELY, NOBUYO MAEDA, AND FRANK M. FARACI

Departments of Internal Medicine and Pharmacology, The Cardiovascular Center, University of Iowa, and Veterans Affairs Medical Center, Iowa City, Iowa 52246

Received 7 September 1999; accepted in final form 12 May 2000

Lamping, Kathryn G., Daniel W. Nuno, Edward G. Shesely, Nobuyo Maeda, and Frank M. Faraci. Vasodilator mechanisms in the coronary circulation of endothelial nitric oxide synthase-deficient mice. Am J Physiol Heart Circ Physiol 279: H1906–H1912, 2000.—Previous studies have demonstrated that responses to endothelium-dependent vasodilators are absent in the aortas from mice deficient in expression of endothelial nitric oxide synthase (eNOS /−/− mice), whereas responses in the cerebral microcirculation are preserved. We tested the hypothesis that in the absence of eNOS, other vasodilator pathways compensate to preserve endothelium-dependent relaxation in the coronary circulation. Diameters of isolated, pressurized coronary arteries from eNOS /−/−, eNOS heterozygous (+/−), and wild-type mice (eNOS +/+ and C57BL/6J) were measured by video microscopy. ACh (an endothelium-dependent agonist) produced vasodilation in wild-type mice. This response was normal in eNOS /−/− mice and was largely preserved in eNOS /−/+ mice. Responses to nitroprusside were also similar in arteries from eNOS /−/+, eNOS /+/+, and eNOS /−/− mice. Dilation to ACh was inhibited by G-nitro-L-arginine, an inhibitor of NOS in control and eNOS /−/− mice. In contrast, trifluoromethylphenylimidazol, an inhibitor of neuronal NOS (nNOS), decreased ACh-induced dilation in arteries from eNOS-deficient mice but had no effect on responses in wild-type mice. Indomethacin, an inhibitor of cyclooxygenase, decreased vasodilation to ACh in eNOS-deficient, but not wild-type, mice. Thus, in the absence of eNOS, dilation of coronary arteries to ACh is preserved by other vasodilator mechanisms.

nitric oxide synthase; acetylcholine; cyclooxygenase

Although the use of inhibitors of the different NOS isoforms potentially will provide insight into the contribution of NO from different sources, interpretation of these studies must be made with caution, because most agents inhibit all forms of NOS. Currently, there are no selective inhibitors of eNOS.

Development of genetic models with altered expression of genes encoding the different NOS isoforms allows more precise studies of the role of NO in the regulation of vascular tone. Studies of both the aorta and pulmonary and carotid arteries from eNOS-deficient mice (eNOS /−/− mice) have demonstrated that NO derived from eNOS is the primary mediator of relaxation to ACh and A-23187, because responses to these agonists were absent in these vessels from eNOS /−/− mice (9, 16, 22, 24, 38). NO derived from eNOS is not mandatory for responses to ACh in all vascular beds, however. In cerebral arterioles of eNOS /−/− mice, dilation to ACh was normal but mediated by other mechanisms (26, 27). These differences in responses of vascular tissue to ACh in eNOS /−/− mice may reflect differences in the contribution of NO in mediating responses or the ability of other vasodilator mechanisms to compensate in the face of eNOS deficiency.

Previous studies from our laboratory have demonstrated that in genetic models of hypercholesterolemia, responses of coronary arteries to ACh are preserved despite impaired responses of aorta (2, 25). Results from those studies suggest that the coronary circulation is resistant or able to compensate in the presence of a risk factor for vascular disease. The first objective of the present study was to test the hypothesis that responses to ACh are preserved in coronary arteries from eNOS /−/− mice. We previously demonstrated that responses of coronary arteries from normal mice to ACh are mediated primarily by NO (25). To determine the role of NO derived from eNOS in responses of coronary arteries to ACh, we measured changes in diameter of isolated, pressurized segments of coronary arteries from wild-type control mice, eNOS heterozygote mice (eNOS /−/+ mice), and eNOS /−/− mice with...
video microscopy. The second goal of this study was to test the hypothesis that nNOS or cyclooxygenase compensates for the loss of eNOS and mediates responses to ACh, the classic endothelium-dependent agonist.

METHODS

Animals. The animal protocol used in these experiments was reviewed and approved by the University of Iowa Animal Care and Use Committee. Three groups of mice were studied: wild-type control mice (C57BL/6J or eNOS +/+ littermates), eNOS heterozygous mice (eNOS +/-), and eNOS-deficient mice (eNOS –/–). These mice were originally generated as a hybrid of 129 × C57BL/6J (35). Mice used in this study were derived from three to four generations of backcross breeding to C57BL/6J mice. Mice were fed regular chow, and water was available ad libitum. The ages of mice in the different groups were similar.

Genotyping of mice was performed by Southern blotting DNA from tail biopsies. High-molecular-weight genomic DNA was isolated from tail biopsies, and identification of eNOS +/+ , eNOS +/-, and eNOS –/– mice was accomplished as described previously (9, 24, 35).

General preparation. Mice (40 males and 51 females) were heparinized and anesthetized with pentobarbital sodium (75–100 mg/kg ip) or α-chloralose (300–400 mg/kg ip). Hearts were rapidly removed and placed in cold Krebs buffer consisting of (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.0 glucose. Left anterior descending or left circumflex coronary arteries from the left ventricle (62–207 nm in diameter) were isolated from myocardium under a microscope, placed in an organ chamber filled with cold Krebs, cannulated with dual micropipettes, and secured with 10–0 monofilament suture. The organ chamber (20 ml) was continuously circulated with Krebs solution bubbled with 20% O2, 5% CO2, and 75% N2. Vessels were pressurized to 40 mmHg under no-flow conditions using two reservoirs filled with Krebs solution. Images of microvessels were displayed on a video monitor using a microscope connected to a camera. An electronic video dimension analyzer measured luminal diameter at steady state. The distending pressure of the vessels was measured with a pressure transducer connected to a sidearm of the cannula connected to one of the micropipettes. Vessels were allowed to equilibrate for 60 min before study. Viability of the vessels was assessed as a minimum of 30–50% constriction in response to 100 mM KCl from resting diameter.

Protocols. Vessels segments were preconstricted with the thromboxane mimetic U-46619 (9,11-dideoxy-11a,9a-epoxy-thromboxane mimetic U-46619 (9,11-dideoxy-11a,9a-epoxy-thromboxane B2, 7–17 × 10−8 M) to 30–60% of the initial vessel diameter. Cumulative dose-response curves to ACh (10−9–10−5 M) were performed. At the completion of the dose-response curve, nitroprusside (10 μM) or papaverine (200 μM) was added to the bath. To compare responses of vascular smooth muscle in arteries from wild-type, eNOS +/+ , and eNOS –/– mice, cumulative dose-response curves to nitroprusside (10−9–10−5 M) were performed. To determine the role of NO in responses of coronary arteries to ACh, dose-response curves to ACh were performed in the presence of Nω-nitro-L-arginine (L-NNA, 10 or 100 μM), an inhibitor that is not specific for a single isoform of NO. The concentrations of L-NNA were chosen on the basis of previous experiments (2, 9, 16, 24, 38). To determine the role of cyclooxygenase in responses of coronary arteries to ACh, dose-response curves to ACh were performed in the presence of indomethacin (10 μM), an inhibitor of cyclooxygenase. Finally, to determine the role of nNOS in responses of coronary arteries to ACh, dose-response curves to ACh were performed in the presence of trifluromethylphenylimidazole (TRIM, 100 μM), an inhibitor of nNOS (28). All inhibitors were added to the organ bath for a minimum of 30 min before the dose-response curves were performed.

Because it was suggested that pentobarbital sodium may alter the relative contribution of NO and endothelium-derived hyperpolarization factor(s) in endothelium-dependent responses to ACh (6), we also examined the effect of L-NNA on the responses to ACh in arteries obtained from control mice anesthetized with α-chloralose.

Drugs. U-46619 was obtained from Biomol Research Laboratories and dissolved in 100% ethanol. ACh, nitroprusside, L-NNA, and indomethacin were obtained from Sigma Chemical and dissolved in distilled water. TRIM was obtained from RBI. All concentrations are final molar concentrations in the organ chamber.

Statistical analysis. Data are presented as percent change in diameter from the preconstricted diameter and are presented as means ± SE. One vessel was obtained per mouse, and n represents the number of mice per group. One dose-response curve was performed per vessel. Comparisons were made using a two-way ANOVA with repeated measures followed by Student-Newman-Keuls test to detect individual differences. A P < 0.05 was defined as being statistically significant.

RESULTS

Responses of coronary arteries from wild-type mice. Baseline diameters of coronary arteries from wild-type mice (eNOS +/+ littermates and C57BL/6J) were 118 ± 10 μm (n = 12). ACh produced dose-dependent dilation of coronary arteries from wild-type mice (maximal dilation to ACh was 49 ± 6%, n = 12, Fig. 1). Dilation in response to ACh was inhibited by 10 μM L-NNA (maximal dilation to ACh with L-NNA was 17 ± 4%, n = 8, Fig. 2). A higher concentration of L-NNA (100 μM, n = 4) inhibited over 90% of the maximal response, suggesting that dilation to ACh in coronary arteries from wild-type mice is mediated predominantly by NO. Vasodilation to nitroprusside (10 μM) was similar in the absence and presence of L-NNA (control = 69 ± 7%, n = 6; L-NNA = 61 ± 17%, n = 4).

To examine possible effects of anesthesia, responses to ACh were measured in arteries obtained from mice anesthetized with pentobarbital sodium or α-chloralose. Responses to ACh were similar whether mice were anesthetized with pentobarbital sodium or α-chloralose (Fig. 2). In addition, L-NNA (10−4 M) inhibited over 90% of the response to the maximal dose of ACh whether vessels were obtained from mice anesthetized with pentobarbital sodium (n = 4) or α-chloralose (n = 4, Fig. 2). Thus the contribution of NO in the response to ACh is similar in coronary arteries obtained from mice anesthetized with pentobarbital sodium or α-chloralose.

Responses of coronary arteries from eNOS +/+ and eNOS −/− mice. Baseline diameters for coronary arteries from eNOS +/+ and eNOS −/− mice were similar to diameters of wild-type mice (eNOS +/+ mice = 120 ± 16 μm, n = 6; eNOS −/− mice = 103 ± 9 μm, n = 9). Arteries from eNOS −/− mice dilated in response to ACh (maximal dilation to ACh was 36 ± 7%) (Fig. 1),
Although slightly reduced compared with eNOS +/+ mice, dilation of arteries from eNOS +/− mice to ACh was similar to the wild-type mice (maximal response to ACh at 10 μM was 59 ± 12%). Dilation of arteries from eNOS +/− (n = 7) and eNOS −/− (n = 4) in response to nitroprusside was similar to wild-type mice (n = 5, Fig. 1). Thus, although the aortas and carotid arteries from eNOS −/− mice do not relax to ACh, coronary arteries from eNOS +/− and eNOS −/− mice dilate in response to ACh.

To determine possible mechanisms involved in the dilation to ACh in coronary arteries from eNOS −/− mice, responses to ACh were measured in the presence of L-NNA. Similar to arteries from wild-type mice, dilation of coronary arteries from eNOS −/− mice to ACh was inhibited by L-NNA (maximal response to ACh was 13 ± 7%, n = 5; Fig. 3). Dilation of coronary arteries from eNOS −/− mice to nitroprusside was not altered by L-NNA (data not shown).

Effect of TRIM on responses to ACh. To determine the possible role of nNOS in mediating responses of coronary arteries to ACh, dose-response curves were performed in the presence of TRIM, a selective inhibitor of nNOS (13, 14, 28). In arteries from wild-type mice, TRIM had no effect on dilation in response to ACh (maximal response to ACh was 50 ± 16%, n = 4, Fig. 4). In contrast, in arteries from eNOS −/− mice, dilation in response to ACh was inhibited by TRIM (maximal response to ACh was 18 ± 8%, n = 4, Fig. 4). TRIM had no effect on dilation of coronary arteries from eNOS +/+ or eNOS −/− mice in response to papaverine (data not shown). These findings suggest that in coronary arteries from eNOS −/− mice, dilation to ACh is dependent on activity of nNOS.

Effect of indomethacin on responses to ACh. To determine the role of cyclooxygenase in responses of coronary arteries to ACh, dose-response curves were performed in the presence of indomethacin (10 μM). In arteries from wild-type mice, dilation in response to ACh was not significantly altered by indomethacin (maximal response to ACh was 59 ± 10%, n = 6) (Fig. 5). In contrast to responses in eNOS +/+ mice, dilation of coronary arteries from eNOS −/− mice in response to ACh was decreased by indomethacin (maximal response to ACh at 10 μM was 4 ± 4%, n = 6) (Fig. 5). Indomethacin had no effect on dilation of coronary arteries from eNOS +/+ or eNOS −/− mice to papaverine (data not shown). These findings suggest that in coronary arteries from eNOS −/− mice, dilation in response to ACh is dependent in part by activity of cyclooxygenase.

**Discussion**

There are several major findings in this study. First, deletion of a single copy of the gene for eNOS (in eNOS +/− mice) had no significant effect on responses of coronary arteries to ACh. Dilation of coronary arteries from eNOS −/− mice in response to ACh was largely preserved. The latter results are in marked contrast to
previous finding that in coronary arteries from normal mice, dilation to ACh is primarily mediated by NO (25). In the present study, indomethacin and TRIM did not alter dilation of coronary arteries from wild-type mice to ACh, suggesting cyclooxygenase and nNOS are not involved in the response. Responses to ACh in mouse coronary arteries are similar to responses in the aortas, carotid, and pulmonary arteries and cerebral arterioles that are largely mediated by NO in normal mice (2, 9, 26, 27, 37, 38). These findings are also consistent with studies in both experimental animals and humans in which NO was found to be the primary mediator of responses to endothelium-dependent agonists (8, 11, 12, 21, 23, 24, 33). Because vasodilation to ACh was inhibited by ~90% by an inhibitor of NOS (present study) or 1H-(1,2,4)oxadiazolo[4,3-a]quinazolin-1-one, an inhibitor of soluble guanylate cyclase (25, 29–31), these findings suggest that other vasodilator mechanisms play a very minimal role in this response in normal murine coronary arteries. These findings are similar regardless of which anesthetic is used.

**Role of eNOS in dilation to ACh.** Several studies have demonstrated abnormal vascular responses in arteries isolated from mice deficient in the expression of the gene for eNOS (4, 9, 16, 22, 24, 38). In the aortas and carotid and pulmonary arteries from eNOS−/− mice, relaxation in response to ACh was absent (4, 9, 16, 22, 24, 38). Thus studies of aorta and other large arteries from normal and eNOS−/− mice have provided direct evidence that release of NO from eNOS is the primary mechanism of relaxation to ACh.

In contrast to studies of the mouse aorta and other large arteries, responses of coronary arteries to ACh are largely preserved in eNOS−/− mice. These results are surprising, because acute inhibition of NOS significantly attenuated dilation to ACh in both our previous study (25) and in the present study. Because acute inhibition of NOS significantly attenuated dilation to ACh, we concluded that responses to ACh are primarily mediated by NO under normal conditions. However, studies of coronary arteries from eNOS−/− mice suggest that in a state of chronic eNOS deficiency, other vasodilator pathways can compensate for the loss of eNOS. We assumed that responses to ACh are endo-

**Reactivity of normal coronary arteries.** In a previous study from our laboratory, we examined mechanisms of vascular reactivity of coronary arteries from normal mice. Depending on species, organ, or location within the vascular tree, endothelium-dependent relaxation to ACh can be mediated by NO, prostaglandins, or endothelium-derived hyperpolarizing factors (40). Results of the present study confirmed and extended our reports that responses to ACh are absent in the aortas and pulmonary and carotid arteries from eNOS−/− mice (9, 16, 24, 38). Second, dilation of coronary arteries from wild-type mice to ACh was mediated very predominantly by NO. This finding confirms our previous study in which dilation of coronary arteries from C57BL/6J mice in response to ACh was inhibited markedly by L-NNA or an inhibitor of soluble guanylate cyclase (25). Third, in contrast to wild-type mice, dilation of coronary arteries from eNOS−/− mice in response to ACh was inhibited by either indomethacin or TRIM, suggesting that responses to ACh were dependent on activity of cyclooxygenase and nNOS. These inhibitors had no effect on dilator responses of coronary arteries from wild-type mice, indicating selectivity of the inhibitory effect in eNOS−/− mice. Thus, unlike other vascular tissue (9, 16, 22, 24, 38), in the absence of eNOS, other vasodilator pathways compensate to maintain near-normal coronary arterial function.

**Fig. 3.** Percent change in diameter of coronary arteries from eNOS−/− mice to ACh in the absence (n = 9) and presence of L-NNA (10 μM, n = 5). Dilation to ACh was decreased in arteries from eNOS−/− mice in the presence of L-NNA (*P < 0.05 vs. eNOS−/−).

**Fig. 4.** Percent change in diameter of coronary arteries from normal (A) and eNOS−/− mice (B) to ACh in the absence and presence of trifluoromethylphenylimidazole (TRIM) (100 μM), an inhibitor of neuronal NOS (nNOS). Dilation to ACh was decreased in arteries from eNOS−/− mice (control n = 9, TRIM n = 4) but not in arteries from eNOS+/+ mice (control n = 12, TRIM n = 4) in the presence of TRIM (*P < 0.05 vs. eNOS−/−).
In addition, responses of cerebral arterioles to ACh in eNOS−/− mice were blocked by an inhibitor of nNOS (7-nitroindazole) (26). Thus Meng et al. (26, 27) suggested that vasodilation to ACh is mediated by nNOS in eNOS−/− mice. These findings suggest that responses of cerebral and coronary vessels in eNOS−/− mice to ACh are likely because of a compensatory mechanism expressed in response to chronic eNOS deficiency. Although inhibition of nNOS (7-nitroindazole) attenuated dilation to ACh in eNOS−/− mice, effects of indomethacin were not tested in the previous studies of cerebral arterioles (26, 27).

In the present study, an nNOS inhibitor (TRIM) attenuated responses to ACh in arteries from eNOS−/− mice. These data suggest that during complete eNOS deficiency, dilation to ACh is mediated by nNOS. Our finding that a selective inhibitor of nNOS (TRIM) abolished dilation to ACh in coronary arteries from eNOS−/− mice is consistent with the studies of Meng at al. (26) in cerebral arterioles from the same mice. The coupling between ACh and production of NO by nNOS in the coronary circulation is unknown, and additional studies will be needed to examine expression and mechanisms of activation of nNOS in coronary arteries from eNOS−/− mice. Although one approach to this question would be to examine expression of nNOS by Northern or Western blotting, the small size of the mouse coronary arteries makes such measurements unrealistic or at least extremely difficult.

Because the conclusion related to nNOS in this study is based largely on the findings obtained with TRIM, it is important to consider the specificity of this inhibitor. Both TRIM and 7-nitroindazole are known to be effective inhibitors of nNOS when used in vivo and in vitro (28). In vitro, however, 7-nitroindazole also inhibits eNOS (28). In contrast, TRIM does not inhibit eNOS (13, 28), and TRIM did not inhibit responses to ACh in vessels from wild-type mice in the present study. Although TRIM has some inhibitory effect on iNOS (13, 28), iNOS is not present in blood vessels normally, and we are not aware of any data suggesting that iNOS is upregulated in eNOS−/− mice. More importantly, TRIM was used to test the role of NOS in responses to ACh, which is a calcium-dependent, receptor-mediated response. Activity of iNOS is calcium independent and is not activated by receptor-mediated stimuli such as ACh. Thus TRIM appears to be the best available

Role of cyclooxygenase in dilation to ACh. While the present study was underway, a study of isolated perfused hearts was published that suggested that vasodilation to ACh was preserved in eNOS−/− mice (10). However, in contrast to the present study, ACh-induced vasodilation in both normal and eNOS−/− mice was dependent (in part) on activity of cyclooxygenase (10). Flow-mediated dilation of skeletal muscle arterioles is also, in part, dependent on activity of cyclooxygenase in normal mice, but in eNOS−/− mice, the response to flow is entirely mediated by a cyclooxygenase-dependent mechanism (39). Dilation to ACh in the present study was dependent on activity of cyclooxygenase only in eNOS−/− mice. Inhibition of cyclooxygenase had no effect on responses to ACh in coronary arteries from wild-type mice. This observation is important because it demonstrates that the effects of indomethacin on responses to ACh in eNOS−/− mice were selective. This difference in the role of cyclooxygenase in mediating responses to ACh may be related to differences in the segment of vasculature studied in the different preparations in the present study and the previous study. In the present study, we measured responses of isolated coronary arteries from mice. Vasodilator responses to intravascularly administered ACh in an isolated perfused Langendorff preparation may reflect responses of more distal vessels in the mouse heart. In other species, 100-μm vessels (similar in size to mouse coronary arteries used in these experiments) contribute to the control of vascular resistance (5). The distribution of microvascular resistance in the mouse heart is not known.

Role of nNOS in dilation to ACh. In addition to the coronary circulation, responses to ACh are preserved in at least some other vascular beds in eNOS−/− mice. ACh also produces dilation of cerebral arterioles in eNOS−/− mice similar to responses in normal mice (26, 27, and our unpublished observations). Similar to the present study, dilation of cerebral arterioles in eNOS−/− mice to ACh was inhibited by l-NNA (27). In addition, responses of cerebral arterioles to ACh in eNOS−/− mice were blocked by an inhibitor of nNOS (7-nitroindazole) (26). Thus Meng et al. (26, 27) suggested that vasodilation to ACh is mediated by nNOS in eNOS−/− mice. These findings suggest that responses of cerebral and coronary vessels in eNOS−/− mice to ACh are likely because of a compensatory mechanism expressed in response to chronic eNOS deficiency. Although inhibition of nNOS (7-nitroindazole) attenuated dilation to ACh in eNOS−/− mice, effects of indomethacin were not tested in the previous studies of cerebral arterioles (26, 27).
inhibitor to examine the role of nNOS in responses of vessels from eNOS −/− mice. Although there is little evidence for expression of nNOS in blood vessels (in the endothelium or vascular smooth muscle) under normal conditions, nNOS expression may occur in vessels in some disease states. For example, nNOS is expressed in vascular muscle from spontaneously hypertensive rats (3) and in atherosclerotic vessels (including humans) (41). Collectively, these studies suggest that in disease states associated with decreased expression or activity of eNOS (or NO), expression of nNOS may occur. The present results in eNOS −/− mice are consistent with this concept.

Responses of coronary arteries from eNOS −/− mice to ACh were inhibited by indomethacin or TRIM. These findings suggest an interaction between cyclooxygenase and nNOS in the response to ACh in coronary arteries from eNOS −/− mice. Interactions between cyclooxygenase and NOS have been described in several studies but are very complex and not well defined particularly in blood vessels. For example, a similar interaction may be involved in the cerebral vascular response to hypercapnia where indomethacin or inhibitors of nNOS attenuate increases in cerebral blood flow (17, 18). In the coronary and renal circulation, there is also evidence that acute or chronic inhibition of NOS enhances the role of cyclooxygenase in the regulation of vascular resistance (19, 32, 34) and stimulates cyclooxygenase production of vasodilator prostaglandins (1, 15, 29). In the coronary circulation of humans with atherosclerosis or coronary risk factors, inhibitors of both NOS or cyclooxygenase greatly reduce flow-mediated responses (an endothelium-dependent response) (7). Our data are consistent with other studies, including in humans, suggesting a potential interaction between cyclooxygenase and NOS. The mechanism that accounts for this interaction has not been determined. However, cyclooxygenase is heme containing, and NO can interact with heme-containing proteins and increase cyclooxygenase activity (19, 20, 36). Thus interactions between products of cyclooxygenase and NOS involved in regulating vascular responses have been described, but the mechanisms involved in this interaction are unknown.

In summary, we demonstrated that, in both eNOS +/- and eNOS −/− mice, responses of coronary arteries to ACh are largely preserved. In coronary arteries from wild-type mice, dilation to ACh is mediated primarily by NO and not cyclooxygenase or nNOS. In contrast, in coronary arteries from eNOS −/− mice, dilation to ACh appears to be dependent on activity of nNOS and/or cyclooxygenase. The data suggest that the coronary circulation has the ability to compensate for the loss of normal vasodilator mechanisms.

We acknowledge Dr. Curt Sigmund and the University of Iowa Transgenic Core for genotyping the mice used in these studies. This work was supported by grants from the National Institutes of Health (HL-39050, HL-38901, NS-24621, HL-62984) and a grant from the American Heart Association. K. G. Lamping and F. M. Faraci are Established Investigators of the American Heart Association.

REFERENCES


