Lamping, Kathryn G., Daniel W. Nuno, David A. Chappell, and Frank M. Faraci. Agonist-specific impairment of coronary vascular function in genetically altered, hyperlipidemic mice. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1023–R1029, 1999.—The objectives of the present study were to 1) examine mechanisms involved in endothelium-dependent responses of coronary arteries from normal mice and 2) determine whether vascular responses of coronary arteries are altered in two genetic models of hypercholesterolemia [apolipoprotein E (apoE)-deficient mice (apoE−/−) and combined apoE and low-density lipoprotein receptor (LDLR)-deficient mice (apoE + LDLR−/−)]. Plasma cholesterol levels were higher in both apoE−/− and apoE + LDLR−/− compared with normal mice on normal and high-cholesterol diets (normal chow: normal 110 ± 5 mg/dl, apoE−/− 680 ± 40 mg/dl, apoE + LDLR−/− 810 ± 40 mg/dl; high-cholesterol chow: normal 280 ± 60 mg/dl, apoE−/− 2,490 ± 310 mg/dl, apoE + LDLR−/− 3,660 ± 290 mg/dl). Coronary arteries from normal (C57BL/6J), apoE1, apoE2, and combined apoE and low-density lipoprotein receptor (LDLR)-deficient mice (apoE + LDLR−/−) were isolated and cannulated, and diameters were measured using videomicroscopy. In normal mice, vasodilation in response to ACh and serotonin was markedly reduced by 10 µM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; an inhibitor of soluble guanylate cyclase). Vasodilation to nitroprusside, but not papaverine, was also inhibited by ODQ. Dilation of arteries from apoE−/− and apoE + LDLR−/− mice on normal diet in response to ACh was similar to that observed in normal mice. In contrast, dilation of arteries in response to serotonin from apoE−/− and apoE + LDLR−/− mice was impaired compared with normal. In arteries from both apoE−/− and apoE + LDLR−/− mice on high-cholesterol diet, dilation to ACh was decreased. In apoE + LDLR−/− mice on high-cholesterol diet, dilation of coronary arteries to nitroprusside was increased. These findings suggest that dilation of coronary arteries from normal mice in response to ACh and serotonin is dependent on production of nitric oxide and activation of soluble guanylate cyclase. Hypercholesterolemia selectively impairs dilator responses of mouse coronary arteries to serotonin. In the absence of both apoE and the LDL receptor, high levels of cholesterol result in a greater impairment in coronary endothelial function.

Coronary artery; acetylcholine; serotonin; gene-targeted mice; endothelium; nitric oxide; soluble guanylate cyclase

PREVIOUS STUDIES of vascular reactivity in experimental models of atherosclerosis have generally relied on diet-induced hyperlipidemia (10, 13, 14, 34). Watanabe rabbits, a genetic model of hyperlipidemia and atherosclerosis, have also been studied (36, 37). Because both genetics and diet may play a role in the development of vascular disease, models incorporating both are advantageous. Recent development of murine models with known defects in cholesterol metabolism provide potentially valuable models for studies of the effects of hypercholesterolemia on vascular biology. Mice deficient in apolipoprotein E (apoE−/−) (2, 25, 39) or combined apoE and low-density lipoprotein receptor (LDLR)-deficient mice (apoE + LDLR−/−) (15) develop spontaneous hypercholesterolemia and atherosclerotic lesions similar to lesions that develop in humans (31, 32, 38).

Several studies have demonstrated that atherosclerosis is associated with impaired endothelium-dependent relaxation in dietary-induced models of atherosclerosis in animals (10, 13, 14, 34) and in humans with arterial atherosclerosis (12, 30). Studies from our laboratory have demonstrated that abnormal vascular function in aorta from apoE−/− and apoE + LDLR−/− mice correlated with the presence of atherosclerotic lesions (1). Vascular function was greatly impaired in proximal segments of aorta from combined apoE + LDLR−/− mice, whereas in distal segments, with minimal evidence of atherosclerotic lesions, endothelium-dependent relaxation to ACh was normal. In contrast, in apoE−/− deficient mice with similar levels of plasma cholesterol, intimal lesions were minimal and vascular function was not impaired. Atherosclerosis appears to be accelerated in apoE + LDLR-deficient mice compared with apoE deficiency alone, and this suggests that absence of LDLR and the resulting increased levels of plasma LDL contribute to the development of disease and abnormal vascular function (1).

In the present study, we examined vascular reactivity in the coronary circulation of normal mice and two genetic models of hypercholesterolemia (apoE−/− and apoE + LDLR−/− mice). Mechanisms that mediate endothelium-dependent relaxation in coronary arteries from normal mice are not known. Before alteration in vascular reactivity could be assessed in models of disease, we felt it was important to begin to understand mechanisms involved in the regulation of reactivity in normal arteries. Thus the first goal of this study was to examine mechanisms that mediate responses of coronary arteries from normal mice to ACh and serotonin (5-HT). The second goal of this study was to compare responses of coronary arteries from apoE−/− and apoE + LDLR−/− mice to determine if genetic deficiency of apoE or apoE and LDLR receptors is associated with vascular dysfunction in these murine models. In addition, we determined whether the effects of...
genetically induced hypercholesterolemia are exacerbated by a high-cholesterol diet.

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: normal mice (C57BL/6J), homozygous apoE-deficient mice (apoE\(^{-/-}\)), and homozygous apoE and LDL receptor-deficient mice (apoE\(^{-/-}\) + LDLR\(^{-/-}\)). Mice were a third- or fourth-generation hybrid from a colony at The Jackson Laboratory. Both male and female mice were fed regular chow. A high-cholesterol diet supplemented with 1% cholesterol (wt/wt) for 10 wk beginning at 5 wk of age. Water was available ad libitum. Ages of normal 16 mice in the different groups were similar (regular chow: normal 16 \pm 1 wk, apoE\(^{-/-}\) \pm 1 wk, apoE \pm LDLR \pm 1 wk; high-cholesterol chow: normal 23 \pm 1 wk, apoE \pm 21 \pm 1 wk, apoE \pm LDLR \pm 29 \pm 1 wk). Levels of plasma cholesterol and fast protein liquid chromatography fractions were determined by colorimetric enzymatic assays (Boehringer Mannheim kit no. 126012). After an overnight fast, 100-μl samples of mouse plasma were withdrawn, fractionated on a Superose 6 column (Pharmacia) with 10 mmol/l Tris-buffered saline (pH 8.0), pumped at 0.5 ml/min, and collected in 0.5-ml fractions as previously described (4).

General preparation. Mice were anesthetized with Avertin (240 mg/kg) and heparinized intraperitoneally. A sample of blood was drawn from caudal vena cava for measurement of total serum cholesterol. Hearts were rapidly removed and placed in cold Krebs buffer of the following composition (in mM/l): 118.3 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), and 11.1 glucose. Epicardial arteries (62–160 μm in diameter) were isolated from myocardium under a microscope (×40), placed in a Plexiglas organ chamber filled with cold Krebs solution, 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% O\(_2\), 5% CO\(_2\), and 75% N\(_2\). Vessels were pressurized to 20 mmHg under no-flow conditions using two reservoirs filled with Krebs solution. Images of microvessels were displayed on a video monitor using a Leitz microscope (×100) connected to a Hitachi camera. An electronic video dimension analyzer (Living Systems Instrumentation, Burlington, VT) continuously measured luminal diameter. The distending pressure of the vessels was measured with a pressure transducer connected to a side arm 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 from a resting diameter in response to 100 mM KCl.

Protocols. Vessel segments were preconstricted with the thromboxane mimetic 9,11-dideoxy-11a,9α-epoxy-methanoprostaglandin F\(_2\alpha\) (U-46619; \(\approx 7–17 \times 10^{-8}\) M) to 30–60% of the initial vessel diameter. Baseline diameters were the diameters of arteries before preconstriction with U-46619. In coronary arteries from C57BL/6J mice, cumulative dose-response curves to ACh \(10^{-9}\)–\(10^{-5}\) M, 5-HT \(10^{-9}\)–\(10^{-5}\) M, nitroprusside \(10^{-5}\)–\(10^{-3}\) M, a nitric oxide donor) were measured in the presence of ODQ.

RESULTS

Responses of coronary arteries from normal mice to ACh. ACh produced dose-dependent dilation of coronary arteries from normal mice (baseline diameter \(119 \pm 8\) μm, \(n = 10\), Fig. 1). The maximal dilation in response to ACh was 45 \pm 7%. Dilation in response to ACh was inhibited substantially by L-NNA (10 µM, \(n = 5\)) and ODQ (10 µM, \(n = 3\), Fig. 1), suggesting that the response is dependent on formation of nitric oxide and activation of guanylate cyclase. Dilation in response to ACh was inhibited by 10.2 \pm 0.3 on October 22, 2017 http://ajpregu.physiology.org/ Downloaded from
Responses to 5-HT were measured in the presence of ketanserin to separate endothelium-mediated effects from direct effects on vascular muscle (due to activation of 5-HT$_2$ receptors). In the presence of ketanserin, 5-HT produced dose-dependent dilation of coronary arteries (baseline diameter 116 ± 12 µm, n = 6, Fig. 2). Dilation to 5-HT was inhibited by L-NNA (10 µM, n = 7, Fig. 2) and ODQ (10 µM, n = 5, Fig. 2). These findings suggest that in mouse coronary arteries, dilation to 5-HT is mediated by release of nitric oxide and stimulation of soluble guanylate cyclase.

Response of coronary arteries from normal mice to nitroprusside and papaverine. To determine whether effects of ODQ were selective for responses mediated through activation of guanylate cyclase, we measured responses to papaverine, a nonspecific vasodilator, in the absence and presence of ODQ. Papaverine produced dose-dependent dilation of coronary arteries (n = 6) that was not altered by ODQ (n = 5, Fig. 3). As part of these experiments, we also examined the effects of ODQ on responses to nitroprusside. Nitroprusside produced dose-dependent dilation of coronary arteries from normal mice (n = 5, Fig. 4). Dilation to nitroprusside was markedly reduced by ODQ (n = 5, Fig. 4) but not L-NNA (n = 6, Fig. 4).

Responses of apoE$^−/−$ and apoE$^+/−$ LDLR$^−/−$ mice to ACh and 5-HT. Next, we studied effects of hyperlipidemia on responses of coronary arteries from apoE and apoE$^+/−$ LDLR$^−/−$ mice compared with arteries from normal mice. On normal chow, dilation in response to ACh was similar in control, apoE$^+/−$ (baseline diameter 120 ± 10 µm, n = 9), and apoE$^+/−$ + LDLR$^−/−$ mice (baseline diameter 117 ± 4 µm, n = 6, Fig. 5A). Maximal responses to ACh were not altered in either apoE$^+/−$ or apoE + LDLR$^−/−$ mice (normal, 49 ± 7%; apoE$^+/−$, 45 ± 7%; apoE + LDLR$^−/−$, 40 ± 5%). In contrast to animals on normal diet, dilation in response to ACh of coronary arteries from apoE$^+/−$ (baseline diameter 111 ± 11 µm, n = 6) and apoE + LDLR$^−/−$ mice (baseline diameter 127 ± 13 µm, n = 7) on high-cholesterol diet was less than dilation of coronary arteries from normal mice (Fig. 5B). Maximal dilation to ACh was decreased in arteries from apoE$^+/−$ and apoE + LDLR$^−/−$ mice (normal, 49 ± 7%; apoE$^+/−$, 32 ± 5%; apoE + LDLR$^−/−$, 16 ± 6% P < 0.05 vs. normal). The response of coronary arteries to ACh from normal mice on high-cholesterol diet was similar to mice on normal diet (maximal dilation 55 ± 4%, n = 3).

Dilation of coronary arteries from apoE$^+/−$ (baseline diameter 111 ± 7 µm, n = 6) and apoE + LDLR$^−/−$ mice (baseline diameter 117 ± 5 µm, n = 6) on normal diet in response to 5-HT was reduced compared with arteries from normal mice (Fig. 6). The decreased dilation to 5-HT of arteries from apoE$^+/−$ and apoE + LDLR$^−/−$ mice was in marked contrast to the response to ACh in arteries from the same mice. Maximal dilation of arteries to 5-HT was decreased in apoE$^+/−$ and apoE + LDLR$^−/−$ compared with normal mice (normal, 75 ± 7%; apoE$^+/−$, 43 ± 11%; apoE + LDLR$^−/−$, 44 ± 6%; P < 0.05 vs. normal).

Responses of apoE$^+/−$ and apoE + LDLR$^−/−$ mice to nitroprusside. To determine whether the decreased dilation to ACh and 5-HT was due to a nonspecific impairment of vascular muscle function in apoE$^+/−$...
and apoE + LDLR −/− mice, we examined responses to the endothelium-independent dilator nitroprusside. Nitroprusside produced similar dilation of coronary arteries from normal, apoE −/− (n = 4), and apoE + LDLR −/− mice (n = 4) on normal diet (ED50 normal, 6.8 ± 0.3; apoE −/−, 6.7 ± 0.3; apoE + LDLR −/−, 7.2 ± 0.3, Fig. 7A). Dilation to 10−5 M nitroprusside was also similar in all groups (normal, 65 ± 9%; apoE −/−, 70 ± 8%; apoE + LDLR −/−, 68 ± 11%). Dilation to nitroprusside was shifted to the left in arteries from apoE + LDLR −/− mice on high-cholesterol (n = 6) compared with normal diet (ED50 normal, 6.8 ± 0.3; apoE −/−, 6.8 ± 0.1; apoE + LDLR −/−, 7.5 ± 0.1; P < 0.05 vs. normal, Fig. 7B). Maximal dilation to nitroprusside was similar in arteries from all groups on high-cholesterol diet (normal, 65 ± 9%; apoE −/−, 71 ± 9%; apoE + LDLR −/−, 69 ± 7%).

Plasma cholesterol levels. The total plasma cholesterol levels were higher in apoE −/− (680 ± 40 mg/dl, n = 19) and apoE + LDLR −/− mice (810 ± 40 mg/dl, n = 16) compared with normal mice (110 ± 5 mg/dl, P < 0.05 vs. apoE −/− and apoE + LDLR −/−, n = 16) on normal chow. Levels of plasma cholesterol were higher in each group on high-cholesterol compared with normal chow (normal, 280 ± 60 mg/dl, n = 7; apoE −/−, 2,490 ± 310 mg/dl, n = 11; apoE + LDLR −/−, 3,660 ± 290 mg/dl, n = 16; P < 0.05 vs. normal chow). We and others have previously published that normal mice do not have a prominent peak of cholesterol-containing particles in the very low density lipoprotein (VLDL) and LDL ranges when measured by fast protein liquid chromatography. The most prominent fraction of cholesterol is in the high-density lipoprotein (HDL) range (1). By comparison, the apoE −/− and apoE + LDLR −/− mice have a prominent fraction of cholesterol in the VLDL and LDL ranges (1). The apoE + LDLR −/− mice have an additional prominent peak of LDL particles (1, 15).

DISCUSSION

This study is the first to examine mechanisms that mediate responses of coronary arteries from normal mice to ACh, 5-HT, and nitroprusside. Coronary arteries from normal mice dilate to ACh, 5-HT, nitroprusside, and papaverine. Vasodilation in response to ACh and 5-HT is inhibited by both l-NNa and ODQ. Dilation to nitroprusside, a nitric oxide donor, is also mediated by production of nitric oxide and activation of soluble guanylate cyclase.

This study also demonstrated that dilation of coronary arteries from genetic models of hypercholesterolemia is selectively impaired to 5-HT, but not ACh, unless mice are fed a high-cholesterol diet. These results differ from a previous study in which abnormal responses of the aorta to ACh in genetic models of hypercholesterolemia were correlated with the presence of atherosclerotic lesions (1). In that study, endothelium-dependent relaxation was intact in vessels with minimal disease. Another interesting finding in the current study is that responses to nitroprusside were enhanced in mice on a high-cholesterol diet.

Reactivity of normal coronary arteries from mice. Understanding mechanisms that regulate vascular tone in coronary vessels from normal mice produces a foundation for subsequent studies of murine models with specific genetic alterations. Endothelium-dependent relaxation to ACh can be mediated by nitric oxide, endothelium-derived hyperpolarizing factor, or prostacyclin, depending on the species, organ, or location of the vessel within the vascular tree (7, 9, 19, 29). Mechanisms that mediate responses to ACh and 5-HT
in the mouse coronary circulation were unknown previously. Results of the present study suggest that dilation of mouse coronary arteries in response to ACh and 5-HT is mediated primarily by nitric oxide. In humans with no angiographic evidence of coronary artery disease, responses of both large coronary arteries and resistance vessels to ACh (5) and bradykinin (17) are mediated by a similar mechanism because responses to these stimuli were inhibited markedly by N-nitro-L-arginine methyl ester. However, in the presence of inhibitors of NOS, dilation to endothelium-dependent agonists in the present study in mice and previous studies in humans (5, 17) were not completely inhibited, suggesting that other mechanisms may in part contribute to the response.

We also tested the contribution of activation of soluble guanylate cyclase in the dilation to ACh and 5-HT. Previous studies have demonstrated that NO binds to the heme portion of soluble guanylate cyclase to increase cGMP production. ODQ markedly inhibited dilation of mouse coronary arteries in response to ACh and nitroprusside, suggesting that responses to exogenously applied and endogenously produced nitric oxide are mediated by soluble guanylate cyclase. Responses to papaverine were not altered by ODQ, providing evidence for the selectivity of the drug. We have shown previously that ODQ does not decrease vasodilator responses to adenosine or an analog of cGMP (35). Similarly, in porcine coronary arteries and murine cerebral arteries, nitric oxide primarily activates soluble guanylate cyclase to produce relaxation because production of cGMP and responses to NO donors were markedly decreased in the presence of inhibitors of soluble guanylate cyclase (28, 35).

Effect of hypercholesterolemia on vascular reactivity. Several studies have demonstrated abnormal vascular reactivity in diet-induced animal models and humans with atherosclerosis (10, 13, 14, 34). Mechanisms that may be involved in the decreased endothelium-dependent response in mice with high cholesterol are unknown. On the basis of previous studies, we could speculate that decreased nitric oxide-mediated responses may be due to an increased degradation of nitric oxide, decreased synthesis and/or release of nitric oxide, or impaired responses of vascular smooth muscle to nitric oxide. It seems unlikely that reactivity of vascular smooth muscle is decreased, because the dilation to nitroprusside was similar in coronary arteries from normal and hypercholesterolemic mice on normal chow and even enhanced in arteries from mice on a high-cholesterol diet. Impaired dilation to endothelium-dependent agents may be due to increased degradation and/or inactivation of nitric oxide by superoxide anion (22, 24). Increased production of superoxide has been reported in several studies, and endothelium-dependent responses in atherosclerotic arteries can be restored toward normal by treatment with superoxide dismutase (23, 24).

In the present study, dilation to 5-HT was impaired in coronary arteries from apoE and apoE + LDLR −/− mice on normal chow, whereas dilation to ACh was normal in arteries from the same mice. Abnormal dilation to ACh was evident in coronary arteries from mice only when they were on a high-cholesterol diet. Reasons for the difference in responses to 5-HT and ACh in arteries from mice on normal chow are unclear but may be related to differences in mechanisms of agonist-mediated release of nitric oxide. Dilation to 5-HT seems to involve activation of a pertussis toxin-sensitive G protein (8), whereas ACh activates multiple G proteins (27). Hypercholesterolemia may selectively impair dilation to pertussis toxin-sensitive G proteins to a greater extent than other G protein-mediated responses (33), making responses to 5-HT more abnormal in atherosclerosis. These data suggest that responses to 5-HT may be a more sensitive indicator of abnormal vascular function.

ApoE mediates cellular uptake of cholesterol from serum lipoprotein particles such as VLDL, intermediate density lipoprotein (IDL), and β-VLDL through interactions with LDLR (21). Interactions of apoE with circulating lipoproteins result in a decreased plasma cholesterol concentration. In murine models of apoE or LDLR deficiency, plasma VLDL, IDL, and LDL are all increased, and atherosclerotic lesions characteristic of those seen in humans develop. Clinically, deficiency of apoE or LDL receptors leads to hypercholesterolemia and atherosclerosis although the cholesterol character-
istics are different. ApoE deficiency leads to elevated remnant cholesterol fraction (type III hyperlipoproteinemia) (12, 21), and LDL deficiency leads to an increase in serum LDL (familial hypercholesterolemia) (3). ApoE deficiency results in elevated levels of VLDL and IDL in serum LDL (familial hypercholesterolemia) (12, 21), and LDL deficiency leads to an increase in lipid profile in the two models.

Recent studies have suggested that vascular reactivity in models of hypercholesterolemia is altered, at least in part, after oxidation of LDL and generation of reactive oxygen species (11, 16, 18, 26). Oxidized LDL is a potent inhibitor of endothelial-dependent relaxation. The present study suggests that in genetic models of hypercholesterolemia, alterations in coronary vascular reactivity are selectively altered. In coronary arteries from apoE−/− and apoE + LDLR−/− mice on a normal diet, responses of coronary arteries to 5-HT are impaired, whereas responses to ACh are not different. Explanations for these differences in responses to 5-HT versus ACh may reflect differences in specific receptors, the role of G protein in the responses, or other compensatory vasodilator pathways in the presence of atherosclerosis.

In summary, these findings suggest that in normal mice, dilation of coronary arteries to ACh and 5-HT are primarily mediated by release of nitric oxide and activation of soluble guanylate cyclase. In two genetic models of hypercholesterolemia, dilation to 5-HT was impaired, whereas dilation to ACh was only impaired in mice with very high levels of serum cholesterol. These studies of vascular reactivity in normal and hypercholesterolemic mice models demonstrate the potential use of murine models with defects in expression of specific gene products to study mechanisms involved in regulation of coronary vascular reactivity. In addition, differences in reactivity of coronary arteries in the present study compared with responses in other vascular beds demonstrate the need to perform studies comparing mechanisms of reactivity in multiple vascular tissue types in these models.

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