Endothelium-derived 2-arachidonylglycerol: an intermediate in vasodilatory eicosanoid release in bovine coronary arteries


1Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin; and 2Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas

Submitted 10 June 2004; accepted in final form 1 November 2004

Address for reprint requests and other correspondence: K. M. Gauthier, Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (E-mail: kgauth@mcw.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2-Arachidonylglycerol (2-AG), like anandamide, is an endocannabinoid derivative of arachidonic acid. It is an endogenous ligand for both CB1 and CB2 receptors and is the most abundant monoacylglycerol in the brain and is found in numerous other tissues, including platelets, macrophages, and endothelial cells (ECs) (2, 11, 17, 18, 29, 30, 32). Previous
evaluation of EC 2-AG production in the rat aorta and human umbilical vein ECs relied on TLC-HPLC or TLC-gas chromatography-mass spectrometry (MS) for quantification (18, 29). In the present study, we isolated 2-AG from bovine coronary ECs using HPLC and confirmed the chemical identity of 2-AG by liquid chromatography-electrospray ionization MS (LC/ESI-MS). Furthermore, stimulation of ECs with methacholine increased the release of 2-AG. Similar to anandamide, we report that 2-AG causes endothelium-dependent relaxations of bovine coronary arteries, which are not dependent on CB1 receptor activation but require hydrolysis of 2-AG to arachidonic acid. The liberated arachidonic acid is further metabolized by the endothelium to the vasodilatory eicosanoids EETs and PGI2, which mediate 2-AG-induced relaxations. The results from these studies demonstrate that 2-AG could contribute to eicosanoid activation and synthesis, representing an important mechanism of agonist-stimulated arachidonic acid liberation in the coronary endothelium.

METHODS

Cultured bovine coronary EC assays. Bovine coronary ECs were cultured in 75-cm² flasks as previously described (27). After 75% confluency was reached, the medium was removed and the cells were washed twice with HEPES buffer containing (in mmol/l) 150 NaCl, 5 KCl, 1.8 CaCl2, 1 MgCl2, and 5.5 glucose, pH 7.4. The cells were incubated for 5 min with [U-14C]arachidonic acid (0.5 μCi/mmol) and 10 μmol/l unlabeled arachidonic acid (in 10 ml of HEPES buffer at 37°C in 95% air–5% CO2). The calcium ionophore A-23187 (5 μmol/l) was added, and the incubation continued for an additional 25 min. The buffer and cells were collected, separated by centrifugation (1,500 rpm for 3 min), and frozen (−40°C). Metabolites were isolated using solid-phase extraction (C-18 columns, Varian) as previously described (26, 27). The extract was resolved by reverse-phase HPLC, using a 40-min linear gradient from 50% acetonitrile in water to 100% acetonitrile and flow rate of 1 ml/min. Column fractions (0.2 ml) were collected and analyzed for radioactivity. In parallel analyses, column eluate was analyzed by LC/ESI-MS as described below. Additionally, the migration times of known standards [6-keto-PGF1α, 14,15-EET, 14,15-EET glycerol ester (14,15-GEET), 14,15-dihydroxyeicosatrienoic acid (14,15-DHET), and 2-AG] were determined. Because of chemical instability, glycerol-PGI2 could not be synthesized.

For the 2-AG assays, ECs were washed and incubated in HEPES buffer containing DAK (10 μmol/l) for 10 min. Methacholine (10 μmol/l) or vehicle was added and cells were incubated for an additional 10 min. In some instances, the cells were pretreated for 10 min with the phospholipase C (PLC) inhibitor U-73122 (10 μmol/l) or the diacylglycerol lipase inhibitor RHC-80267 (10 μmol/l). Currents were recorded using patch-clamp procedures and instrumentation as previously described (1, 14, 15). Currents were sampled at 3 kHz and filtered at 1 kHz at a membrane potential of +60 mV. Perfuse and pipette solutions contained (in mmol/l) 145 KCl, 1.0 MgCl2, 1.0 EGTA, 10 HEPES, and 100 mmol/l ionized Ca2+ (pH 7.4). To determine the effects of 14,15-GEET on K+ channel activity, channel recordings (2–4 min) were obtained in cells perfused with either vehicle or increasing concentrations of 14,15-GEET (100 nmol/l to 10 μmol/l). All recordings were performed at room temperature.

Patch-clamp studies. Bovine coronary smooth muscle cells were cultured on glass coverslips as described above. Single channel K+ currents were recorded using patch-clamp procedures and instrumentation as previously described (1, 14, 15). Currents were sampled at 3 kHz and filtered at 1 kHz at a membrane potential of +60 mV. Perfuse and pipette solutions contained (in mmol/l) 145 KCl, 1.0 MgCl2, 1.0 EGTA, 10 HEPES, and 100 mmol/l ionized Ca2+ (pH 7.4). To determine the effects of 14,15-GEET on K+ channel activity, channel recordings (2–4 min) were obtained in cells perfused with either vehicle or increasing concentrations of 14,15-GEET (100 nmol/l to 10 μmol/l). All recordings were performed at room temperature.

Fatty acid amidohydrolase and monooacylglycerol lipase assays. Fatty acid amidohydrolase (FAAH) activity was determined in rat forebrain membranes as previously described (9). Briefly, membranes were incubated in TME buffer (50 mM Tris–HCl, 3.0 mM MgCl2, and 1.0 mM EDTA, pH 7.4, final volume of 0.5 ml) containing 1.0 mg/ml fatty acid-free BSA and [3H]anandamide (0.2 mM) with or without 10 μmol/l indomethacin, 10 μmol/l RHC-80267, or vehicle 10 min before the addition of U-46619. When required, the endothelium was removed by gently rubbing the luminal surface with metal forceps. Removal of the endothelium was verified by the lack of a relaxation response to the endothelium-dependent agonist bradykinin. Tension was represented as percent relaxation where 100% relaxation was basal pre-U-46619 tension.

Vascular reactivity. Bovine hearts were purchased from a local slaughterhouse, and sections of the left anterior descending coronary artery were dissected and cleaned of connective tissue. The artery was cut into 2-mm-diameter rings (3 mm width), and isometric tension was measured as previously described (1, 15, 22, 26, 27). Arterial rings were equilibrated in Krebs buffer consisting of (in mmol/l) 119 NaCl, 4.8 KCl, 24 NaHCO3, 3.2 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 11 glucose, and 0.02 EDTA at 37°C and aerated with 5% CO2–95% air. Basal tension was set at the length-tension maximum of 3.5 g and equilibrated for 1.5 h. KCl (40–60 mmol/l) was added to the chamber until reproducible maximal contractions were maintained. U-46619 (10–20 nmol/l), a thromboxane receptor agonist, was used to precontract the vessels from basal tension to between 50 and 90% of the maximal KCl contraction.

In the present study, we isolated 2-AG from bovine coronary artery were dissected and cleaned of connective tissue. The artery was cut into 2-mm-diameter rings (3 mm width), and isometric tension was measured as previously described (1, 15, 22, 26, 27).

Assay. Samples were analyzed by use of LC/ESI-MS (Agilent 1100 LC/MSD, SL model). Internal standard (81 ng 2-[3H]2AG), ethanol (175 μl/ml of sample), and glacial acetic acid (20 μl/ml of sample) were added, and the sample was mixed. Samples were centrifuged at 1,500 rpm for 3 min. The supernatant was applied to the C18 Bond Elut SPE columns, which had been preconditioned with 5 ml of ethanol and 15 ml of water. The columns were washed with 20 ml of water and allowed to run dry. The sample was eluted from the column with 5 ml of ethyl acetate. The ethyl acetate layer was removed from the water layer. The water layer was then extracted twice with 1 ml of ethyl acetate. The ethyl acetate portions were combined for each sample and dried under the stream of nitrogen gas. The sample was redissolved in 20 μl of acetonitrile, transferred to an insert in the sample vial, and analyzed or stored frozen at −80°C until analyzed. The extracts (5 μl) were separated into their components on a reverse-phase C18 column (Kromasil, 250 × 2 mm) using water-acetonitrile with 0.005% acetic acid as a mobile phase at a flow rate of 0.2 ml/min. The mobile phase started at 60% acetonitrile, linearly increased to 80% acetonitrile in 30 min, held for 5 min, increased to 100% acetonitrile in 5 min, and held for 10 min. Drying gas flow was 12 l/min, drying gas temperature was 350°C, nebulizer pressure was 35 l/min, vaporizer temperature was 325°C, capillary voltage was 3,000 V, and fragmentor voltage was 120 V. The detection was made in the positive ion mode. 2-AG eluted from the column at 31.93 min and produced a major ion of 379 mass/charge (m/z; M+1). For quantitative measurements, m/z = 379 and 387 were used for 2-AG and 2-[3H]2AG, respectively. The standard curves were typically constructed over the range of 250 pg to 500 ng of 2-AG per injection. The concentrations of 2-AG in the samples were calculated by comparing the ratios of peak areas of 2-AG to 2-[3H]2AG with the standard curves.
at 1,000 rpm for 10 min. The amount of $^3$H in 1 ml each of the aqueous and organic phases was determined by liquid scintillation counting.

Monoacylglycerol lipase (MAGL) activity was performed with protocols similar to the FAAH assay described above using rat whole brain cytosolic fraction protein. Cytosolic proteins (100 μg) were incubated for 15 min at 25°C in TME buffer containing 1.0 mg/ml fatty acid-free BSA and 0.2 nM $[^3]$H mono-oleoyl glycerol with 10 μmol/l 14,15-GEET amide, 10 μmol/l 2-AG amide, or vehicle (DMSO) (final volume of 0.5 ml). Incubations were stopped by the addition of 2 ml of chloroform-methanol (1:2), and 0.67 ml of chloroform and 0.6 ml of water were added. Aqueous and organic phases were separated as described above. Protein concentrations were determined by the Bradford method.

Materials. Noladin ether, 2-AG amide, 14,15-GEET, 14,15-GEET amide, DAK, 14,15-EEZE, and 14,15-EET were provided by J. R. Falck. SR-141716 was obtained from the Drug Supply Program of the National Institute on Drug Abuse. 2-AG was purchased from Cayman Chemical (Ann Arbor, MI), MAFP was purchased from Tocris Cookson, [U-14]$^C$arachidonic acid was purchased from New England Nuclear, and U-73122 and RHC-80267 were purchased from BioMol. All solvents were HPLC grade and purchased from Burdick Jackson or Sigma. Indomethacin, methacholine, 1-NNA, and SKF-525a were purchased from Sigma.

Statistics. Statistical analysis was performed with ANOVA to determine the significant differences within groups, with subsequent Student-Neuman-Keul’s post hoc analysis used to determine the significance between groups. Data are expressed as means ± SE.

RESULTS

Bovine coronary ECs were incubated with $[^14]$C arachidonic acid, and radioactive metabolites were resolved by reverse-phase HPLC (Fig. 1). Metabolites comigrated with 6-keto-PGF$_{1α}$ (fractions 18–22), 14,15-DHET (fractions 76–79), 14,15-GEET (fractions 93–96), and 14,15-EET (fractions 133–137). Nonmetabolized arachidonic acid eluted at fractions 172–179. An unknown nonpolar metabolite (fractions 147–152) comigrated with the 2-AG standard. $[^14]$C 2-AG accounted for ~4% of the total $^{14}$C. Fractions containing the nonpolar metabolite were collected and analyzed by LC/ESI-MS. Using a different HPLC solvent system, we eluted the unknown metabolite at 31.93 min (Fig. 2A) and presented a mass spectra with major ions of 401 (M+Na), 379 (M+H), and 361 (M+H$_2$O) (Fig. 2B). 2-AG eluted at the same time (31.93 min) (Fig. 2C) and presented an identical mass spectrum as the nonpolar unknown metabolite (Fig. 2D). These data verify that the nonpolar metabolite of arachidonic acid is 2-AG. Therefore, coronary ECs synthesize 2-AG, and endogenous 2-AG could serve as a source of arachidonic acid.

We tested the effect of 2-AG on vascular tone in bovine coronary arteries precontracted with U-46619. In endothelium-intact coronary rings, 2-AG caused concentration-related relaxations (Fig. 3A). These relaxations were nearly abolished by endothelium removal. As a result, all other experiments were performed in arteries with an intact endothelium. Because 2-AG is a cannabinoid agonist, we evaluated the effect of the
CB1 cannabinoid antagonist SR-141716 on 2-AG-induced relaxations. As shown in Fig. 3B, the 2-AG relaxations were not altered by pretreatment with SR-141716. Thus, in bovine coronary arteries, endothelium-dependent relaxations to 2-AG do not involve activation of the CB1 receptor.

We propose that arachidonic acid release from 2-AG is a critical mechanism of 2-AG-induced relaxation of coronary arteries. To test this hypothesis, we examined the effect of the hydrolase inhibitors DAK and MAFP on 2-AG-induced relaxations. 2-AG-induced relaxations were blocked by pretreatment with DAK or MAFP (Fig. 4A). We also evaluated the ability of 2-AG analogs noladin ether, a CB1 cannabinoid agonist, and 2-AG amide to induce relaxations of the preconstricted coronary arteries. The glycerol-arachidonic acid linkage of noladin ether is an ether bond rather than an ester bond and is resistant to hydrolysis. Similarly, the glycerol-arachidonic acid linkage of 2-AG amide is an amide bond and is not a substrate for hydrolysis. In contrast to 2-AG, noladin ether and 2-AG amide failed to relax preconstricted coronary arteries (Fig. 4B). Pretreatment of the arterial rings with indomethacin to inhibit cyclooxygenase inhibited the 2-AG relaxations (Fig. 4C). The combination of SKF-525a to inhibit cytochrome P-450 and cyclooxygenase to vasodilator eicosanoids. These events explain the mechanisms of 2-AG-induced relaxations of bovine coronary arteries.

In previous studies of bovine coronary ECs, the release of this nonpolar metabolite was stimulated by the addition of the calcium ionophore A-23187 (27). In ECs pretreated with the FAAH inhibitor DAK, 2-AG release was stimulated by methacholine (Fig. 5). This increase was inhibited 88% by pretreatment of the cells with the PLC inhibitor U-73122 and 91% by cytochrome P-450 and cyclooxygenase to vasodilator eicosanoids. These events explain the mechanisms of 2-AG-induced relaxations of bovine coronary arteries.
pretreatment with the diacylglycerol lipase inhibitor RHC-80267. These results indicate that, in coronary ECs, 2-AG is rapidly degraded by a DAK-inhibitable process. Additionally, 2-AG is synthesized by the sequential release of diacylglycerol from membrane phospholipids by PLC with subsequent metabolism by diacylglycerol lipase. Because 2-AG release was stimulated by methacholine and nearly eliminated by PLC and DAG lipase inhibition, we evaluated the effect of these inhibitors on the relaxation response to methacholine. The L-NNA-resistant relaxations to methacholine were attenuated by pretreatment with U-73122 or RHC-80267 (Fig. 6, A and B) and by pretreatment with the hydrolase inhibitor DAK (Fig. 6 C). These results demonstrate that PLC, DAG lipase, and fatty acid hydrolysis contribute to the vascular activity of methacholine in bovine coronary arteries.

In addition to hydrolysis of 2-AG to free arachidonic acid, 2-AG could also serve as a substrate for EC cytochrome P-450s and/or cyclooxygenases and the subsequent GEET or glycerol-prostacyclin esters could contribute to relaxations induced by 2-AG. To this end, we evaluated the ability of 14,15-GEET and 14,15-GEET amide to relax preconstricted bovine coronary arteries. 14,15-GEET induced concentration-dependent relaxations that were similar to relaxations induced by 14,15-EET, whereas 14,15-GEET amide failed to cause relaxation (Fig. 7A). Relaxations to 14,15-GEET were not altered by DAK (Fig. 7B) and, similarly, were not altered by MAFP (data not shown). In contrast, relaxations to 14,15-GEET were attenuated by 14,15-EEZE (Fig. 7B). In FAAH and MAGL assays, 2-AG amide (10 μmol/l) and 14,15-GEET amide (10 μmol/l) showed weak inhibition of anandamide hydrolysis (87.6 and 79.5% of control, respectively, n = 2) and weak inhibition of mono-oleoyl glycerol hydrolysis (90.4 and 93.6% of control, respectively, n = 2). Therefore, 2-AG amide and 14,15-GEET amide do not appear to be good substrates for these hydrolytic enzymes.

Because 14,15-EET relaxations are mediated by the activation of smooth muscle BKCa channels (1), we evaluated the ability of 14,15-GEET to similarly activate BKCa channels. As illustrated in the cell-attached patch of an isolated coronary smooth muscle cell (Fig. 8A), 14,15-GEET induced a concentration-dependent increase in K+ channel activity. Figure 8B summarizes the effect of 14,15-GEET (100 nmol/l to 10 μmol/l) on channel mean open time. 14,15-GEET (10 μmol/l) increased average mean channel open time over 12-fold from vehicle control. Channel unitary conductance averaged 222 ± 11 pS. Thus, similar to EETs, 14,15-GEET activates coronary smooth muscle BKCa channels.

DISCUSSION

This study represents the first evaluation of 2-AG-induced relaxations of isolated coronary arteries. Our results provide evidence that 2-AG is a component of the EC arachidonic acid signaling cascade and represents a new role for 2-AG as an...
intracellular lipid mediator of vascular relaxation. In the vascular endothelium, 2-AG production has been detected in human umbilical vein and rat aortic cells (18, 29). In the endothelium-intact rat aorta, the addition of the muscarinic agonist carbachol increased 2-AG concentrations fivefold as determined by TLC-gas chromatography-MS. In cultured human umbilical vein ECs, thrombin increased the levels of 2-AG in total cellular lipids nearly twofold as measured by TLC-HPLC. Our study identified 2-AG as a major metabolite in the coronary endothelium using LC/ESI-MS. Furthermore, we demonstrated that 2-AG release is stimulated by methacholine.

Infusion of 2-AG decreases blood pressure in rats and mice (8, 18, 32). In the mouse, the decrease in blood pressure induced by 2-AG was not altered by the CB1 receptor antagonist SR-141716 but was secondary to the rapid metabolism of 2-AG to arachidonic acid. The nonhydrolyzable 2-AG ether induced hypotension that was blocked by SR-141716 (8). In contrast, decreased blood pressure induced by 2-AG in the rat was mediated by the CB1 receptor (32). In indomethacin- and l-NAME-treated rabbit mesenteric arterial rings, relaxations to 2-AG were not dependent on an intact endothelium and were inhibited by SR-141716 (10). From these studies, it appears that, in many vascular beds, 2-AG-induced relaxations are mediated, at least in part, through activation of CB1 receptors. In contrast, 2-AG-induced relaxations of bovine coronary arteries were not altered by the CB1 receptor antagonist SR-141716. However, the relaxations were inhibited by endothelial removal, inhibition of 2-AG hydrolysis with DAK or MAFP, cyclooxygenase inhibition by indomethacin, or the combination of indomethacin and cytochrome P-450 inhibition or EET antagonist treatment with indomethacin and SKF-525a or 14,15-EEZE. 14,15-EEZE plus indomethacin inhibition of 2-AG relaxations was slightly but significantly less than the inhibition by SKF-525a plus indomethacin. This difference could be secondary to the partial inhibition of 14,15-GEET relaxations by 14,15-EEZE. Alternatively, 14,15-EEZE nearly eliminated 14,15-EEZE-induced relaxations of bovine coronary arteries (6). This advocates for a possible role of 14,15-GEET in the 2-AG relaxations. Indomethacin alone attenuated the 2-AG relaxations. Because arachidonic acid is metabolized by the bovine coronary endothelium to 6-keto-PGF1α, a role of cyclooxygenase metabolites in the 2-AG relaxations was expected. The CB1 receptor agonist noladin ether (31) or 2-AG amide, which contain nonhydrolyzable arachidonic acid linkages, did not relax the coronary arteries. Thus, in bovine coronary arteries, 2-AG-induced relaxations are dependent on EC metabolism of 2-AG to vasodilatory arachidonic acid metabolites and not activation of CB1 receptors. The reasons for the disparity in the role of CB1 receptors in the relaxations by 2-AG are not clear but could be related to species, vascular bed, the expression of CB1 receptors, and/or 2-AG metabolizing enzymes.

Activation of PLC results in diacylglycerol formation, which is then hydrolyzed by diacylglycerol lipase to 2-AG (5, 23). 2-AG is then hydrolyzed to arachidonic acid and glycerol by FAAH, MAGL, or other esterases (3, 7, 23). In bovine coronary ECs incubated with the hydrolase inhibitor DAK, methacholine stimulated the production of 2-AG. The presence of
DAK allowed the accumulation of 2-AG. The methacholine-induced increase in 2-AG was nearly eliminated by the PLC inhibitor U-73122 or the DAG lipase inhibitor RHC-80267. Similar inhibitions of 2-AG formation by RHC-80267 and U-73122 were observed in primary cultures of cortical neurons (28). These results suggest that EC 2-AG synthesis is mediated by the sequential actions of PLC and DAG lipase. Because preincubation with DAK or MAFP inhibited 2-AG-induced relaxations, the hydrolysis of 2-AG to arachidonic acid appears to be a critical component of the 2-AG-relaxation pathway. Previously, we demonstrated that bovine coronary ECs hydrolyze anandamide (21); therefore, similar hydrolysis of 2-AG by FAAH or other hydrolytic enzymes was projected. Furthermore, the 1-NNAA-resistant relaxations to methacholine were inhibited or eliminated by U-73122, RHC-80267, or DAK. Together, these results implicate 2-AG as a mediator of the methacholine relaxation response.

2-AG serves as a substrate for 12- and 15-lipoxygenases to produce 12-S- and 15-S-hydroxyeicosatetraenoic acid glycerol esters, respectively (12, 20). Additionally, cyclooxygenase 2 metabolizes 2-AG to prostaglandin H$_2$ glycerol esters (13). Therefore, the possibility exists that 2-AG also serves as a substrate for EC cytochrome P-450s and cyclooxygenases to produce glycerol EETs and glycerol prostacyclin (PGI$_2$). Only slight amounts of 14,15-GEET were detected in bovine coronary EC incubations with $[^{14}C]$arachidonic acid (Fig. 1). Although we did not have a glycerol PGI$_2$ standard, products more polar than 6-keto-PGI$_2$ were not detected. However, because hydrolysis of the glycerol esters occurs rapidly and 2-AG would have to compete with endogenous arachidonic acid for metabolizing enzymes, the isolation of small amounts of these metabolites could prove elusive. Nevertheless, we tested 14,15-GEET and 14,15-GEET amide for vascular activity. 14,15-GEET relaxed the coronary arteries and activated smooth muscle large-conductance K$^+$ channels with similar potency as 14,15-EET (1). Relaxations to 14,15-GEET were not altered by DAK or MAFP, suggesting that the 14,15-GEET-induced relaxation is not dependent on hydrolysis to 14,15-EET. Because 14,15-GEET amide failed to induce relaxation, it appears that 14,15-GEET activation of vascular relaxation occurs through a receptor or binding-dependent mechanism, which is obstructed by the amide linkage of 14,15-GEET amide. Additionally, the 14,15-GEET relaxations were inhibited by the EET antagonist 14,15-EEZE (6). Together, our results show that 14,15-GEET induces relaxations of bovine coronary arteries through similar mechanisms as 14,15-EET and could contribute to relaxations induced by 2-AG.

At this time, a physiological role of 2-AG in the regulation of vascular tone and blood pressure remains poorly defined. Besides the endothelium, other tissues in close proximity to the vasculature could produce 2-AG to alter vascular diameter and tissue blood flow. In this regard, a transferable relaxing factor from rat aorta adventitial adipose tissue has been documented (4,16). Relaxations mediated by this factor are independent of CB1 and CB2 receptors and perivascular nerves. It is conceivable that 2-AG could contribute to this effect. Further studies are required to clarify the role of endogenous 2-AG.

In conclusion, the results from this study indicate that 2-AG induces relaxations of bovine coronary arteries by EC hydrolysis to arachidonic acid and the subsequent metabolism to the vasodilatory eicosanoids. The relaxations are not mediated by CB1 receptor activation. Furthermore, endogenous 2-AG may be a key constituent of agonist-stimulated, PLC-dependent arachidonic acid liberation in bovine coronary ECs. Importantly, modulation of 2-AG synthesis and metabolism could alter EC production and release of arachidonic acid eicosanoid metabolites and therefore alter vascular tone.

ACKNOWLEDGMENTS

The authors thank Blythe Holmes, Erik Edwards, Daniel K. Stringer, and Marilyn Isbell for technical assistance and Gretchen Barg for secretarial assistance.

GRANTS

This research was supported by National Institutes of Health Grants HL-51055, DA-09155, and GM-31278 and the Robert A. Welch Foundation. K. M. Gauthier was a postdoctoral fellow of the American Heart Association, Northland Affiliate.

REFERENCES


