Acetylcholine causes endothelium-dependent contraction of mouse arteries

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Submitted 8 March 2005; accepted in final form 4 May 2005

Zhou, Yingbi, Saradhadevi Varadharaj, Xue Zhao, Narasimham Parinandi, Nicholas A. Flavahan, and Jay L. Zweier. Acetylcholine causes endothelium-dependent contraction of mouse arteries. Am J Physiol Heart Circ Physiol 289: H1027–H1032, 2005.—The goal of this study was to determine whether acetylcholine evokes endothelium-dependent contraction in mouse arteries and to define the mechanisms involved in regulating this response. Arterial rings isolated from wild-type (WT) and endothelial nitric oxide (NO) synthase knockout (eNOS−/−) mice were suspended for isometric tension recording. In abdominal aorta from WT mice contracted with phenylephrine, acetylcholine caused a relaxation that reversed at the concentration of 0.3–3 μM. After inhibition of NO synthase [with Nω-nitro-L-arginine methyl ester (l-NAME), 1 mM], acetylcholine (0.1–10 μM) caused contraction under basal conditions or during constriction to phenylephrine, which was abolished by endothelial denudation. This contraction was inhibited by the cyclooxygenase inhibitor indomethacin (1 μM) or by a thromboxane A2 (TXA2) and/or prostaglandin H2 receptor antagonist SQ-29548 (1 μM) and was associated with endothelium-dependent generation of the TXA2 metabolite TxB2. Also, SQ-29548 (1 μM) abolished the reversal in relaxation evoked by 0.3–3 μM acetylcholine and subsequently enhanced the relaxation to the agonist. The magnitude of the endothelium-dependent contraction to acetylcholine (0.1–10 μM) was similar in aortas from WT mice treated in vitro with l-NAME and from eNOS−/− mice. In addition, we found that acetylcholine (10 μM) also caused endothelium-dependent contraction in carotid and femoral arteries of eNOS−/− mice. These results suggest that acetylcholine initiates two competing responses in mouse arteries: endothelium-dependent relaxation mediated predominantly by NO and endothelium-dependent contraction mediated most likely by TXA2.

endothelial nitric oxide synthase: gene knockout; cyclooxygenase; endothelium-derived contracting factor

THE ENDOTHELIUM REGULATES vascular tone through the synthesis and release of vasoactive mediators (7, 13, 22). Nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor contribute to the endothelium-dependent relaxation (7, 22). In contrast, thromboxane A2 (TXA2) and/or prostaglandin H2 (PGH2) synthesized by cyclooxygenase (COX) can mediate endothelium-dependent contraction (4, 7, 11–15, 20, 24, 25, 28, 32). In rat blood vessels, endothelium-dependent contraction to acetylcholine is not observed under normal physiological conditions but is observed under pathological conditions, for example, hypertension and diabetes (4, 15, 17, 21, 24, 28, 32).

Genetically altered mice have been increasingly used as experimental models to study cardiovascular diseases. In mice, the deletion of the endothelial NO synthase (NOS) gene (eNOS−/−) has been found to cause hypertension and abnormal vasoreactivity (10, 23). Several studies have demonstrated that endothelial agonists such as acetylcholine can still cause vasodilation in arteries from eNOS−/− mice (2, 3, 5, 8, 9, 19, 21, 27, 29). These studies in common emphasize the importance of NO and other endothelial-relaxing mediators in the regulation of vascular function. In contrast, the vasoconstrictor activity of the endothelium has not yet been characterized in mouse blood vessels. We noted that in the abdominal aorta of wild-type (WT) mice, the relaxation induced by acetylcholine appeared to be reversed by a biphasic tension development (33, 34), which might suggest the existence of competing contractile and relaxation responses. In addition, acetylcholine was previously found to cause contraction in the carotid artery of eNOS−/− mice (6). Therefore, the present study was performed to determine whether acetylcholine initiates endothelium-dependent contraction of arteries (abdominal aorta, carotid artery, and femoral artery) from WT and/or eNOS−/− mice.

METHODS

Solution and chemicals. The composition of the physiological salt solution (PSS) was (in mM) 123 NaCl, 4.7 KCl, 15.5 NaHCO3, 1.2 KH2PO4, 1.2. MgCl2, 1.25 CaCl2, and 11.5 d-glucose. High-potassium PSS (60 mM K+*) was prepared by replacing equal molar NaCl with KCl. Acetylcholine, phenylephrine, indomethacin, and Nω-nitro-L-arginine methyl ester (l-NAME) were obtained from Sigma (St. Louis, MO), and SQ-29548 was obtained from ICN Pharmaceuticals (Costa Mesa, CA).

Animals and tissue preparation. Male eNOS−/− and WT C57BL/6J mice (12–16 wk) from Jackson Laboratory were euthanized with 95% CO2 inhalation, which was approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University. The abdominal aorta, femoral, and carotid arteries were rapidly and carefully excised and placed in ice-cold PSS. The fat and the adventitia were dissected free under a binocular microscope. Arteries were cut transversely into ~1.0-mm-long arterial rings.

Isometric force measurement. Isometric force measurement was performed as described previously (33, 34). Briefly, the vascular ring was mounted onto two tungsten wires with a diameter of 50 μm in a 37°C water-circulating tissue bath, of which one was fixed and the other was connected to a force transducer (AE 801; Sensor One, Horten, Norway). During the equilibration period, tissues were stimulated with 60 mM K+ every 15 min, and the resting tension was increased in a stepwise manner. After the equilibration, the resting tension was adjusted to ~300 mg, at which point the maximal 60 mM K+ response was obtained.

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Measurement of TxA2 metabolite TxB2. Aortic segments (10 mm) were cut open and washed with PSS. The endothelium was washed off under the binocular microscope with a moistened cotton swab. Endothelium-denuded or intact vessels were first incubated in PSS at 37°C for 30 min and then exposed to acetylcholine in 150 μl PSS for an additional 10 min. The elution was taken out and then snap frozen with liquid nitrogen and stored at −80°C. TxB2 was measured with an enzyme immunoassay kit (Amersham International) according to the manufacturer’s instruction.

Experimental protocols. Because of its biphasic property, the contractile response to a given concentration of acetylcholine was determined singly on each arterial specimen. The relaxation responses to acetylcholine were examined on the contraction induced by phenylephrine (adjusted with 2 or 3 μM to reach 60–80% of contraction induced by 60 mM K⁺) in a cumulative manner by increasing the concentration of the agonist in half-log increments once the response to the previous concentration had stabilized. Unless otherwise indicated, the arteries were incubated with indomethacin (1 μM) or SQ-29548 (1 μM) 5 min before acetylcholine or phenylephrine was applied, whereas l-NAME (1 mM) was administered 20 min before application. In some experiments, the endothelium was removed by moving the arterial ring around two tungsten wires, with a passive tension kept at ~100 mg.

Data analysis. Contraction was expressed as a percentage of the response to 60 mM K⁺, whereas relaxation was expressed as a percentage of the contraction to phenylephrine. Data are means ± SE, where n equals the number of animals from which blood vessels were isolated. Student’s t-test was used to determine the statistical significance. P < 0.05 was considered to indicate statistical significance.

RESULTS

Abdominal aorta. During constriction to the α1-adrenergic agonist phenylephrine, acetylcholine caused relaxation of WT mouse abdominal aorta, which was reversed at the concentration of 0.3–3 μM (Fig. 1, A and B). In the presence of the NOS inhibitor L-NAME (1 mM), which alone was unable to cause any contraction (data not shown), acetylcholine-induced relaxation was abolished. Instead of reversal of relaxation, acetylcholine increased phenylephrine-induced constriction in vessels with intact endothelium but did not increase constriction in vessels denuded of endothelial cells (Fig. 2A). Under resting baseline conditions, acetylcholine (10 μM) had no effect on the contractile tone of control WT aorta (Fig. 2B) but caused constriction in the presence of L-NAME (Fig. 2B). Similar concentration-dependent contractions to the agonist were observed in the abdominal aorta from eNOS−/− mice (0.1–10 μM) (Fig. 2C). Therefore, acetylcholine initiates both contractile and relaxant responses in mouse abdominal aorta.

To facilitate analysis of the contractile response, experiments were performed in eNOS−/− mice aorta under resting baseline conditions. Similar to results obtained in WT mice, the contractile response to acetylcholine (0.1–100 μM) was abolished by endothelial denudation (Fig. 3). This did not result from injury to the smooth muscle cells because contractions to K⁺ (60 mM) were not reduced by the procedure (Fig. 3). These results indicate that the contraction to acetylcholine is mediated by the endothelium rather than by a direct action of the agonist on vascular smooth muscle. Because endothelium-dependent contractions are generally mediated by COX-derived metabolites and activation of TxA2/PGH2 receptors (7, 13, 28), experiments were performed to assess this possibility. The contractions evoked by acetylcholine were abolished by the COX inhibitor indomethacin (1 μM) or by the TxA2/PGH2 receptor antagonist SQ-29548 (1 μM; 0.01–30 μM) in the abdominal aorta of wild-type (WT) mice. Aortas were contracted with 2–3 μM phenylephrine (to achieve 60–80% of the contraction induced by 60 mM K+)

DISCUSSION

The results of the present study demonstrate that acetylcholine initiates two distinct responses in mouse arteries: endothelium-dependent contraction and relaxant responses in mouse abdominal aorta. During constriction to the α1-adrenergic agonist phenylephrine, acetylcholine caused relaxation of WT mouse abdominal aorta, which was reversed at the concentration of 0.3–3 μM (Fig. 1, A and B). In the presence of the NOS inhibitor L-NAME (1 mM), which alone was unable to cause any contraction (data not shown), acetylcholine-induced relaxation was abolished. Instead of reversal of relaxation, acetylcholine increased phenylephrine-induced constriction in vessels with intact endothelium but did not increase constriction in vessels denuded of endothelial cells (Fig. 2A). Under resting baseline conditions, acetylcholine (10 μM) had no effect on the contractile tone of control WT aorta (Fig. 2B) but caused constriction in the presence of L-NAME (Fig. 2B). Similar concentration-dependent contractions to the agonist were observed in the abdominal aorta from eNOS−/− mice (0.1–10 μM) (Fig. 2C). Therefore, acetylcholine initiates both contractile and relaxant responses in mouse abdominal aorta.

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Carotid and femoral arteries. The ability of acetylcholine to initiate endothelium-dependent contractions was also investigated in carotid arteries and femoral arteries. Experiments were performed using arteries from eNOS−/− mice under baseline resting conditions (Fig. 6). When compared with the abdominal aorta (maximal response of 45.6 ± 3.6%, n = 5), acetylcholine-induced contractions were significantly increased in the carotid artery (maximal response of 79.5 ± 2.9%, n = 5) but significantly reduced in the femoral artery (maximal response of 12.5 ± 1.0%, n = 5). In both arteries, the acetylcholine-induced contraction was abolished by indomethacin (1 μM; n = 3) or by SQ-29548 (1 μM; n = 3).
lum-dependent relaxation mediated predominantly by NO and endothelium-dependent contraction mediated by COX metabolism of arachidonic acid and activation of TxA2 or PGH2 receptors. The contractile response to acetylcholine was most evident when the activity of NOS was inhibited either by pharmacological (L-NAME) or molecular (eNOS knockout) intervention, and in these conditions the contraction was observed under resting conditions or during contraction to the α1-adrenergic agonist phenylephrine. However, inhibition of the contractile mechanism (by inhibiting TxA2/PGH2 receptors) amplified endothelium-dependent relaxation to acetylcholine in control arteries from WT mice. These results indicate that the normal physiological response to acetylcholine comprises both components and that the net effect reflects functional competition between them. Although endothelium-dependent contraction to acetylcholine was observed in all the arteries studied, the magnitude of the response was variable among different arteries, being greatest in the carotid artery, followed by the abdominal aorta, and smallest in the femoral artery.

Endothelium-derived contracting factor (EDCF), which mediates agonist-induced endothelium-dependent contraction, has been characterized as superoxide and/or TxA2/PGH2 generated by COX-mediated metabolism of arachidonic acid (2, 4, 7, 11–15, 17, 20, 21, 24, 25, 28, 30–32). This is the first study to demonstrate the presence of EDCF responses in mouse blood vessels. Faraci et al. (6) previously demonstrated that acetylcholine caused contraction in the carotid artery of eNOS knockout mice; however, they did not determine whether the response was mediated by an endothelial or smooth muscle action of the agonist. In some blood vessels, acetylcholine can cause endothelium-independent contraction by activating muscarinic receptors located on vascular smooth muscle cells (1, 26). In the present study, the contractile response to acetylcholine was abolished by endothelial denudation, demonstrating that acetylcholine initiated contraction by activating endothelial, not smooth muscle, receptors. The constriction was also reduced by 10.220.32.247 on October 14, 2017 http://ajpheart.physiology.org/ Downloaded from
by inhibition of COX or TxA2/PGH2 receptors, which is consistent with EDCF activity in other species (4, 7, 11–15, 20, 24, 25, 28, 32) and suggests that the response is intimately linked to arachidonic acid metabolism and generation of TxA2/PGH2. Indeed, constriction to acetylcholine was associated with endothelium-dependent generation of TxA2, suggesting that this mouse EDCF is, in fact, TxA2. However, our studies cannot rule out the possibility that acetylcholine increased the release of an unidentified factor from the endothelium, which then diffused to the smooth muscle and caused constriction by stimulating TxA2 production in smooth muscle cells.

Endothelium-dependent contraction to acetylcholine was most evident after inhibition of NOS with pharmacological (L-NAME) or molecular intervention (eNOS knockout). This confirms that the response is not mediated by inhibition of NOS activity, for example, endothelial generation of superoxide activity (21, 30, 31), but represents a true contractile mechanism. Endothelium-dependent relaxation to acetylcholine in WT mice was increased after inhibition of EDCF activity with the TxA2/PGH2 receptor inhibitor SQ-29548. This suggests that acetylcholine initiates two distinct, competing mechanisms through the endothelium: endothelium-dependent relaxation mediated predominantly by NO and endothelium-dependent contraction most likely by TxA2. Indeed, as revealed by the results from L-NAME-treated arteries, or those from eNOS knockout mice, the contraction to acetylcholine occurred over a similar concentration range (0.1–0.2 μM) to that of relaxation. This suggests that EDCF is generated along with NO in response to acetylcholine stimulation.

In rat arteries, endothelium-dependent contractions in peripheral arteries were not observed in control, physiological conditions and were only observed during vascular disease, for example, hypertension (4, 15, 17, 21, 28, 32). This response is in contrast to mouse arteries in the present study, where EDCF appears to be a normal physiological response of the blood vessels. Indeed, endothelium-dependent contraction to acetylcholine was similar in control arteries treated in vitro with L-NAME and in arteries from eNOS−/− mice. Therefore, the activity of EDCF in mice arteries was not significantly altered after persistent loss of NO (as in eNOS−/− mice). Because eNOS−/− mice develop hypertension might suggest that, unlike in rat blood vessels, EDCF activity is not increased in mouse arteries during hypertension. However, because blood pressure was not measured in the present study, the modulation of EDCF activity by hypertension or by other mouse vascular pathologies remains to be determined.

In addition to the abdominal aorta, acetylcholine also caused endothelium-dependent contraction of mouse carotid and femoral arteries. Whereas this implies that EDCF is not restricted...
to a particular vessel type, the difference in extent of contraction may suggest a differential role for EDCF among mouse vessels. The increased prominence of this response in carotid arteries may reflect a greater role for this endothelium-dependent constrictor mechanism in the cerebral circulation, as originally proposed by Katusic and Vanhoutte (14). Interestingly, in several mouse vessels including mesenteric artery, endothelium-dependent agonists were reported to evoke eNOS or NO-independent relaxation (2, 3, 5, 8, 9, 18, 19, 21, 27, 29), whereas endothelium-dependent relaxation in the mouse thoracic aorta was suggested to depend entirely on eNOS function (2, 14). These results suggest that the involvement of NO-independent endothelial regulatory mechanisms, including relaxant and contractile mechanisms, differs considerably among mouse vessels.

In summary, the results of the present study demonstrate for the first time that acetylcholine causes endothelium-dependent contraction of mouse arteries. The contraction was abolished by inhibition of COX or TxA2/PG H2 receptors and was associated with an endothelium-dependent generation of TxA2. Therefore, the nature of this EDCF is likely to be TxA2. Endothelium-dependent contraction was observed in all mouse arteries examined: abdominal aortas and femoral and carotid arteries from WT and/or eNOS−/− mice. The results indicate that the normal physiological response to acetylcholine in mouse arteries reflects functional competition between endothelium-dependent contraction and endothelium-dependent relaxation.

ACKNOWLEDGMENTS

We thank Jonathan Davis for critical reading of the paper.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-63744, HL-65608, and HL-38324 (to J. L. Zweier), and by an American Heart Association Postdoctoral Fellowship grant (Ohio Valley; to Y. Zhou).

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