Flow-Induced Constriction in Arterioles of Hyperhomocysteinemic Rats Is Due to Impaired Nitric Oxide and Enhanced Thromboxane A₂ Mediation

Zsolt Bagi, Zoltan Ungvari, Lajos Szollár, Akos Koller

Abstract—Hyperhomocysteinemia (HHcy) is thought to promote arteriosclerosis and peripheral arterial disease, in part by impairing the function of endothelium. Because flow-induced dilation is mediated by the endothelium, we hypothesized that HHcy alters this response by interfering with the synthesis/action of NO and prostaglandins. Thus, changes in the diameter of isolated, pressurized (at 80 mm Hg) gracilis skeletal muscle arterioles (diameter \( \approx 170 \) \( \mu \)m) from control and methionine diet–induced HHcy rats were investigated with videomicroscopy. Increases in intraluminal flow (from 0 to 25 \( \mu \)L/min) resulted in dilations of control arterioles (maximum, 34 ± 4 \( \mu \)m). In contrast, increases in flow elicited constrictions of HHcy arterioles (−36 ± 3 \( \mu \)m). In control arterioles, the NO synthase inhibitor \( N^\bullet \)-nitro-L-arginine-methyl ester significantly attenuated (≈50% dilation), whereas the additional administration of indomethacin, an inhibitor of cyclooxygenase, eliminated flow-induced dilation. In the arterioles of HHcy rats, flow-induced constriction was not affected by \( N^\bullet \)-nitro-L-arginine-methyl ester, whereas it was abolished by indomethacin or the prostaglandin \( H_2/thromboxane \) \( A_2 \) receptor antagonist SQ 29,548 or the TXA₂ synthase inhibitor CGS 13,080. Thus, in HHcy, increases in intraluminal flow elicit constrictions of skeletal muscle arterioles due to the impaired NO and enhanced TXA₂, mediation of the response, alterations that likely contribute to the development of peripheral arterial disease. (Arterioscler Thromb Vasc Biol. 2001;21:233-237.)

Key Words: homocysteine • arteriole • flow-induced response • endothelium • nitric oxide • thromboxane A₂

Increasing epidemiological and experimental evidence suggests that an elevated plasma level of homocysteine is associated with the development of peripheral arterial disease.1–5 Homocysteine is a sulfur-containing amino acid that is formed during the metabolism of the essential amino acid methionine.4 Plasma homocysteine concentration can be increased through alterations in genetic and nutritional factors, such as various enzyme (cystathionine-β-synthase, methyltetrahydrofolate reductase) abnormalities and deficiency of vitamins (folic acid, cyanocobalamin, pyridoxal phosphate), all of which participate in the metabolism of homocysteine and methionine (for further references, see Lentz et al4). Previously, several mechanisms have been suggested by which elevated homocysteine levels promote peripheral arterial disease, such as vascular smooth muscle proliferation,6 platelet activation,7 and endothelial injury.7

It is likely that hyperhomocysteinemia (HHcy) impairs the vasoactive function of the endothelium of arteries and arterioles before the development of morphological changes.8–11 Indeed, the diminished increase in hindlimb circulation to acetylcholine in monkeys with diet-induced HHcy8,9 suggests that the dilator capacity of small arteries and arterioles responsible for tissue vascular resistance is altered by HHcy. Also, we recently demonstrated that endothelium-dependent acetylcholine- and histamine-induced NO-mediated dilations are impaired in isolated skeletal muscle arterioles of HHcy rats.10 In addition, bradykinin elicits an enhanced constriction of arterioles of HHcy rats due to the augmented release of thromboxane \( A_2 \) (TXA₂) from the endothelium.11 Moreover, in the same HHcy rats, we found an enhanced platelet aggregation that could be inhibited by blocking TXA₂ receptors. These findings suggest that the agonist-induced synthesis of NO and prostaglandins by the resistance vessels is altered in HHcy.

One of the primary in vivo stimuli for the endothelial synthesis/release of NO and prostaglandins in arterioles is an increase in intraluminal flow.12 This mechanism is important because unlike the responses to acetylcholine and bradykinin, it is known to be involved in the moment-to-moment regulation of arteriolar diameter in vivo.13 On the basis of the aforementioned studies, it is logical to hypothesize that flow-induced arteriolar dilation is altered in HHcy rats. Thus, in the present study we sought to investigate the responses of isolated gracilis muscle arterioles of rats with HHcy10,11,14,15 to increases in intraluminal flow, to contrast the responses to those of rats with normal plasma homocysteine levels, and to characterize the alterations in the mediation of responses by endothelial factors.

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Methods

In male Wistar rats (weight 325 to 345 g; purchased from Charles River Co), moderate HHcy was induced by the administration of L-methionine (1 g · kg body wt \(-1 \cdot \text{d} \)) in the drinking water for a period of 4 weeks \((n = 35)\), as described previously.8-11 The doses administered were based on average daily fluid intake. This diet is known to increase plasma total homocysteine levels in rats from \(\sim 6\) to \(\sim 21 \mu\text{mol/L}\).10,11 The water consumption and body weight did not differ significantly between methionine-fed rats and age-matched control rats \((n = 35)\).

Isolation of Arterioles

Experiments were conducted on isolated arterioles (inside diameter \(~170 \mu\text{m}\) ) of rat gracilis muscle as described previously.8-11 Briefly, on the fourth week after overnight fasting, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The gracilis muscle was dissected and placed in a silicone-lined Petri dish containing cold \((0\text{° to } 4\text{°C})\) physiological salt solution (PSS) composed of \((\text{in mmol/L})\) NaCl 110, KCl 5.0, CaCl\(_2\) 2.5, MgSO\(_4\) 1.0, KH\(_2\)PO\(_4\) 1.0, dextrose 10.0, and NaHCO\(_3\) 24.0 and was equilibrated with a gas mixture of 10% O\(_2\) and 5% CO\(_2\), balanced with nitrogen, at pH 7.4. With microsurgery instruments and an operating microscope, a segment of 1.5 mm in length of an arteriole running intramuscularly was isolated and transferred into an organ chamber containing 2 glass micropipettes filled with PSS. From a reservoir, the vessel chamber (15 mL) was continuously supplied with PSS at a rate of 20 mL/min. After the vessel had been mounted on the proximal (inflow) pipette and was secured with sutures, the perfusion pressure was raised to 20 mm Hg to clear the lumen. Then the other end of the vessel was mounted on the distal (outflow) pipette.16 Both micropipettes were connected with silicone tubing to an adjustable PSS reservoir. Inflow and outflow pressures were measured with an electromanometer. The intraluminal pressure was slowly increased to 80 mm Hg. The temperature was set at 37°C by a temperature controller (Grant Instruments), and the vessel was allowed to develop spontaneous tone in response to intraluminal pressure under no-flow conditions (equilibration period \(~1 \text{ hour}\)). The inner arteriolar diameter was measured with videomicroscopy with a microangiometer and recorded on a chart recorder. Perfusion flow was measured with a ball flowmeter (Omega Engineering Inc).

Experimental Protocols

Changes in the diameter of arterioles were assessed in response to step increases in intraluminal flow \((0 \text{ to } 25 \mu\text{L/min})\). Flow was established at a constant intravascular pressure \((80 \text{ mm Hg})\) by changing the inflow and outflow pressures to an equal degree, but in opposite directions, to keep midpoint luminal pressure constant.16 Arterioles were then incubated with \(10^{-7} \text{ mol/L N}-\text{nitro-L-arginine-}

Discussion

The salient findings of the present study are that increases in intraluminal flow resulted in the constriction of arterioles isolated from rats with an elevated plasma homocysteine concentration. The constrictions were not affected by inhibition of NO synthesis but were abolished by inhibition of prostaglandin synthesis, PGH\(_2\)/TXA\(_2\) receptor antagonists (Figure 3B). L-NNAME, indomethacin, and TXA\(_2\) synthase inhibited CGS 13,080 had no significant effect on the basal arteriolar diameter of control and HHcy rats (Table).
dilated in response to increases in flow, a response that is mediated by NO and dilator prostaglandins.

Our previous studies showed that plasma homocysteine concentrations in rats fed a methionine diet are increased by 3-fold, reaching a concentration similar to that shown to be associated with an increased risk of vascular disease in humans. In these studies, we demonstrated that acetylcholine and histamine–induced, NO-mediated endothelial dilations are reduced in skeletal muscle arterioles of HHcy rats. In addition, bradykinin induces an enhanced constriction of HHcy arterioles via increased TXA2 production in the endothelium. Also, it has been reported that elevated plasma homocysteine concentrations are associated with an impaired dilation of conduit arteries in response to the release of an NO synthase inhibitor L-NAME (10^(-5) mol/L), the cyclooxygenase inhibitor indomethacin (Indo; 10^(-5) mol/L), or both. Data are mean±SEM. *Significant difference (P<0.05).

The underlying mechanism for the simultaneous impaired NO release and enhanced TXA2 production in the endothelium of arterioles of HHcy rats is not known. Several previous studies in endothelial cells in culture suggest a role for an increased level of reactive oxygen species (ROS). Because endothelial cells have a limited capacity to metabolize homocysteine, they are particularly vulnerable to elevated levels of homocysteine. Homocysteine is thought to promote the generation of ROS via the auto-oxidation of the sulfhydryl group or by decreasing the intracellular levels of glutathione and glutathione peroxidase that are involved in the detoxification of ROS. Enhanced levels of ROS can interfere with NO and reduce the release of NO in response to increases in flow and agonists in HHcy. Indeed, recent studies showed that in humans after methionine loading, impaired brachial artery dilations in response to acetylcholine and to the release of forearm occlusion are restored with the antioxidant ascorbic acid. The presence of ROS may also favor the formation of TXA2, in part through an increased formation of arachidonic acid. Also, it is possible that superoxide can form peroxynitrite with NO, which then may inhibit PGL2 synthase and result in an enhanced formation of constrictor prostaglandins, especially when the level of arachidonic acid is elevated. Inter-

Figure 2. Changes in diameter of skeletal muscle arterioles isolated from control (n=15; A) and HHcy (n=15; B) rats as a function of perfusate flow in the presence of the NO synthase inhibitor L-NAME (10^(-5) mol/L), the cyclooxygenase inhibitor indomethacin (Indo; 10^(-5) mol/L), or both. Data are mean±SEM. *Significant difference (P<0.05).

In the present study, we sought to investigate the flow-induced response of arterioles isolated from HHcy rats because it is known that increases in intraluminal flow stimulate the endothelium to release vasoactive mediators and because the flow-sensitive mechanism is important in the moment-to-moment regulation of peripheral vascular resistance. First, we confirmed that increases in intraluminal flow elicit substantial endothelium-dependent dilations in arterioles of control rats (Figure 1.). In contrast, in arterioles isolated from HHcy rats, increases in intraluminal flow elicited significant constrictions in the presence of the endothelium (Figure 1.). In the absence of the endothelium, both responses were abolished. In control arterioles, flow-induced dilation is mediated by NO and dilator prostaglandins because the inhibition of NO synthesis significantly reduced the response, whereas additional inhibition of the prostaglandin synthesis abolished this response (Figure 2A), confirming our previous findings. In contrast, inhibition of NO synthesis did not affect flow-induced constriction in arterioles of the HHcy rats (Figure 2B). These alterations are likely due to impaired endothelial synthesis or bioavailability of NO rather than to altered dilatory capacity of the smooth muscle, because arteriolar dilations in response to the endothelium-independent NO donor sodium nitroprusside are not affected by HHcy.

The finding that indomethacin (Figure 2 B) or the PGH2/TXA2 receptor antagonist SQ 29,548 (Figure 3A) abolished flow-induced constrictions of HHcy arterioles indicates that increases in flow activate the arachidonic acid cascade in the endothelium; however, predominantly constrictor (PGH2/TXA2) instead of dilator prostaglandins are released. Because the TXA2 synthase inhibitor CGS 13,080 also abolished flow-induced constrictions, it seems that TXA2 is synthesized (and released) in HHcy in response to flow (Figure 3B). These results are in accordance with recent data that show methionine load–induced HHcy acutely increases TXA2 synthesis in rats and with our recent findings that demonstrate enhanced TXA2 production in the endothelium of HHcy arterioles in response to bradykinin. Vascular endothelium represents a large surface that is continuously exposed to changes in blood flow. Thus, it is likely that the flow-induced arteriolar release of TXA2 together with that released from platelets contributes to the development of increased vascular tone and urinary excretion of TXB2, the stable end metabolite of TXA2 shown to be present in patients with genetic HHcy.

The underlying mechanism for the simultaneous impaired NO release and enhanced TXA2 production in the endothelium of arterioles of HHcy rats is not known. Several previous studies in endothelial cells in culture suggest a role for an increased level of reactive oxygen species (ROS). Because endothelial cells have a limited capacity to metabolize homocysteine, they are particularly vulnerable to elevated levels of homocysteine. Homocysteine is thought to promote the generation of ROS via the auto-oxidation of the sulfhydryl group or by decreasing the intracellular levels of glutathione and glutathione peroxidase that are involved in the elimination of free radicals. Enhanced levels of ROS can interfere with NO and reduce the release of NO in response to increases in flow and agonists in HHcy. Indeed, recent studies showed that in humans after methionine loading, impaired brachial artery dilations in response to acetylcholine and to the release of forearm occlusion are restored with the antioxidant ascorbic acid. The presence of ROS may also favor the formation of TXA2, in part through an increased formation of arachidonic acid. Also, it is possible that superoxide can form peroxynitrite with NO, which then may inhibit PGL2 synthase and result in an enhanced formation of constrictor prostaglandins, especially when the level of arachidonic acid is elevated. Inter-
that they precede structural changes in the vascular wall. By arteriolar endothelium represent early effects of HHcy and endothelial dysfunction. It is likely that these changes in the vasoactive function of arteriolar endothelium represent early effects of HHcy and that they precede structural changes in the vascular wall. By adjusting the diameter, a flow-dependent mechanism plays an important role in the dilation of precapillary vessels during increased demand, such as exercise. It is thought that flow-dependent dilation amplifies increases in blood flow to working skeletal muscle to prevent the development of relative oxygen deficiency and an increase in wall shear stress. In HHcy, flow-induced constriction of the arterioles may limit tissue blood supply, whereas the impaired NO release and upregulated synthesis of TXA₂ in the endothelium, together with the simultaneously increased TXA₂ synthesis in platelets, can substantially enhance platelet aggregation as well, favoring thrombus formation and leading to occlusive peripheral vascular disease, such as intermittent claudication.

In conclusion, the present study is the first to demonstrate that flow-dependent arteriolar dilation observed under normal healthy conditions is converted to constriction in HHcy rats. The constriction is due to the simultaneously impaired NO and enhanced TXA₂ mediation of arteriolar responses to increases in flow. Such alterations in the vasoactive function of endothelium in HHcy could limit or reduce tissue perfusion, thereby promoting symptomatic peripheral arterial disease.

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References


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