Chronic Endothelin-1 Improves Nitric Oxide–Dependent Flow-Induced Dilation in Resistance Arteries From Normotensive and Hypertensive Rats

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Abstract—Endothelin-1 (ET-1) is released on stimulation by shear stress of the vascular wall. In several pathological situations, an involvement of ET-1 is suspected. Nevertheless, the effect of a chronic increase in circulating ET-1 on vascular tone in resistance arteries is not yet fully understood. We investigated the response to tensile stress (pressure-induced myogenic tone) and shear stress (flow-induced dilation, FD) of rat mesenteric resistance arteries cannulated in an arteriograph. Intraluminal diameter was measured continuously. Rats (normotensive Wistar-Kyoto rats [WKYs] and spontaneously hypertensive rats [SHRs]) were treated for 2 weeks with ET-1 (5 pmol · kg⁻¹ · min⁻¹ SC; n=8 to 16 per group). Systolic arterial blood pressure increased significantly in ET-1–treated rats (171.7 ± 196.6 mm Hg in WKYs and 216.±8 versus 245.±6 mm Hg in SHRs, P<0.05). Passive arterial diameter in isolated resistance arteries ranged from 78.9 ± 169.4 μm in WKYs and from 62.6 ± 149.7 μm in SHRs (pressure from 10 to 150 mm Hg). Myogenic tone was not significantly affected by chronic ET-1. Flow (9 to 150 μL/min) significantly increased the arterial diameter by 2.±0.5 to 22.±2 μm in WKYs and by 1.3.±0.7 to 8.3.±0.8 μm in SHRs (P<0.001 versus WKYs). The NO synthesis blocker N⁶-nitro-L-arginine methyl ester (L-NAME; 100 μmol/L) attenuated FD in WKYs (eg, 22.±2 versus 15.±3 μm after L-NAME, flow=150 μL/min) and, to a lesser extent, in SHRs (P<0.001 versus WKYs). The cyclooxygenase inhibitor indomethacin (3 μmol/L) attenuated the remaining FD in WKYs (eg, 15.±3 versus 8.±3 μm, flow=150 μL/min) and in SHRs (eg, 7.5.±0.5 versus 5.0.±0.6 μm). Chronic ET-1 significantly increased FD in SHRs but not in WKYs. In both strains, NO-dependent FD was significantly increased by chronic ET-1. Furthermore, indomethacin-sensitive FD was increased by chronic ET-1 in SHRs only. Thus, chronic ET-1 increased NO-dependent FD in resistance mesenteric arteries from both WKYs and SHRs and increased indomethacin-sensitive FD in SHRs only. (Arterioscler Thromb Vasc Biol. 1999;19:2148–2153.)

Key Words: myogenic tone ■ shear stress ■ resistance arteries ■ endothelin ■ hypertension

Pressure-induced tone (myogenic tone) is a characteristic of small resistance arteries and of some veins.1–5 It is opposed by flow-induced dilation in vitro as well as in vivo.1,2,6–8 These 2 mechanical stimuli determine a basal vascular tone in resistance arteries and allow a rapid adaptation to changes in flow and pressure.1,8 Whereas myogenic tone is mainly independent of endothelial factors,1,5 flow produces shear stress and triggers an endothelium-dependent dilation.1,7,8 Flow-induced dilation depends in part on the production of NO9–11 and cyclooxygenase (COX) products10–13 by endothelial cells.

Endothelial cells stimulated by hormonal14 or mechanical15–17 factors can produce endothelin-1 (ET-1). Mechanical factors such as pressure or wall stretch increase ET-1 gene expression and ET-1 production.16,17 Flow, or shear stress, exerts a flow rate–dependent effect on the production of ET-1. A low flow rate or a short-term increase in flow would enhance ET-1 production, whereas a high flow rate or a long-term decrease in flow would diminish ET-1 production.13 Endothelin-1 might be involved in several pathological situations such as preeclampsia,18 renal dysfunction,19,20 sepsis,21 heart failure,22 atherosclerosis,16 cerebral vasospasm,23,24 and aging.25 In spontaneous hypertension, the role of ET-1 is still a matter of debate. No role for ET-1 was found in spontaneously hypertensive rats (SHRs) by Li et al,26 although in mesenteric resistance arteries from SHRs, ETₐ receptor activation led to higher increases in intracellular calcium.27 Blockade of ETₐ receptors decreases blood pressure in SHRs.28 In experimental hypertension the importance of ET-1 is better understood. In deoxycorticosterone acetate salt–hypertensive rats, ET-1 is a determinant of hypertension.29,30 Angiotensin II–induced hypertension31 as well as the hypertension due to the chronic inhibition of NO synthesis32 is counteracted by the blockade of ETₐ receptors. Nevertheless, no study has yet clearly determined the consequences of a chronic increase in ET-1 on vascular reactivity.
In the different pathological situations given above, ET-1 is involved in a pathological context in which its specific role is difficult to distinguish. Thus, we used a model of chronic infusion of ET-1 in rats to determine the specific effect of ET-1 on basal vascular tone in mesenteric resistance arteries. Because pressure and flow are important in ET-1 production,\textsuperscript{15,16} we hypothesized that a chronic infusion of ET-1 might interact with pressure-induced (myogenic) tone and flow-induced dilation. Furthermore, both responses to flow and to ET-1\textsuperscript{32,33} involve NO and COX derivatives. Thus, chronic ET-1 might change the proportion of NO and/or COX products in response to flow. We also used hypertensive rats, in which flow-induced dilation in mesenteric resistance arteries involves minimal NO and vasodilator COX derivatives.\textsuperscript{13} Thus, the effect of chronic ET-1 was investigated under conditions in which NO and COX derivatives were involved (Wistar-Kyoto rats [WKYs]) and under conditions in which NO and COX derivatives were not involved (SHRs).

**Methods**

Ten-week-old male WKYs (Ifa-Credo, Lyon, France) weighing 250 g were treated for 2 weeks with ET-1 (5 pmol \( \cdot \) kg\(^{-1} \cdot \) min\(^{-1} \) SC) by osmotic minipumps; Alzet model 2002, n=7).\textsuperscript{32} In the control group, rats were given the solvent (physiological salt solution) subcutaneously. After 2 weeks, systolic blood pressure was measured by the tail-cuff method (BP recorder 8006, Ugo Basile) and then anesthetized with pentobarbital (50 mg/kg IP). A median laparotomy was performed, and a segment of mesenteric artery \( \approx 100 \mu m \) in internal diameter was isolated, cannulated at both ends, and mounted in a video-monitored perfusion system.\textsuperscript{33} The artery was bathed in a 5-mL organ bath containing a physiological salt solution of the following composition (in mmol/L): 135.0 NaCl, 15.0 NaHCO\(_3\), 4.6 KCl, 1.5 CaCl\(_2\), 1.2 MgSO\(_4\), 11.0 glucose, and 5.0 HEPES. The pH was 7.4, the Po\(_2\) 160 mm Hg, and the PC\(_2\) 37 mm Hg. The bath solution was changed continuously at a rate of 4 mL/min. Perfusion of the artery with a similar physiological salt solution was set at a rate ranging from 0 to 150 \( \mu L \cdot \) min\(^{-1} \) (under a pressure of 75 mm Hg). The pressure in both ends of the artery segment was monitored by pressure transducers (Figure 1). As previously described,\textsuperscript{34,35} flow can be generated through the distal pipette with a peristaltic pump. Pressure in the proximal end of the vessel was controlled by a servo perfusion system. When flow was increased, the difference in pressure between the distal and proximal ends of the vessel increased, but the average pressure remained constant. This supposes that the 2 pipettes oppose the same resistances. Therefore, pairs of pipettes were selected to satisfy the following conditions in which NO and COX derivatives were not involved (SHRs).

![Figure 1. Arterial diameter measured in mesenteric resistance artery segments isolated from rats treated for 2 weeks with ET-1 (5 pmol \( \cdot \) kg\(^{-1} \cdot \) min\(^{-1} \))](image)

Diameter (active diameter) was measured on stepwise increases in pressure in the absence of flow in normotensive (WKY) and hypertensive (SHR) rats (n=14 per group). Passive diameter is the diameter measured in a Ca\(^{2+}\)-free physiological salt solution containing EGTA (2 mmol/L) and sodium nitroprusside (10 \( \mu mol/L \)). Diameter values are shown in the upper panel and normalized values (% of passive diameter at 100 mm Hg) in the lower panel. Data are expressed as mean=\( \pm \)SEM. *P<0.001, SHR vs WKY, 2-factor ANOVA for consecutive measurements (pressure steps).

Diameter was determined in the absence of Ca\(^{2+}\)+EGTA (2 mmol/L)+sodium nitroprusside (10 \( \mu mol/L \)). Flow-induced relaxation was expressed as increases in diameter (micrometers) as a function of shear stress due to flow in vessels subjected to an intraluminal pressure of 75 mm Hg, thus developing an optimal level of myogenic tone. Shear stress was calculated for each individual segment of artery as previously described:\textsuperscript{17} \( \tau = 4 \pi Q / \pi r^2 \), where \( \eta \) is viscosity (poise=\( dyne \cdot s / cm^2 \)), \( Q \) is flow (mL/s), and \( r \) is radius (cm).

The viability of each vessel was tested before the experimental protocol. The responsiveness of the smooth muscle was assessed by testing the constrictor effect of KCl (80 mmol/L) after preconstriction of the mesenteric arteries with phenylephrine (0.1 \( \mu mol/L \)). The presence of the endothelium was assessed by testing the vasodilator effect of acetylcholine (1 \( \mu mol/L \)) after preconstriction of the mesenteric arteries with phenylephrine (0.1 \( \mu mol/L \)), under an intraluminal pressure of 50 mm Hg. Vessels that did not fully contract to KCl (80 mmol/L) and did not fully relax on application of acetylcholine (1 \( \mu mol/L \)) were excluded.

The procedure followed in the care and euthanasia of the rats was in accordance with the European Community standards on the care and use of laboratory animals (Ministère de l’agriculture, France, authorization No. 00577).

**Histomorphological Study**

The morphometric analysis of the mesenteric resistance arteries was performed as previously described.\textsuperscript{36} In brief, segments of artery, adjacent to those used in the functional study, were mounted in the arteriorgraph as described above, and pressure was set at 100 mm Hg. Vessels were then fixed in 10% formaldehyde in saline solution for 30 minutes and sectioned (10-\( \mu m \)-thick sections). Morphometric analysis was performed with an automated image processor (NS 15000, Microvision). The total surface area and area of the lumen were measured. This allowed the calculation of the area of the media.\textsuperscript{36}

**Drugs**

HEPES, L-NAME, indomethacin, EGTA, phenylephrine, and acetylcholine were purchased from Sigma Chemical Co. SQ 29548 was purchase from Biomol. Other reagents were purchased from Prolabo.
Results

Chronic ET-1 induced a significant increase in systolic arterial blood pressure, from 171±7 to 196±6 mm Hg in WKYs and from 216±8 to 245±6 mm Hg in SHRs (P<0.05).

In isolated mesenteric resistance arteries, stepwise increases in pressure induced the development of myogenic tone (Figure 1). Myogenic tone was higher in SHRs than in WKYs (Figure 1). Myogenic tone was not significantly affected by chronic ET-1 in both strains (not shown). Passive arterial diameter (Figure 1) ranged from 78±9 to 169±4 μm in WKYs and from 62±6 to 149±7 μm in SHRs (P<0.001 versus WKYs) for pressure values ranging from 10 to 150 mm Hg. Chronic ET-1 had no significant effect on passive diameter in both strains (data not shown).

Stepwise increases in flow, under a pressure of 75 mm Hg, significantly attenuated myogenic tone in mesenteric resistance arteries (Figure 2). Flow-induced dilation was significantly lower in SHRs than in WKYs (Figure 2). The NO synthesis blocker L-NAME (100 μmol/L) significantly attenuated flow-induced dilation in WKYs (Figures 3 and 4). L-NAME (100 μmol/L) had a significantly lower effect on flow-induced dilation in SHRs than in WKYs (Figure 4). The COX inhibitor indomethacin (3 μmol/L) significantly attenuated flow-induced dilation in WKYs (Figure 3) and SHRs (Figure 5). Chronic ET-1 significantly increased flow-induced dilation in SHRs (Figure 2), without significantly affecting the response to flow in WKYs (Figure 2). In both WKYs (Figure 4) and SHRs (Figure 6), chronic ET-1 increased the proportion of flow-induced dilation sensitive to L-NAME. The proportion of flow-induced dilation sensitive to indomethacin (Figure 6) was also increased by chronic ET-1 in SHRs (with no effect in WKYs; not shown).

The thromboxane A_2–PGH_2 receptor blocker SQ 29548 (1 μmol/L) significantly increased flow-induced dilation in mesenteric resistance arteries from SHRs (Figure 7). In WKYs, SQ 29548 (1 μmol/L) had no significant effect on flow-induced dilation (Figure 7). The chronic treatment of rats with ET-1 did not significantly affect SQ 29548 (1 μmol/L)-dependent, flow-induced dilation in either strain (Figure 7).

The presence of the endothelium was confirmed by testing the vasodilator effect of acetylcholine (1 μmol/L) after preconditioning of the mesenteric resistance arteries with phenylephrine (0.1 μmol/L). Phenylephrine (0.1 μmol/L at 50 mm Hg) induced a decrease in diameter from 121±12 to 44±8 μm (n=8) in WKYs and from 112±10 to 40±9 μm (n=8) in SHRs. The further addition of acetylcholine (1 μmol/L) induced an increase in diameter from 44±8 to 138±11 μm (n=8) in WKYs and from 40±9 to 103±10 μm (n=8) in SHRs (P<0.05 versus WKYs). Phenylephrine (0.1 μmol/L)-induced tone and acetylcholine (1 μmol/L)-induced dilation were not significantly affected by chronic ET-1 in either WKYs or SHRs (not shown). The constrictor effect of KCl (80 mmol/L) was not significantly affected by chronic ET-1 in both SHRs and WKYs (not shown).
Sodium nitroprusside induced a dilation that was not significantly affected by chronic ET-1. In all groups, the maximal dilation was 100%. In WKYs, the IC₅₀ was 2.6 ± 0.33 nmol/L in controls and 1.5 ± 0.27 nmol/L in the chronic ET-1 group. In SHRs, the IC₅₀ was 5.1 ± 1.1 nmol/L in controls and 4.2 ± 1.0 nmol/L in the chronic ET-1 group.

The histomorphometric analysis of the mesenteric resistance arteries showed that chronic ET-1 significantly increased the thickness of the media tunica (5.7 ± 0.5 versus 7.8 ± 0.7 μm, n=9, in WKYs, P<0.01; and 6.9 ± 0.4 versus 8.5 ± 0.6 μm, n=9, in SHRs, P<0.03). The media to lumen ratio was also significantly higher in ET-1–treated rats (0.19 ± 0.04 versus 0.33 ± 0.04, n=9, in WKYs, P<0.05; and 0.32 ± 0.05 versus 0.48 ± 0.06, n=9, in WKYs, P<0.05).

Discussion

The main new finding of the present study is that chronic administration of ET-1 modified the vascular response to flow (shear stress) without affecting pressure-induced (myogenic) tone. In both WKYs and SHRs, NO production in response to flow was increased, whereas the production of vasodilator COX derivative(s) was increased in SHRs only. In mesenteric resistance arteries isolated from SHRs, we found a higher myogenic tone and a lower response to flow, compared with WKYs. This result is in agreement with previous studies.12,13,36 –38 In addition, in SHRs the response to flow depends less on the production of NO (vide infra and References 12 and 13). In SHRs, the production of COX derivatives in response to flow has been shown to be higher in resistance arteries from gracilis muscle12 and lower in mesenteric resistance arteries. 13 In mesenteric resistance arteries from SHRs, we have previously shown a lower production of vasodilator COX products and a higher production of vasoconstrictor COX products.12 In the present study, indomethacin-sensitive, flow-induced dilation was increased by chronic ET-1 in SHRs but not in WKYs. Different type of COX products are involved in flow-induced dilation in SHRs, as previously shown.13 This could explain the difference between the 2 strains. Moreover, ET-1 has been shown to activate COX derivative production. The production of vasodilator prostanooids is activated by ET-1 in the rat aorta,39 lung vessels,40 mesenteric arteries,41 and afferent arterioles,42
whereas vasoconstrictor prostanoids are involved in ET-1–induced contraction in the rat aorta and in afferent arterioles from SHRs. We found an increased sensitivity of flow-induced dilation to indomethacin in SHRs chronically treated with ET-1. Thus, in SHRs, chronic ET-1 might either activate the production of vasodilator COX derivatives or downregulate vasoconstrictor COX product(s). Nevertheless, this latter possibility may not apply, since the increase in flow-induced dilation due to the thromboxane–PGH₂ receptor blocker SQ 29548 in SHR arteries was not affected by chronic ET-1.

The participation of NO in flow-induced dilation was higher after chronic ET-1. This might be a response to the increased tone due to ET-1. Because ET-1 can participate in the response to flow as a contractile factor, an increased NO production might counterbalance the ET-1–induced tone. In addition, we found that the relaxation to the NO donor sodium nitroprusside was not affected by chronic ET-1. Thus, a nonspecific effect of chronic ET-1 on the cGMP pathway can be excluded.

Finally, chronic ET-1 increased (in SHRs) or did not change (in WKYs) the amplitude of flow-induced dilation, despite an increase in blood pressure in both strains. This increase was significant in SHRs for shear stress values equal to 20 dyn/cm² and higher. In resistance arteries, shear stress may vary greatly, as flow may locally change rapidly from low to high values (up to 100 dyn/cm²) and even to negative values if flow reverses. In all other animal models of hypertension, a decreased endothelium-dependent dilation has been described. Both flow-induced dilation and agonist-induced dilation are affected in hypertensive animals. This emphasizes that the change in NO and prostanoïd production in response to flow is specific to ET-1. Another possibility is that flow-induced dilation may not necessarily be a determinant of blood pressure. In agreement with this possibility, we have shown in mice lacking the gene for vimentin that flow-induced dilation was decreased despite a normal blood pressure. Nevertheless, chronic ET-1 increased the wall to lumen ratio in mesenteric arteries, which is a characteristic of a vascular adaptation to a high blood pressure.

The duration of the treatment with ET-1 (2 weeks) and the dose used (5 ng kg⁻¹ min⁻¹) were validated in previous work. A 1-week-long treatment with concentrations of ET-1 ranging from 1 to 5 ng kg⁻¹ min⁻¹ induced a doubling of the plasma ET-1 concentration. Such an increase in plasma ET-1 has been described in several pathological situations.

In conclusion, the main effect of chronic ET-1 was to increase L-NAME–sensitive, flow-induced dilation in mesenteric resistance arteries in WKYs and SHRs. In addition, in SHRs the dilator response to flow was improved after chronic ET-1.

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