Endothelin-1 Released by Vascular Smooth Muscle Cells Enhances Vascular Responsiveness of Rat Mesenteric Arterial Bed Exposed to High Perfusion Flow

Domenico Russo, Roberto Minutolo, Carla Clienti, Luca De Nicola, Carmela Iodice, Francesco A. Savino, and Vittorio E. Andreucci

Vasodilation of resistance vessels ensues in response to increased perfusion flow to maintain tissue perfusion. The flow-induced vasodilation is mainly dependent on nitric oxide (NO), which also regulates vascular responsiveness to vasoconstrictors. Besides NO, however, high flow increases endothelin-1 (ET-1) production from endothelial cells. It is likely, therefore, that the interaction between NO and ET-1 may play a critical role in the control of arterial vascular tone under high perfusion flow.

In this study, the vascular responsiveness (VR) to high flow rate and the role of ET-1 released by vascular smooth muscle cells (VSMC) were evaluated in isolated and in vitro-perfused mesenteric arteries (MA). MA were perfused at constant (3.5 mL/min; CPF) and increased flow rate (4.5, 5.5, 6.5 mL/min; IPF). VR was evaluated by infusing norepinephrine (NE; 5 μmol/L) and potassium chloride (KCl; 80 mmol/L). Mesenteric vascular resistance (MVR), ET-1, and cGMP release were measured under different flow rates. The role of endothelium-derived ET-1 was evaluated by perfusing MA with phosphoramidon (endothelin converting enzyme inhibitor), whereas the role of other endothelium-derived vasoactive substances was excluded by measuring VR in MA without endothelium. Finally, ETA and ETB receptor antagonists were perfused in disendothelized MA. In the IPF group of intact MA, MVR dropped (P < .05) and both ET-1 and cGMP increased in the perfusate (P < .05). VR was enhanced by high flow after NE (101 ± 9 vs 56 ± 12 mm Hg in CPF, P < .005) and KCl (119 ± 12 vs 51 ± 10 mm Hg in CPF, P < .005) and it was unaffected by either phosphoramidon or endothelium removal. On the contrary, BQ-610 abolished the flow-dependent increase in VR. No further additive effect was achieved with BQ-788. In conclusion, in MA, high flow reduces MVR and concurrently enhances VR, likely through VSMC-derived ET-1. Am J Hypertens 1999;12:1119–1123 © 1999 American Journal of Hypertension, Ltd.

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From the Department of Nephrology, School of Medicine, University “Federico II,” Naples, Italy.

Address correspondence and reprint requests to Domenico Russo, MD, Via Marconi 80, 80024 Cardito, Naples, Italy.
Previously, we observed that flow-dependent vasodilation occurs also in isolated mesenteric arteries and that the stepwise increase of flow was accompanied by an increased release of cGMP and ET-1. In fact, mesenteric vascular resistances (MVR) were significantly lower under high flow, and mesenteric arteries concurrently showed increased VR to norepinephrine (NE) and potassium chloride (KCl).

In the present study we evaluate whether the flow-dependent increased VR is attributable to vasoactive substances produced by endothelium or to ET-1 released from vascular smooth muscle cells (VSMC). The experiments were performed in the isolated and in vitro-perfused arterial mesenteric bed, as it is largely involved in the in vivo regulation of peripheral resistances.

METHODS

Experiments were carried out in Sprague-Dawley rats (250 to 350 g), maintained on regular food intake and with free access to drinking water. On the day of the experiment, rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg). After abdominal laparotomy, the aorta was ligated proximally to the superior mesenteric artery. A catheter (outer diameter 0.75 mm) was inserted into the mesenteric artery and tied in place. Then, the whole mesenteric bed was isolated by carefully cutting its most distal branches from the intestinal border. The isolated mesenteric bed was transferred on a warmed chamber (37°C). A T-shaped connector was attached to the catheter previously inserted into the mesenteric preparation. A line conveying physiologic salt solution (PSS) was plugged in one of two extremities of connector to perfuse the mesenteric bed. PSS was gassed with a mixture of 95% O2 and 5% CO2 and warmed (37°C). PSS composition was (in mmol/L): NaCl 120, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25.0, EDTA 0.026, and glucose 5.5. The scheduled flow rates perfusing mesenteric preparation were: 3.5 (this is the usual value of in vivo flow rate in rat mesenteric bed), 4.5, 5.5, 6.5 mL/min; the changes of flow rate were obtained by increasing the rotation of a peristaltic pump (Gilson Minipuls, Glasgow, United Kingdom). Each period of perfusion lasted 20 min. Pressure was measured through a transducer connected to the other extremity of the T-shaped connector; the pressure was simultaneously recorded on paper. The MVR were obtained by dividing the effective perfusion pressure by the corresponding flow rate value.

For each experimental set isolated mesenteric preparations were divided into two groups according to perfusion flow rate: constant flow rate (3.5 mL/min; CPF) and increased flow rate (4.5, 5.5, 6.5 mL/min; IPF).

In the first set of experiments MVR, VR, ET-1, and cGMP were measured in two groups of rats (15 rats each) to confirm the previous observation. Subsequently other sets of experiments were carried out as follows. To ascertain the role of ET-1 produced from endothelial cells, some mesenteric arteries (CPF n = 6; IPF n = 6) were perfused with phosphoramidon (10 μmol/L), which inhibits endothelin converting enzyme. To verify the role of other endothelium-derived vasoactive substances, in some mesenteric arteries (CPF n = 11; IPF n = 11) endothelium was removed with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS). The mesenteric preparations were perfused with a solution containing the detergent (0.3%) for 2 min; then the perfusion continued with PSS for 30 min. This procedure immediately causes the complete loss of endothelium; nevertheless, in each mesenteric artery the complete removal of endothelium was ascertained by the absence of acetylcholine-dependent vasodilation (Ach; 1 μmol/L) of the mesenteric bed preconstricted with NE. To evaluate the role of ET-1 produced by VSMC, the peptide was measured in homogenates of disendothelized arteries (CPF n = 4; IPF n = 4), whereas in other mesenteric preparations, BQ-610 (1 μmol/L), an ETA receptor antagonist, was singly (CPF n = 6; IPF n = 6) or concurrently (CPF n = 6; IPF n = 6) administered to BQ-788 (1 μmol/L), an ETB receptor antagonist, in disendothelized arteries. To evaluate VR all mesenteric preparations were challenged with NE (5 μmol/L) and KCl (80 mmol/L). These agents were chosen because NE acts through specific receptors, whereas the vasoconstriction of KCl is due to direct membrane depolarization. The vasoconstrictors were randomly administered; the second was added when the artery had completely recovered its basal tone after the previous vasoconstrictor.

Analysis of variance for repeated measurements was applied for intragroup comparisons; Newmann–Keuls was used as a post-hoc test. Student’s t test was also performed to assess intergroup differences. The level of statistical significance was defined as a two-tailed P value of .05.

RESULTS

The release of cGMP and ET-1 is shown in Figure 1. In CPF arteries, cGMP release (top) was constant throughout the study, whereas in IPF arteries cGMP excretion progressively increased and was always higher (P < .05) than that of CPF arteries. ET-1 release (lower) remained unchanged in CPF arteries, and it slowly increased in the presence of increasing perfusion flow, with the value measured at a rate of 6.5 mL/min being different (P < .05) from baseline as well as from CPF value.

In IPF arteries MVR (measured at 6.5 mL/min flow rate) decreased (P < .05) to a value of 6.6 ± 0.6 mm
Hg/mL/min, whereas in CPF arteries MVR was 9.8 ± 1.2 mm Hg/mL/min. VR to NE and KCl was markedly higher (P < .005) in IPF compared to CPF (Figure 2).

No changes of ET-1 release were observed in CPF arteries pretreated with phosphoramidon. ET-1 in the perfusate of IPF arteries before phosphoramidon administration was 6.1 ± 0.9, 5.9 ± 1.4, 6.5 ± 1.0, 10.1 ± 2.7 pg/min at flow rate of 3.5, 4.5, 5.5, 6.5 mL/min). The concentration of ET-1 in artery homogenate was strikingly greater in IPF than in CPF (0.32 ± 0.14 and 1.92 ± 0.08 pg/g of tissue; P < .005).

VR in the absence and presence of the ET-1 receptor antagonists is shown in Figure 4. The flow-dependent enhancement of VR in IPF arteries was completely abolished by infusing BQ-610 and no further additive effect was achieved with BQ-788.

**DISCUSSION**

The data of this study show that a critical association exists between flow and release of vasoactive substances. In fact, high flows decrease MVR on one hand and increase VR to agonists on the other. A flow-dependent vasodilation has been already observed in many experimental models and it has been linked to increased release of NO.3–8

The increased VR observed in the present study is likely due to ET-1 produced by VSMC. This hypothe-
sis is suggested by the following data. In intact arteries ET-1 release was abolished by phosphoramidon, but VR remained unchanged. It should be taken in account that phosphoramidon is a nonspecific metalloproteinase inhibitor and, therefore, it may influence other endothelial vasoactive system-regulating vascular tone in addition of endothelin converting enzyme. VR was, however, still increased after endothelium removal and this strongly supports a critical role for VSMC rather than for endothelium. In addition, the release of ET-1 from IPF arteries was similar both in arteries with and without endothelium. Tissue content of ET-1 was greater in IPF than in CPF arteries. Finally, with infusion of BQ-610, VR of disendothelized IPF arteries reached a level similar to that observed in CPF arteries. An additive role of the ETB receptor could be excluded as the simultaneous block of both receptors did not further attenuate VR.

Taken together the data of this study show that in mesenteric beds high flow rate on one hand causes vasodilation and increases ET-1 production in VSMC on the other. The increased release of the peptide makes mesenteric vessels more responsive to vasoconstrictors.

It is possible to speculate that the flow-dependent ET-1 may cause, over the time, irreversible alterations in VSMC leading to a stable increase in systemic blood pressure.

REFERENCES


