Modulation by 20-HETE of Phenylephrine-Induced Mesenteric Artery Contraction in Spontaneously Hypertensive and Wistar-Kyoto Rats

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Abstract—Small mesenteric arteries of spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY) were compared for the production of 20-HETE and the effects of 20-HETE and N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS, 30 μmol/L), a 20-HETE synthesis inhibitor, on contractile responsiveness to phenylephrine (0.1 to 50.0 μmol/L). 20-HETE production was higher in vessels of SHR compared with WKY (1.34±0.16 versus 0.27±0.09 pmol/mg tissue, P<0.05). Phenylephrine elicited concentration-dependent vascular contraction; the R max was similar in vessels of SHR and WKY, but the former were more sensitive as denoted by the lower EC 50 (1.10±0.14 versus 1.89±0.33 μmol/L, P<0.05). DDMS caused a rightward shift in the concentration-response curve to phenylephrine, increasing (P<0.05) the EC 50 by 258% and 134% in vessels of SHR and WKY, respectively. In contrast, in DDMS-treated vessels, 20-HETE (0.01 to 10.0 μmol/L) caused a leftward shift in the phenylephrine concentration-response curve, decreasing (P<0.05) the EC50 without affecting the R max. Importantly, the minimal concentration of 20-HETE that decreased the EC 50 of phenylephrine was much smaller in vessels of SHR that of WKY (0.01 versus 1.0 μmol/L). We conclude that 20-HETE increases the sensitivity of mesenteric arterial vessels to phenylephrine, vessels of SHR are more sensitive to this action of the eicosanoid than vessels of WKY, and vessels of SHR produce more 20-HETE than do vessels of WKY. Hence, 20-HETE of vascular origin may be a determinant of the increased reactivity to constrictor agonists in the vasculature of SHR. (Hypertension. 2001;38:1311-1315.)

Key Words: eicosanoids ▪ vascular reactivity ▪ 20-HETE ▪ vasoconstriction

R at arterial vessels express cytochrome P450 (CYP) 4A enzymes, which catalyze the ω-hydroxylation of arachidonic acid to 20-HETE.1,2 20-HETE constricts isolated vessels, arterioles, or small arteries that were previously pressurized and/or subjected to partial constriction with an agonist.3–6 Endogenous 20-HETE contributes to the mechanisms underlying myogenic constrictor responses in renal and cerebral arterial vessels,7,8 oxygen-induced constriction of cremaster arterioles,9–11 and the renal vasoconstriction brought about by endothelin12 and NO synthesis inhibition.13 The ability of 20-HETE to foster vasoconstrictor mechanisms was attributed to inhibition of large conductance calcium-activated potassium channels14 and to activation of L-type calcium channels15 in vascular smooth muscle.

CYP4A expression and activity are increased in kidneys of spontaneously hypertensive rats (SHR).16 In these animals, inhibitors of 20-HETE production lower blood pressure,17 dilate renal arterial vessels,18 and decrease vasoconstrictor responsiveness to angiotensin II in the mesenteric vasculature.19 Recently, treatment of SHR with CYP4A1 antisense oligonucleotides was found to lower blood pressure and to reduce the reactivity of small mesenteric arteries to phenylephrine.20 These observations suggest that CYP4A-derived products contribute to the mechanism of hypertension in SHR, perhaps by amplifying the vasoconstrictor responsiveness to neurohormonal pressor systems.

The present study was undertaken to test the hypothesis that 20-HETE of vascular origin sensitizes vascular smooth muscle to constrictor agonists and that this sensitizing mechanism is expressed more prominently in the vasculature of SHR compared with Wistar-Kyoto rats (WKY).

Methods

Protocols using 12-week old SHR and WKY (Harlan, Indianapolis, Ind) were approved by the Institutional Animal Care and Use Committee. Rats were anesthetized with sodium pentobarbital (60 mg/kg IP), arterial pressure was measured, and second- or third-order branches of the superior mesenteric artery were obtained for assessment of 20-HETE production and vascular contractility studies.

Measurement of Vascular 20-HETE

Mesenteric arteries (40 mg wet tissue) were placed inside vials containing 1 mL of Krebs’ buffer complemented with NADPH...
(1 mmol/L) and indomethacin (10 μmol/L). The samples were incubated for 1 hour at 37°C in an atmosphere of 95% O2/5% CO2. At the end of the incubation period, both media and vessels were extracted with ethyl acetate, and 20-HETE present in the extract was purified and quantitated by negative chemical ionization gas chromatography–mass spectrometry.20

Vascular Contractility Studies

Vessels were cut into ring segments 2 mm in length and mounted on 25-μm stainless steel wires in the chambers of a myograph (J.P. Trading) for measurement of isometric tension21; they were bathed in Krebs’ buffer (37°C) gassed with 95% O2/5% CO2, unless indicated otherwise. The internal diameter for mesenteric arteries of WKY and SHR was 216±13 μm and 202±10 μm, respectively. Isometric tension is expressed as millinewtons per millimeter of vessel length (mN/mm).

The following protocols were implemented after 30 minutes of equilibration. Protocol 1 compared vessels of SHR and WKY in terms of responsiveness to phenylephrine in the presence and absence of N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS, 30 μmol/L), an inhibitor of 20-HETE synthesis;22 concentration-response curves were constructed by cumulatively increasing the concentration of phenylephrine (0.1 to 50.0 μmol/L) every 2 minutes and recording the resulting changes in tension at the end of the 2-minute period. Protocol 2 contrasted the effect of 20-HETE (0.01 to 10.0 μmol/L) on responsiveness to phenylephrine (0.1 to 50.0 μmol/L) in vessels of SHR and WKY exposed to DDMS (30 μmol/L). Protocol 3 examined the effect of 20-HETE (0.001 to 1.0 μmol/L) on isometric tension in vessels precontracted with phenylephrine (1 μmol/L) and then exposed to DDMS (30 μmol/L), as well as in vessels without preconstriction but exposure to DDMS. Protocol 4 examined the effect of vasopressin (0.01 to 500 nmol/L) on isometric tension in vessels of SHR and WKY, both in the absence and presence of DDMS (30 μmol/L) alone and with 20-HETE (1.0 μmol/L).

Data Analysis

Data are expressed as mean±SEM. Concentration-response data were fitted to a logistic function by nonlinear regression, and the maximum asymptote of the curve (maximal response, Rmax) and EC50 were calculated. Concentration-response data were analyzed by a 2-way ANOVA followed by the Tukey test; other data were analyzed by a 1-way ANOVA or Student’s t test.

Results

Mean arterial pressure was 156±5 and 101±2 mm Hg in SHR and WKY (P<0.05), respectively. Estimates of 20-HETE in mesenteric arteries of SHR (1.34±0.16 pmol/mg tissue, n=4) surpassed (P<0.05) corresponding values in WKY (0.27±0.09 pmol/mg tissue, n=4).

Figure 1 illustrates the effect of the CYP4A inhibitor DDMS (30 μmol/L) on phenylephrine-induced contraction of mesenteric artery bathed in Krebs’ buffer gassed with 95% O2/5% CO2. In preparations not exposed to DDMS, the Rmax for phenylephrine was similar in SHR and WKY (1.83±0.25 versus 1.89±0.33 μmol/L). However, in preparations exposed to DDMS, the EC50 for phenylephrine in vessels of SHR did not differ from that in vessels of WKY (3.94±0.71 versus 4.43±0.81 μmol/L). DDMS caused a rightward displacement in the concentration-response curve to phenylephrine in vessels of both SHR and WKY. This agent did not affect the Rmax for phenylephrine but caused the EC50 to increase (P<0.05) by 258% and 134% in vessels of SHR and WKY, respectively.

Comparable results were obtained in preparations bathed in Krebs’ buffer gassed with 95% air/5% CO2, an experimental setting reported to be less than optimal for 20-HETE synthesis.10 DDMS increased (P<0.05) the EC50 for phenylephrine in mesenteric artery rings of both WKY (1.78±0.22 versus 4.03±0.43 μmol/L, n=4) and SHR (0.97±0.23 versus 5.42±0.19 μmol/L, n=4) without affecting the Rmax (WKY, 5.12±0.18 versus 5.15±0.24 mN/mm; SHR, 4.31±0.25 versus 4.67±0.26 mN/mm). DDMS also increased (P<0.05) the EC50 for phenylephrine in vascular rings bathed in Krebs’ buffer containing indomethacin (10 μmol/L) to inhibit cyclooxygenase (WKY: 1.75±0.34 versus 3.90±0.78 μmol/L, n=6; SHR: 1.83±0.25 versus 3.51±0.35 μmol/L, n=4) without altering the Rmax (WKY, 5.02±0.71 versus 5.14±0.79 mN/mm; SHR, 4.47±0.53 versus 4.44±0.74 mN/mm).

Figure 1. Concentration-response curves to phenylephrine in mesenteric artery rings of WKY (A) and SHR (B). Experiments were conducted in vessels bathed in buffer with (○) or without (□) DDMS. Results are the mean±SEM, n=number of experiments. *P<0.05 vs vessels not exposed to DDMS.

Shown in Figure 2A and 2B, in preparations exposed to DDMS (30 μmol/L), 20-HETE added to the buffer caused a leftward shift in the concentration-response curve to phenylephrine, decreasing the EC50 without altering the Rmax. Interestingly, the minimal concentration of 20-HETE that modifies the concentration-response curve for phenylephrine was much smaller in vessels of SHR (0.01 μmol/L) compared with WKY (1.0 μmol/L). As illustrated in Figure 2C, vessels of SHR are more sensitive than vessels of WKY to 20-HETE–induced reduction in the EC50 for phenylephrine.

Figure 3A shows data obtained in SHR and WKY on the concentration of 20-HETE in mesenteric arteries of both SHR and WKY (0.14 versus 1.89 pmol/mg tissue, n=6), respectively. Estimates of 20-HETE in mesenteric arteries of WKY (1.78±0.22 versus 4.03±0.43 μmol/L, n=4) and SHR (0.97±0.23 versus 5.42±0.19 μmol/L, n=4) without affecting the Rmax (WKY, 5.12±0.18 versus 5.15±0.24 mN/mm; SHR, 4.31±0.25 versus 4.67±0.26 mN/mm). DDMS also increased (P<0.05) the EC50 for phenylephrine in vascular rings bathed in Krebs’ buffer containing indomethacin (10 μmol/L) to inhibit cyclooxygenase (WKY: 1.75±0.34 versus 3.90±0.78 μmol/L, n=6; SHR: 1.83±0.25 versus 3.51±0.35 μmol/L, n=4) without altering the Rmax (WKY, 5.02±0.71 versus 5.14±0.79 mN/mm; SHR, 4.47±0.53 versus 4.44±0.74 mN/mm).

Figure 3B shows data obtained in SHR and WKY on the concentration of 20-HETE in mesenteric arteries of both SHR and WKY (0.14 versus 1.89 pmol/mg tissue, n=6), respectively. Estimates of 20-HETE in mesenteric arteries of WKY (1.78±0.22 versus 4.03±0.43 μmol/L, n=4) and SHR (0.97±0.23 versus 5.42±0.19 μmol/L, n=4) without affecting the Rmax (WKY, 5.12±0.18 versus 5.15±0.24 mN/mm; SHR, 4.31±0.25 versus 4.67±0.26 mN/mm). DDMS also increased (P<0.05) the EC50 for phenylephrine in vascular rings bathed in Krebs’ buffer containing indomethacin (10 μmol/L) to inhibit cyclooxygenase (WKY: 1.75±0.34 versus 3.90±0.78 μmol/L, n=6; SHR: 1.83±0.25 versus 3.51±0.35 μmol/L, n=4) without altering the Rmax (WKY, 5.02±0.71 versus 5.14±0.79 mN/mm; SHR, 4.47±0.53 versus 4.44±0.74 mN/mm).
duced contraction in vessels of SHR and WKY. In this experimental setting, cumulative addition of authentic 20-HETE to the buffer resulted in concentration-dependent development of isometric tension that was more intense ($P < 0.05$) in vessels of SHR compared with WKY. In contrast, as depicted in Figure 3B, 20-HETE did not stimulate tension development in preparations exposed to DDMS (30 μmol/L) but not to phenylephrine. Also, 20-HETE did not stimulate contraction of SHR vessels (n=4) treated with DDMS (30 μmol/L) and exposed to 30 mmol/L KCl. KCl increased isometric tension from 0.41±0.09 to 1.05±0.30 mN/mm ($P < 0.05$); when 20-HETE was added to the buffer to establish concentrations of 0.001, 0.01, 0.1, and 1.0 μmol/L, respectively, isometric tension was 1.04±0.29, 1.05±0.29, 1.05±0.27, and 1.15±0.31 mN/mm.

Data illustrating the effect of DDMS (30 μmol/L) on vasopressin-induced contraction of mesenteric artery rings are shown in Figure 4. In preparations not treated with DDMS, the EC$_{50}$ for vasopressin-induced contraction of vascular rings taken from SHR (●) and WKY (○), in the absence and the presence of 20-HETE. Results are the mean±SEM, n=number of experiments. *$P<0.05$ vs absence of 20-HETE.

![Figure 2](image2.png)

**Figure 2.** Effect of 20-HETE on phenylephrine-induced contraction of mesenteric artery rings taken from WKY (A) and SHR (B). The experiments were conducted in vessels bathed in buffer containing DDMS. C. The EC$_{50}$ for phenylephrine-induced contraction of vascular rings taken from SHR (●) and WKY (○), in the absence and the presence of 20-HETE. Results are the mean±SEM, n=number of experiments. *$P<0.05$ vs absence of 20-HETE.

![Figure 3](image3.png)

**Figure 3.** Effect of 20-HETE on isometric tension development in mesenteric artery rings precontracted with phenylephrine and subsequently exposed to DDMS (A) and in rings exposed to DDMS but not to phenylephrine (B). The experiments were conducted in vessels taken form SHR (●) and WKY (○). Results are the mean±SEM, n=number of experiments. *$P<0.05$ vs vessels exposed to phenylephrine and DDMS but not to 20-HETE. †$P<0.05$ vs SHR.

**Discussion**

This study demonstrates that DDMS decreases the sensitivity of small mesenteric arteries from SHR and WKY to constrictor agonists. In support of this conclusion, we found that ex vivo treatment of mesenteric arterial vessels with DDMS causes a rightward displacement in the concentration-response curve to both phenylephrine and vasopressin, increasing the EC$_{50}$ for the agonists without altering the maximal response. As DDMS inhibits CYP4A enzymes, it is reasonable to attribute the reduction in vascular sensitivity to phenylephrine and vasopressin after treatment with this agent to diminished formation of a CYP4A product that sensitizes the vessels to the constrictor agonists. This interpretation is consistent with a report that the sensitivity of small mesenteric arteries to phenylephrine is decreased in SHR treated with antisense oligonucleotides, which attenuate the vascular expression of CYP4A1.

CYP4A enzymes catalyze the ω-hydroxylation of arachidonic acid to 20-HETE. Mesenteric arterial vessels express CYP4A enzymes and manufacture 20-HETE. We found, in vessels of SHR and WKY treated with DDMS ex vivo, that authentic 20-HETE causes a leftward displacement of the concentration-response to vasopressin, decreasing the EC$_{50}$ without altering the R$_{max}$. 

This study demonstrates that DDMS decreases the sensitivity of small mesenteric arteries from SHR and WKY to constrictor agonists. In support of this conclusion, we found that ex vivo treatment of mesenteric arterial vessels with DDMS causes a rightward displacement in the concentration-response curve to both phenylephrine and vasopressin, increasing the EC$_{50}$ for the agonists without altering the maximal response. As DDMS inhibits CYP4A enzymes, it is reasonable to attribute the reduction in vascular sensitivity to phenylephrine and vasopressin after treatment with this agent to diminished formation of a CYP4A product that sensitizes the vessels to the constrictor agonists. This interpretation is consistent with a report that the sensitivity of small mesenteric arteries to phenylephrine is decreased in SHR treated with antisense oligonucleotides, which attenuate the vascular expression of CYP4A1.
Concentration of the contractile agonists without alteration of the maximal response. These findings indicate that 20-HETE enhances the sensitivity of mesenteric arterial vessels to these constrictor agonists. Therefore, desensitization to phenylephrine- and vasopressin-induced vascular contraction after treatment with DDMS is attributable to diminished vascular synthesis of 20-HETE, which appears to serve as a facilitatory modulator of vasoconstrictor responsiveness rather than as a directly acting constricting mediator.

In our study, the minimal concentration of 20-HETE that reduces the EC\textsubscript{50} of phenylephrine-induced contraction of SHR vessels was much smaller than that needed to achieve a comparable response in vessels of WKY. The mechanism underlying the greater sensitivity of SHR vessels to 20-HETE is not known. The increased sensitivity may reflect a primary increase in the reactivity of SHR vessels to 20-HETE, or augmented access of the eicosanoid to its cellular site of action. One possible site of action is the high conductance calcium-activated potassium channel, which is overexpressed in arterial vessels of SHR.\textsuperscript{24} 20-HETE decreases the activity of such a channel in vascular smooth muscle,\textsuperscript{18} which promotes cell depolarization and thus may account for the sensitizing effect of this eicosanoid.

That DDMS reduces isometric tension in mesenteric arteries contracted with 1 \textmu{}mol/L phenylephrine, a submaximal concentration, suggests that a product of vascular CYP4A activity promotes the expression of phenylephrine-induced vascular tone. Along this line of thinking, we found that exogenous 20-HETE elicits isometric tension development in vessels contracted with phenylephrine and then treated with DDMS, but not in DDMS-treated vessels without phenylephrine-induced contraction or in DDMS-treated vessels with KCl-induced constrictor tone. It appears then that the vascular contraction induced by 20-HETE in the former experimental setting is linked to amplification of the phenylephrine-induced tone rather than to a direct contractile action of the eicosanoid. As expected, the contractile response to 20-HETE in vessels preconditioned by exposure to phenylephrine and DDMS was more intense in SHR than in WKY.

Confirming previous reports,\textsuperscript{25,26} we found that mesenteric arteries of SHR are more sensitive to phenylephrine and vasopressin than are the mesenteric arteries of WKY. Importantly, after ex vivo treatment with DDMS, the sensitivity of mesenteric arteries to the constrictor agonists became comparable in SHR and WKY. It is plausible that the greater sensitivity of mesenteric arterial vessels of SHR to phenylephrine, vasopressin, and perhaps angiotensin II\textsuperscript{19,26} reflects the sensitizing influence of vascular 20-HETE on vascular smooth muscle responsiveness to the constrictor agonists. In this regard, mesenteric arterial vessels of SHR not only display a greater responsiveness to 20-HETE than do the vessels of WKY but also, according to the present study, produce more 20-HETE.

In summary, this study demonstrates that the sensitivity of small mesenteric arteries to phenylephrine and vasopressin is increased by 20-HETE and decreased by DDMS. Mesenteric arterial vessels from SHR manufacture 20-HETE, more so than corresponding vessels of WKY. Vessels of SHR are more responsive to the sensitizing action of 20-HETE on phenylephrine-induced vascular contraction. Hence, 20-HETE of vascular origin appears to be a key component of a mechanism regulating vascular reactivity to neurohormonal pressor systems. Such a mechanism is overexpressed in SHR and may contribute to increase blood pressure in this model of hypertension.

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References


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