

Vascular CO Counterbalances the Sensitizing Influence of 20-HETE on Agonist-Induced Vasoconstriction

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Abstract—We examined the influence of interactions between CO and 20-hydroxyeicosatetraenoic acid (20-HETE) on vascular reactivity to phenylephrine and vasopressin. Renal interlobar arteries incubated in Krebs buffer released CO at a rate that is decreased (from 125.0 ± 15.2 to 46.3 ± 8.8 pmol/mg protein per hour, $P < 0.05$) by the heme oxygenase inhibitor chromium mesoporphyrin (CrMP; $30 \mu\text{mol/L}$). The level of 20-HETE in vessels was not affected by CrMP (74.3 ± 6.1 versus 72.5 ± 16.2 pmol/mg protein), but was decreased ($P < 0.05$) by CO ($1 \mu\text{mol/L}$; 33.2 ± 7.9 pmol/mg protein) or the cytochrome P450-4A inhibitor *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS; $30 \mu\text{mol/L}$; 11.4 ± 3.3 pmol/mg protein). Phenylephrine elicited development of isometric tension in vascular rings mounted on a wire-myograph (EC_{50} , $0.29 \pm 0.02 \mu\text{mol/L}$; R_{max} , 3.78 ± 0.19 mN/mm). The sensitivity to phenylephrine was decreased ($P < 0.05$) by CO ($1 \mu\text{mol/L}$; EC_{50} , $0.60 \pm 0.04 \mu\text{mol/L}$) or DDMS (EC_{50} , $0.71 \pm 0.12 \mu\text{mol/L}$) and increased ($P < 0.05$) by 20-HETE ($10 \mu\text{mol/L}$; EC_{50} , $0.08 \pm 0.02 \mu\text{mol/L}$) or CrMP (EC_{50} , $0.11 \pm 0.02 \mu\text{mol/L}$). Notably, neither CO nor CrMP changed the sensitivity to phenylephrine in vessels treated with DDMS. Refractoriness to CO and CrMP in such a setting was eliminated by inclusion of 20-HETE ($1 \mu\text{mol/L}$) in the bathing buffer. The aforementioned interventions affected the vascular reactivity to vasopressin in a similar manner. These data indicate that the reactivity of renal arteries to phenylephrine and vasopressin is reciprocally influenced by CO and 20-HETE of vascular origin and that CO desensitizes the vascular smooth muscle to constrictor agonists by interfering with the sensitizing influence of 20-HETE. (*Hypertension*. 2004;44:210-216.)

Key Words: adrenergic receptor agonists ■ vasopressins ■ potassium channels ■ renal circulation

Recent studies indicate that CO and 20-hydroxyeicosatetraenoic acid (20-HETE) of vascular origin influence the reactivity of rat arterial vessels to constrictor agonists in divergent directions.^{1,2} CO, a product of heme metabolism by heme oxygenase (HO) isoforms -1 and -2,³ reduces the sensitivity of vascular smooth muscle to phenylephrine and vasopressin.¹ In contrast, 20-HETE, a product of arachidonic acid ω -hydroxylation by isoforms of the cytochrome P450 4A family (CYP4A),^{4,5} increases the sensitivity of vascular smooth muscle to phenylephrine² and vasopressin.⁴ The reciprocal modulatory actions of CO and 20-HETE on vascular reactivity to phenylephrine are linked, respectively, to stimulation and inhibition of large conductance calcium-activated potassium (K_{Ca}) channels in vascular smooth muscle.^{1,5-8}

In view of the aforementioned observations, it is conceivable that the sensitivity of arterial vessels to constrictor agonists is determined, at least in part, by the interplay between CO and 20-HETE manufactured by the vessels. One possibility is that the modulatory interplay simply results from the balance between regulatory substances with oppo-

site actions on vascular reactivity. Another possibility is that the inhibitory action of CO on vasomotor responsiveness to phenylephrine relies on interference with the vascular production of 20-HETE. The latter possibility is supported by reports that CO inhibits many cytochrome P450 enzymes, including the CYP4A isoforms that catalyze 20-HETE synthesis.^{5,9}

This study was undertaken to test the hypothesis that an interplay between CO and 20-HETE of vascular origin influences the reactivity of renal vascular smooth muscle to phenylephrine and vasopressin. We examined whether vascular CO influences the production of vascular 20-HETE. We also examined whether the ability of CO and HO inhibitors to decrease and increase, respectively, the sensitivity of rat renal interlobar arteries to phenylephrine and vasopressin is influenced by the status of 20-HETE synthesis or the level of exogenous 20-HETE.

Methods

Chemicals and Solutions

20-HETE and the CYP4A inhibitor *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) were synthesized by J.R.

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Falck,¹⁰ and stock solutions were prepared using punctilious ethanol. The HO inhibitor chromium mesoporphyrin (CrMP) was purchased from Porphyrin Products and stock solutions were prepared in 50 mmol/L Na₂CO₃. CO was purchased from Tech Air and a saturated solution (1 mmol/L) was prepared using ice-cold Krebs buffer composed of (in mmol/L) 118.5 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25.0 NaHCO₃, and 11.1 dextrose. All other chemicals were obtained from Sigma.

Animals

Studies were conducted on male Sprague-Dawley rats (body weight 250 to 300 g; Charles-River, Wilmington, Mass) according to protocols approved by the Institutional Animal Care and Use Committee. Rats were anesthetized with pentobarbital sodium (60 mg/kg, IP), the kidneys were excised and sectioned sagittally, and the interlobar arteries were dissected out for immediate use in studies on production of 20-HETE and CO, contractile responsiveness to agonists, and assessment of K⁺ currents in vascular smooth muscle cells.

Assessment of 20-HETE Production

Renal interlobar artery specimens were transferred into glass vials (5 mL) containing 1 mL of Krebs buffer saturated with 95% O₂-5% CO₂ and complemented with NADPH (1 mmol/L), *N*^ω-nitro-L-arginine methyl ester (L-NAME; 1 mmol/L) and indomethacin (10 μmol/L). The vials were capped tightly with rubberized Teflon liners, and the samples were incubated at 37°C for 60 minutes in the absence and presence of DDMS (30 μmol/L), CrMP (30 μmol/L), or CO (1 μmol/L). At the end of the incubation period, both media and vessels were extracted with acidified ethyl acetate (pH 4.0) and the organic phase was evaporated to dryness. The 20-HETE present in the extract was purified by reverse-phase high-pressure liquid chromatography on a C₁₈ Beckman Ultrasphere column (4.6 mm × 24 cm; 5 μm particle size) using a linear gradient from acetonitrile:water:acetic acid (75:25:0.05) to acetonitrile (100%) over 20 minutes at a flow rate of 1 mL/min. Fractions containing 20-HETE were dried under N₂ and derivatized for quantification by negative chemical ionization gas chromatography-mass spectroscopy according to a published method.¹¹

Assessment of CO Production

Renal interlobar arteries were transferred into amber vials (2 mL) containing 1.0 mL of Krebs buffer saturated with 95% O₂-5% CO₂, the vials were capped tightly with rubberized Teflon liners, and the samples were incubated at 37°C for 60 minutes in the absence and presence of CrMP (30 μmol/L). Subsequently, internal standards made of isotopically labeled CO (¹³C¹⁶O and ¹³C¹⁸O) were injected into samples, and the CO content of the headspace gas was determined by gas chromatography-mass spectroscopy analysis as described.¹

Assessment of Agonist-Induced Vascular Contraction

Renal interlobar arteries 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 tension.^{1,2} The rings were bathed in Krebs buffer (37°C) containing L-NAME (1 mmol/L) and gassed with 95% O₂-5% CO₂, unless indicated otherwise. After a 30-minute equilibration interval, concentration-response curves to phenylephrine (10⁻⁹ to 5 × 10⁻⁵ mol/L) or vasopressin (10⁻¹¹ to 10⁻⁷ mol/L) were constructed in the absence and the presence of test agents by cumulatively increasing the concentration of agonist every 2 to 3 minutes and recording the resulting changes in tension at the end of the period. Isometric tension is expressed in millinewtons per millimeter of vessel length (mN/mm). The effect of exogenous CO (1 μmol/L) on constrictor responsiveness to agonists was studied in vascular preparations pretreated and not pretreated with DDMS (30 μmol/L), in the absence and the presence of exogenous 20-HETE (10 μmol/L). The effect of exogenous CO on responsiveness to phenylephrine also was

investigated in preparations pretreated with 20-HETE (10 μmol/L) and the K_{Ca} channel blocker tetraethylammonium (TEA; 1 mmol/L). The effect of the HO inhibitor CrMP (30 μmol/L) on constrictor responsiveness to phenylephrine and vasopressin was examined in preparations pretreated and not pretreated with DDMS (30 μmol/L), or with DDMS and exogenous 20-HETE (1 μmol/L) in combination.

Recording of K⁺ Channel Currents

K⁺ channel currents were studied in smooth muscle cells isolated from rat renal interlobar arteries.¹ The experiments were conducted within 4 hours of completing isolation of the cells, using the patch-clamp technique in the cell-attached configuration as previously described.¹ Open-state probability (NP_o) was calculated from data sampled over a 30- to 60-second interval in the steady state during control and experimental periods.¹ Recordings were conducted during the control period and during sequential exposure of the vascular smooth muscle cells to DDMS (30 μmol/L) only, DDMS plus 20-HETE (1 μmol/L), and DDMS plus 20-HETE and CO (10 μmol/L) in combination. As previously reported,¹ a 105-pS K channel was identified in cell-attached patches of renal interlobar arteries. The activity of this channel is inhibited by TEA (0.1 mmol/L) and stimulated by exposure to Ca²⁺.¹

Data Analysis

Data are expressed as means ± SEM. Concentration-response data were fitted to a logistic function by nonlinear regression, and the maximum asymptote of the curves (maximal response, R_{max}) and concentration of agonist producing 50% of the maximal response (EC₅₀) were calculated as described.¹ Concentration-response data were analyzed by 2-way ANOVA followed by a Duncan multiple range test. All other data were analyzed by a 1-way ANOVA or the Student *t* test. The null hypothesis was rejected at *P* < 0.05.

Results

Production of CO and 20-HETE by Renal Interlobar Artery

Renal interlobar arteries released CO into the headspace during incubation in Krebs buffer (125.0 ± 15.2 pmol/mg protein per hour; n = 6); the amount released decreased (*P* < 0.05) to ≈ 37% of control when the vessels were incubated in buffer containing the HO inhibitor CrMP (30 μmol/L, 46.3 ± 8.8 pmol/mg protein per hour; n = 6). The level of 20-HETE in renal interlobar arteries incubated for 60 minutes in Krebs buffer was 74.3 ± 6.1 pmol/mg protein (n = 7), decreasing (*P* < 0.05) to ≈ 15% of control when the vessels were incubated in buffer containing DDMS (30 μmol/L, 11.4 ± 3.3 pmol/mg protein; n = 6). The level of 20-HETE also was reduced (*P* < 0.05) in renal interlobar arteries incubated in buffer containing CO (1 μmol/L, 33.2 ± 7.9 pmol/mg protein; n = 6), but was unchanged in vessels incubated in buffer containing CrMP (30 μmol/L, 72.5 ± 16.2 pmol/mg protein; n = 8).

CO and 20-HETE as Determinants of Renal Vascular Reactivity to Phenylephrine

Phenylephrine elicited concentration-dependent augmentation of isometric tension in rings of rat renal interlobar arteries. Figure 1 illustrates the effects of CO and 20-HETE, alone and in combination, on contractile responsiveness to phenylephrine in arteries bathed in media without (Figure 1A) and with (Figure 1B) DDMS (30 μmol/L). The concentration-response curve to phenylephrine was shifted to the right in vessels exposed to DDMS only, resulting in augmentation of the phenylephrine EC₅₀ (from 0.29 ± 0.02 to

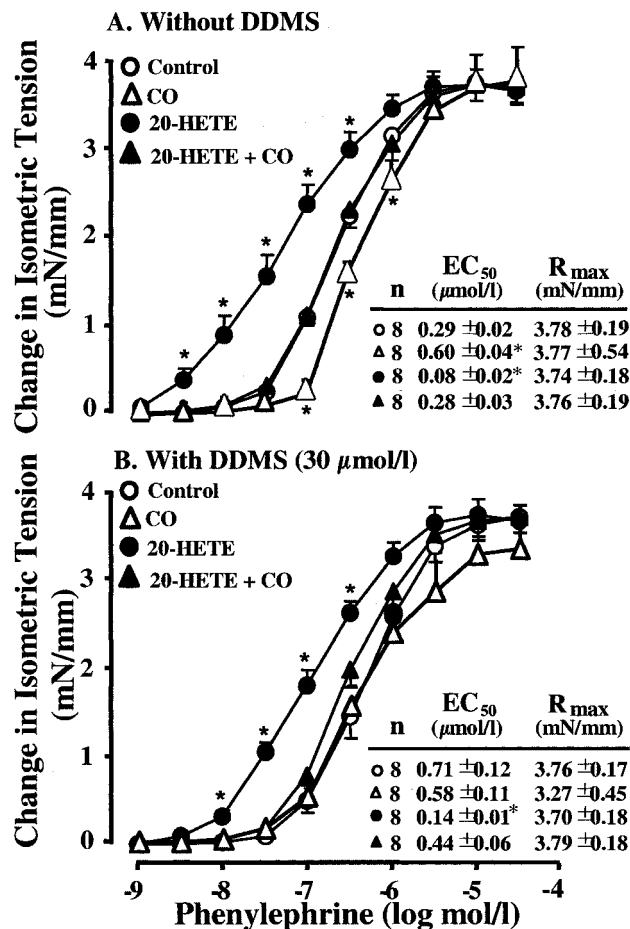


Figure 1. Concentration-response curves to phenylephrine in rat renal interlobar arteries bathed in buffer containing (B) and not containing (A) DDMS (30 μmol/L). The studies were conducted in the absence and presence of CO (1 μmol/L), 20-HETE (10 μmol/L), or CO and 20-HETE in combination. Results are mean ± SEM, n = number of experiments. **P* < 0.05 relative to control.

0.71 ± 0.12 μmol/L; *P* < 0.05) without alteration of R_{max} values (3.78 ± 0.19 versus 3.76 ± 0.17 nM/mm), which indicates desensitization to the agonist. Conversely, 20-HETE caused a leftward shift in the concentration-response curve to phenylephrine, implying vascular sensitization to the agonist, decreasing (*P* < 0.05) the EC₅₀, both in vessels bathed in media not containing (from 0.29 ± 0.02 to 0.08 ± 0.02 μmol/L) and containing (from 0.71 ± 0.12 to 0.14 ± 0.01 μmol/L) the CYP4A inhibitor DDMS. In vessels not exposed to DDMS, the inclusion of CO (1 μmol/L) into the bathing buffer elicited a rightward displacement in the concentration-response curve to phenylephrine, increasing (*P* < 0.05) the EC₅₀ (from 0.29 ± 0.02 to 0.60 ± 0.04 μmol/L). CO also caused desensitization to phenylephrine in vessels previously sensitized by exposure to 20-HETE, increasing (*P* < 0.05) the EC₅₀ from 0.08 ± 0.02 μmol/L in 20-HETE-exposed vessels to 0.28 ± 0.03 μmol/L in vessels exposed to both 20-HETE and CO. In contrast, CO did not increase the EC₅₀ for phenylephrine in vascular preparations exposed to DDMS only (0.71 ± 0.12 versus 0.58 ± 0.11 μmol/L), although it did so (*P* < 0.05) in preparations concurrently exposed to the

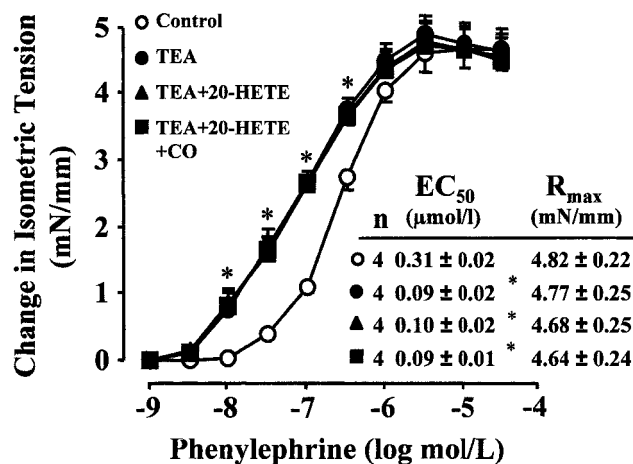


Figure 2. Concentration-response curves to phenylephrine in rat renal interlobar arteries bathed in buffer alone and buffer containing TEA (1 μmol/L), TEA plus 20-HETE (10 μmol/L), or a combination of TEA, 20-HETE, and CO (10 μmol/L). Results are mean ± SEM, n = number of experiments. **P* < 0.05 relative to control.

CYP4A inhibitor and 20-HETE (from 0.14 ± 0.01 to 0.44 ± 0.06 μmol/L). The R_{max} for phenylephrine was not affected by 20-HETE, CO, or both substances combined in either vessels exposed or not exposed to DDMS.

As shown in Figure 2, treatment of renal interlobar artery rings with TEA produced, like treatment with 20-HETE alone, a leftward displacement in the concentration-response curve to phenylephrine and a reduction of EC₅₀ values (*P* < 0.05) without altering the maximal response. The sensitization to phenylephrine in TEA-treated vessels was not enhanced further by concurrent treatment with 20-HETE. Moreover, exogenous CO did not decrease the sensitivity to phenylephrine in vessels pretreated with both TEA and 20-HETE.

Figure 3 (top graph) illustrates the effects of CrMP (30 μmol/L), alone and in combination with DDMS (30 μmol/L), on contractile responsiveness to phenylephrine. The HO inhibitor CrMP elicited a leftward shift in the concentration-response curve to phenylephrine in vascular preparations not exposed to the CYP4A inhibitor DDMS, decreasing (*P* < 0.05) the EC₅₀ from 0.31 ± 0.01 to 0.11 ± 0.02 μmol/L. In contrast, CrMP did not decrease the EC₅₀ for phenylephrine in preparations exposed to DDMS (0.49 ± 0.05 versus 0.52 ± 0.04 μmol/L). Yet, CrMP did decrease (*P* < 0.05) the EC₅₀ for phenylephrine in vessels concurrently exposed to DDMS and 20-HETE (1 μmol/L) (from 0.30 ± 0.04 to 0.13 ± 0.02 μmol/L). 20-HETE at 1 μmol/L was effective in offsetting the desensitizing effect of DDMS, decreasing (*P* < 0.05) the EC₅₀ for phenylephrine from 0.49 ± 0.05 μmol/L in vessels treated with DDMS only to 0.30 ± 0.04 μmol/L in vessels treated concurrently with DDMS and 20-HETE, which is not different from that value obtained in control untreated vessels (0.31 ± 0.01 μmol/L). The R_{max} for phenylephrine was not affected by any of the aforementioned experimental manipulations.

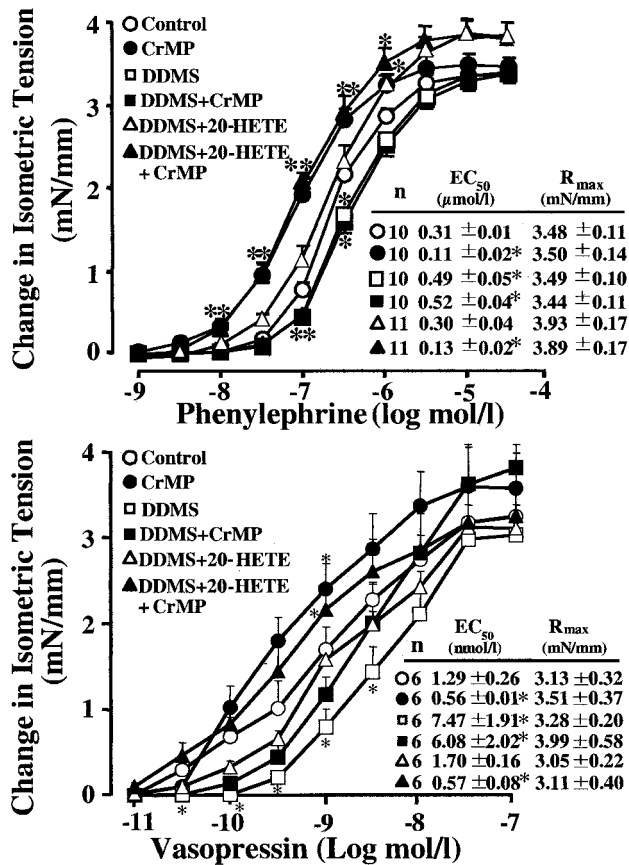


Figure 3. Concentration-response curves to phenylephrine (top) and vasopressin (bottom) in rat renal interlobar arteries bathed in buffer alone and buffer containing either DDMS (30 μmol/L), CrMP (30 μmol/L), DDMS plus CrMP, DDMS plus 20-HETE (1 μmol/L), or DDMS plus 20-HETE plus CrMP. Results are mean ± SEM, n = number of experiments. **P* < 0.05 relative to control.

CO and 20-HETE as Determinants of Renal Vasculature Reactivity to Vasopressin

Figure 4 shows data on the effects of CO (1 μmol/L) and 20-HETE (10 μmol/L), alone and in combination, on responsiveness to vasopressin in renal interlobar arteries bathed in media without (Figure 4A) and with (Figure 4B) DDMS (30 μmol/L). Vasopressin elicited concentration-dependent development of isometric tension in all the experimental groups. The *R*_{max} for the agonist was not affected by 20-HETE, CO, or both agents combined in vessels exposed or not exposed to DDMS. Vessels exposed to DDMS displayed increased (*P* < 0.05) EC₅₀ for vasopressin (2.00 ± 0.65 versus 6.18 ± 1.82 nmol/L), denoting desensitization to the agonist after CYP4A inhibition. Treatment with 20-HETE sensitized the vessels to vasopressin-induced contraction, decreasing (*P* < 0.05) the EC₅₀ for the agonist in preparations not exposed (from 2.00 ± 0.65 to 0.30 ± 0.09 nmol/L) and exposed (from 6.18 ± 1.82 to 1.70 ± 0.41 nmol/L) to DDMS. Treatment with CO of vessels not exposed to DDMS increased (*P* < 0.05) the EC₅₀ for vasopressin both in the absence (from 2.00 ± 0.65 to 9.54 ± 1.41 nmol/L) and the presence of exogenous 20-HETE (from 0.30 ± 0.09 to 3.41 ± 1.34 nmol/L). Of note, CO did not increase the EC₅₀ for vasopressin in preparations exposed to

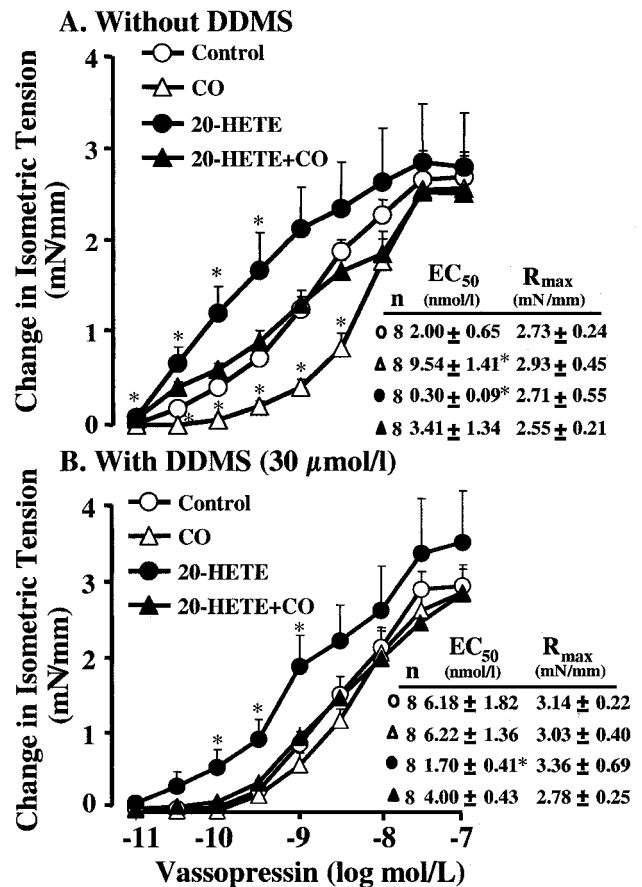


Figure 4. Concentration-response curves to vasopressin in rat renal interlobar arteries bathed in buffer containing (B) and not containing (A) DDMS (30 μmol/L). The studies were conducted in the absence and presence of CO (1 μmol/L), 20-HETE (10 μmol/L), or CO and 20-HETE in combination. Results are mean ± SEM, n = number of experiments. **P* < 0.05 relative to control.

DDMS only (from 6.18 ± 1.82 to 6.22 ± 1.36 nmol/L), but did so (*P* < 0.05) in preparations concurrently exposed to DDMS and exogenous 20-HETE (from 1.70 ± 0.41 to 4.00 ± 0.43 nmol/L).

As shown on Figure 3 (lower graph), renal interlobar arteries treated with CrMP (30 μmol/L) displayed increased sensitivity to vasopressin as denoted by the reduction (*P* < 0.05) in the EC₅₀ for the agonist (from 1.29 ± 0.26 to 0.56 ± 0.01 nmol/L). However, CrMP did not decrease the EC₅₀ for vasopressin in vascular preparations exposed to DDMS (30 μmol/L) (7.47 ± 1.91 versus 6.08 ± 2.02 nmol/L), unless the vessels were concurrently exposed to DDMS and 20-HETE (1 μmol/L) (from 1.70 ± 0.16 to 0.57 ± 0.08 nmol/L; *P* < 0.05).

Reciprocal Influence of 20-HETE and CO on K Channel Currents in Smooth Muscle Cells of Renal Interlobar Arteries

As shown in Figure 5, cell-attached patches display little or no K⁺ channel activity in smooth muscle cells bathed in control media (NP₀, 0.03 ± 0.02; n = 4). Addition of the CYP4A inhibitor DDMS (30 μmol/L) to the bath greatly

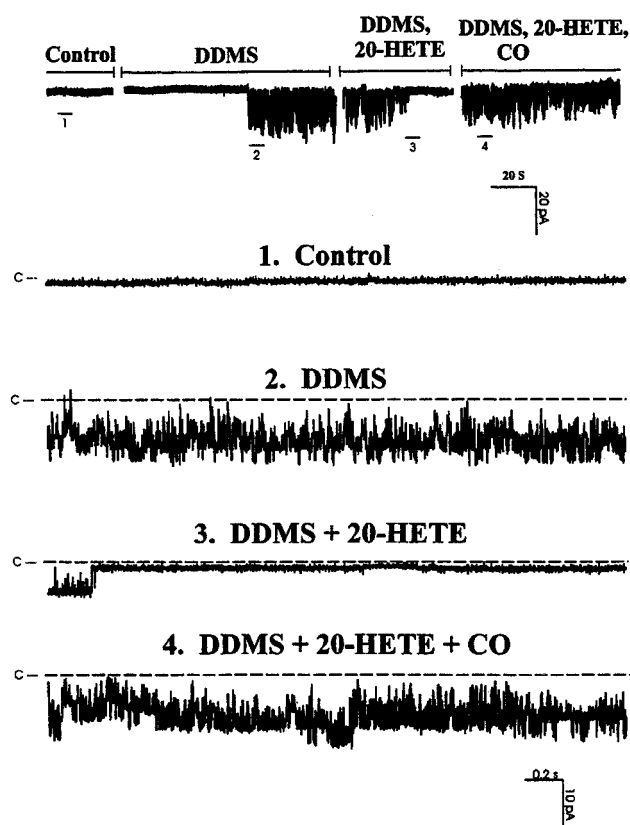


Figure 5. Channel recording showing the effect of DDMS (30 $\mu\text{mol/L}$) alone, DDMS plus 20-HETE (1 $\mu\text{mol/L}$), and DDMS plus 20-HETE plus CO (10 $\mu\text{mol/L}$) on the activity of K_{Ca} channels in a smooth muscle cell isolated from rat renal interlobar artery. The experiment was performed in a cell-attached patch bathed in media containing (in mmol/L) 140 NaCl, 5 HCl, 1.8 MgCl_2 , 1.8 CaCl_2 , and 10 HEPES (pH 7.4). The holding potential was 0.0 mV. The channel closed level is indicated by C. The top recording shows the time course of the experiment; the segments of the recording indicated by the numbers 1 to 4 are presented below at fast time resolution.

stimulated ($P < 0.05$) channel activity (NP_0 , 1.67 ± 0.03 ; $n = 4$). In the face of continuous exposure of the cells to DDMS, K^+ channel activity decreased (NP_0 , 0.30 ± 0.19 ; $n = 4$, $P < 0.05$) following the addition of exogenous 20-HETE to the media (1 $\mu\text{mol/L}$). In the face of continuous exposure to DDMS and 20-HETE, K^+ channel activity was increased (NP_0 , 1.20 ± 0.23 ; $n = 4$, $P < 0.05$) on the inclusion of exogenous CO (10 $\mu\text{mol/L}$) into the buffer. Hence, exogenous CO is effective in offsetting the inhibitory action of exogenous 20-HETE on K^+ channel activity.

Discussion

This study provides information on the influence of CO on vascular 20-HETE production and on whether vasoregulatory mechanisms involving CO and 20-HETE are interconnected. The salient conclusion derived from the study is that an interplay between CO and 20-HETE of vascular origin regulates constrictor responsiveness to phenylephrine and vasopressin in rat renal interlobar arteries.

Rat renal interlobar arteries incubated in Krebs buffer were found to manufacture CO and 20-HETE. Confirming a

previous report,¹ vascular CO production was inhibited by CrMP, which is suggestive of HO dependency. The level of 20-HETE in renal interlobar arteries was reduced by treatment with DDMS, implying that vascular 20-HETE production is dependent on the activity of 1 or more CYP4A oxygenases.^{2,5} CO is known to interfere with the activity of cytochrome P450 enzymes and therefore is expected to decrease 20-HETE synthesis.⁹ In keeping with this notion, we found that the level of 20-HETE falls in renal interlobar arteries incubated in buffer containing exogenous CO (1 $\mu\text{mol/L}$). However, 20-HETE levels were not increased in vessels treated with CrMP. The fact that inhibition of vascular HO with CrMP is without effect on vascular 20-HETE levels implies that basal levels of endogenous CO are inconsequential to 20-HETE synthesis. This may not be the case in pathophysiological states featuring increased expression or activity of HO isoforms leading to enhanced vascular production of CO. It is plausible that in such settings the vascular production of 20-HETE is subject to inhibitory regulation by CO of vascular origin.

Observations that the sensitivity of renal interlobar arteries to phenylephrine and vasopressin is reciprocally influenced by CO and 20-HETE are central to the notion that an interplay between these substances regulates the reactivity of renal vascular smooth muscle to constrictor agonists. In this regard, we confirmed previous reports that the sensitivity of small arteries to phenylephrine and vasopressin is decreased by exogenous CO¹ and increased by exogenous 20-HETE.^{2,4} The reactivity of the vessels to the constrictor agonists also is decreased and increased, respectively, by CO and 20-HETE manufactured by the renal vessels themselves because their sensitivity to phenylephrine and vasopressin was found to increase in response to HO inhibition with CrMP¹ and to decrease after CYP4A inhibition with DDMS.^{2,4} Notably, neither exogenous CO nor CrMP effected changes in responsiveness to the constrictor agonist in vascular preparations undergoing CYP4A inhibition with DDMS. Refractoriness to CO and the HO inhibitor in DDMS-treated vessels was eliminated by the inclusion of authentic 20-HETE in the bathing buffer. Hence, these findings suggest that the ability of CO to reduce the sensitivity of rat renal interlobar arteries to phenylephrine and vasopressin is linked to interference with 20-HETE-induced sensitization of the vessels to the constrictor agonists, rather than to inhibition of vascular production of 20-HETE.

K_{Ca} channels in vascular smooth muscle participate in the regulation of membrane potential and hence vascular tone and reactivity.¹² Previous studies established that the activity of K_{Ca} channels in small arterial vessels is reduced by exogenous 20-HETE^{5,8} and increased by exogenous CO.^{1,6,7} 20-HETE and CO manufactured by vascular smooth muscle cells also are believed to decrease and increase the activity of K_{Ca} channels, respectively, because the open state probability of the channel(s) was shown to increase⁸ and decrease^{1,7} during treatment with a CYP4A inhibitor and a HO inhibitor, respectively. According to the present study, in renal interlobar arteries smooth muscle cells undergoing inhibition of 20-HETE synthesis with DDMS, treatment with exogenous 20-HETE suppressed K^+ channel activity; this effect was

offset by concurrent exposure of the cells to exogenous CO. Such an interplay between 20-HETE and CO at the level of K_{Ca} channels in vascular smooth muscle may explain the reciprocal regulatory influence of these substances on vascular reactivity to constrictor agonists. In this respect, we found that blockade of K_{Ca} channels with TEA increases the sensitivity of renal interlobar arteries to phenylephrine, mimicking the sensitizing influence of 20-HETE. Sensitization to this constrictor agonist in TEA-treated vessels was not enhanced further by concurrent treatment with 20-HETE, nor was the degree of sensitization obtained under such condition diminished by the inclusion of exogenous CO into the bathing buffer. Hence, the ability of CO to counteract 20-HETE-induced sensitization of renal interlobar arteries to phenylephrine appears to rely on its capacity to offset the inhibitory action of the eicosanoid on K_{Ca} channel activity.

There is paucity of information on the molecular mechanisms underlying the interplay of 20-HETE and CO in relation to the regulation of K_{Ca} channel activity in vascular smooth muscle cells. Inhibition of K_{Ca} channel activity by 20-HETE was shown to rely on a mechanism involving protein kinase C (PKC) in cerebral vascular smooth muscle¹³ and tyrosine kinase in smooth muscle cells obtained from renal interlobular arteries.¹⁴ Stimulation of vascular smooth muscle K_{Ca} channel activity by CO was attributed to increased sensitivity of the channel to calcium,⁶ along with enhanced coupling of calcium sparks to K_{Ca} channels.¹⁵ CO-induced stimulation of vascular K_{Ca} channels does not rely on increased formation of cGMP.¹ No information is available on whether the stimulatory action of CO on K_{Ca} channels depends on interference with the PKC- or tyrosine kinase-mediated mechanism that reportedly is central to the inhibitory action of 20-HETE on K_{Ca} channel activity.^{13,14}

In summary, this study demonstrates that an interplay between CO and 20-HETE manufactured by rat renal interlobar arteries influences the reactivity of the vessels to the constrictor action of phenylephrine and vasopressin. We found that the ability of CO to reduce the vascular sensitivity to these agents is linked to interference with 20-HETE-induced sensitization of the vessels to agonist-induced vasoconstriction. We also found that the desensitizing action of CO relies on its capacity to stimulate K_{Ca} channel activity in vascular smooth muscle and, thus, offset the inhibitory action of 20-HETE on the K_{Ca} channel. Our study, suggests that the interaction between CO and 20-HETE of vascular origin influences the reactivity of the renal vasculature to constrictor stimuli and, thus, may contribute to the regulation of renal hemodynamic function.

Perspectives

The notion that the HO-CO and CYP4A-20-HETE systems are functionally coupled merits special consideration in view of evidence implicating CO and 20-HETE of renal origin in the regulation of renal circulatory functions. For example, intrarenal generation of an HO product, presumably CO, was shown to support blood flow to the kidney by counteracting constrictor mechanisms dependent on the sympathetic nervous and renin-angiotensin systems.¹⁶⁻¹⁸ On the other hand, intrarenal generation of 20-HETE was reported to promote

renal vasoconstriction via amplification of constrictor mechanisms involving myogenic and neurohormonal stimuli.¹⁹⁻²¹ If, as suggested in the present study, CO serves as a counterbalancing influence to the sensitizing action of 20-HETE on responsiveness to constrictor stimuli, conditions in which vascular CO generation is compromised may also feature reduction of renal blood flow because of amplification of 20-HETE-induced sensitization of the renal vasculature to prevailing constrictor mechanisms. Such may be the case of young spontaneously hypertensive rats, animals which were shown to display diminished vascular HO activity²² and greatly increased responsiveness to the sensitizing action of 20-HETE on phenylephrine-induced constriction of small arterial vessels.^{4,23} The HO-CO and CYP4A-20-HETE systems also may be functionally coupled in relation to their regulatory functions in extrarenal circulatory beds.

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Vascular CO Counterbalances the Sensitizing Influence of 20-HETE on Agonist-Induced Vasoconstriction

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