Contribution of endogenous carbon monoxide to regulation of diameter in resistance vessels

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Contribution of endogenous carbon monoxide to regulation of diameter in resistance vessels. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1087–R1094, 1999.—Endogenous carbon monoxide was proposed to subserve vasodepressor functions. If so, inhibition of heme oxygenase may be expected to promote vascular contraction. This hypothesis was examined in large and small arteries and in isolated first-order gracilis muscle arterioles of rat. The heme oxygenase inhibitors chromium mesoporphyrin (CrMP) and cobalt protoporphyrin (0.175–102 µmol/l) decreased the diameter of pressurized (80 mmHg) gracilis muscle arterioles, whereas magnesium protoporphyrin, a weak heme oxygenase inhibitor, did not. CrMP also elicited development of isometric tension in the muscular branch of the femoral artery but not in the aorta or femoral artery. Arteriolar constrictor responses to CrMP varied in relation to the intravascular pressure, were blunted in preparations exposed to exogenous carbon monoxide (100 µmol/l), and were unaffected by an endothelin receptor antagonist. Importantly, CrMP amplified the constrictor response to increases of pressure in gracilis arterioles. Accordingly, the constrictor effect of heme oxygenase inhibitors is attributable to magnification of myogenic tone due to withdrawal of a vasodilatory mechanism mediated by endogenous carbon monoxide. The study suggests that the vascular carbon monoxide system plays a role in the regulation of basal tone in resistance vessels.

HEM OXYGENASE CATALYZES the conversion of heme to biliverdin and carbon monoxide (20). Three heme oxygenase isoforms have been identified: heme oxygenase-1, heme oxygenase-2, and heme oxygenase-3 (20). Both vascular endothelium and smooth muscle express heme oxygenases (2, 5, 10, 35), and heme oxygenase-catalyzed formation of carbon monoxide has been documented in blood vessels (6, 12).

It is known that carbon monoxide stimulates soluble guanylyl cyclase (2, 17, 21), increases cGMP in vascular tissues (2, 9, 27), and relaxes vascular smooth muscle (4, 9, 13, 32). The vasodilatory effect of carbon monoxide was attributed to activation of smooth muscle relaxing mechanisms mediated by cGMP on the basis of reports that inhibitors of soluble guanylate cyclase attenuate carbon monoxide-induced vasodilation (9, 13). However, a primary role of cGMP in carbon monoxide-induced vasodilation has recently been challenged (4, 32). Moreover, there are reports that the vasodilatory effect of carbon monoxide may involve interaction with calcium-activated potassium channels in vascular smooth muscle (32), as well as increased production of vasoconstrictors such as endothelin (3, 22) or cytochrome P-450-derived products (3, 8).

Recent studies suggest that endogenous carbon monoxide arising from heme via metabolism by heme oxygenase participates in the regulation of circulatory functions (15, 16, 21). For example, a report that treatment of normotensive rats with heme oxygenase inhibitors causes elevation of peripheral vascular resistance and blood pressure suggests that endogenous carbon monoxide subserves a vasodepressor function (15). The same conclusion was derived from a report that treatment of hypertensive rats with heme oxygenase substrates reduces blood pressure via a heme oxygenase-dependent mechanism (16). Also, recent observations that heme elicits heme oxygenase-dependent vasodilation in isolated gracilis muscle arterioles (18) and tail arteries (32) suggested that carbon monoxide of vascular origin can be a mediator of vasodilatory mechanisms.

If endogenous carbon monoxide reduces vascular tone, treatment of arterial vessels with inhibitors of heme oxygenase should cause vasoconstriction. Therefore, the present study was undertaken to characterize the effects of inhibitors of heme oxygenase on isometric tension in isolated ring segments of large and small arteries as well as on the diameter of isolated, first-order gracilis muscle arterioles. Experiments were also conducted to verify the presence of heme oxygenase in arterial vessels.

MATERIALS AND METHODS

Chemicals

Metalloporphyrins were purchased from Porphyrin Products (Logan, UT), carbon monoxide from Tech Air (White Plains, NY), 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) from Calbiochem-Novabiochem (La Jolla, CA), and endothelin-1 (ET-1) from Peninsula Laboratories (Belmont, CA). (−)-N-(4-isopropylbenzenesulfonyl)-a-(4-carboxy-2-n-propylphenoxy)-3,4-methylenedioxyphenylacetaldehyde (L-754, 450) was a gift from Merck Research Laboratories (Rahway, NJ). All other drugs were purchased from Sigma (St. Louis, MO).

Cobalt protoporphyrin (CoPP) stock solution (38 mmol/l) was prepared (30) and stored at −40°C in opaque vials until use. Chromium mesoporphyrin (CrMP) and magnesium protoporphyrin (MgPP) stock solutions (15 mmol/l) were prepared in 50 mmol/l Na2CO3, ET-1 stock solution (10 µmol/l) was in distilled water. CoPP, CrMP, MgPP, and ET-1 were diluted immediately before use in saline and further diluted.
in Krebs buffer to achieve final concentrations. ODQ stock solution (10 mmol/l) was dissolved in DMSO, and further diluted in Krebs buffer. L-754,142 stock solution (100 mmol/l) was prepared in saline and further diluted in Krebs buffer. Carbon monoxide-saturated solution (1 mmol/l) was prepared by bubbling ice-cold Krebs buffer with carbon monoxide gas for 20 min. N\(^{\text{N}}\)-nitro-L-arginine methyl ester (L-NAME) was dissolved immediately before use in Krebs buffer. The composition of Krebs bicarbonate buffer was (in mmol/l) 118.5 NaCl, 4.7 KCl, 2.8 CaCl\(_2\), 1.2 KH\(_2\)PO\(_4\), 1.1 MgSO\(_4\), 25.0 NaHCO\(_3\), and 11.1 dextrose.

**Animals**

Male Sprague-Dawley rats (200–300 g; Charles River, Wilmington, DE) were used in these studies approved by the Institutional Animal Care and Use Committee. Rats had free access to commercial rat chow and tap water. On the days of the experiment, animals were anesthetized (pentobarbital sodium, 60 mg/kg ip) and injected with heparin (1,000 U/kg iv), followed immediately by removal of arterial vessels for assessment of heme oxygenase protein expression and studies on vascular contractility.

**Assessment of Heme Oxygenase in Arterial Vessels**

Analysis of heme oxygenase-1 and -2 by immunoblotting was performed in each of three pools of aortas (2 or 3 vessels per pool) and gracilis muscle arteries (20–40 vessels per pool). The vessels were isolated in ice-cold Krebs buffer, snap-frozen in liquid nitrogen, pooled and stored at −70°C until used. The samples were homogenized in ice-cold 20 mmol/l Tris·HCl buffer, pH 7.5, containing 0.5 mmol/l EDTA, 0.5 mmol/l EGTA, 25 µg/ml aprotinin, 25 µg/ml leupeptin, and 10 mmol/l mercaptoethanol. Homogenates were incubated on ice for 1 h and centrifuged at 10,000 g for 30 min. The supernatant was assayed for protein with a kit (Bio-Rad Laboratories, Hercules, CA) and saved for Western blot analysis of heme oxygenase-1 and -2 using polyclonal heme oxygenase-1 and heme oxygenase-2 antibodies (Stress Gen, Victoria, BC, Canada). This to endogenous proteins were subjected to electrophoresis on sodium dodecyl sulfate-12% polyacrylamide gels and then transferred to a nitrocellulose membrane.

**Measurement of Isometric Tension in Vascular Rings**

The aorta, femoral artery, and the muscular branch of the femoral artery of rats were placed on a dish containing ice-cold Krebs buffer, dissected free of surrounding connective tissue, and cut into ring segments ~2 mm in length. Vascular rings were mounted on wires in the chambers of a multivessel myograph (J. P. Trading, Aarhus, Denmark) filled with Krebs buffer (37°C) containing the nitric oxide synthase inhibitor L-NAME (1 mmol/l) to avoid potential interactions between the nitric oxide and carbon monoxide systems (1, 20). This concentration of L-NAME was previously shown to maximally inhibit purified nitric oxide synthase (26). The buffer was gassed with 95% O\(_2\)-5% CO\(_2\). After equilibration for 30–60 min, the vessels were set to an internal circumference equivalent to 90% of that they would have in vitro when relaxed under a transmural pressure of 100 mmHg for aorta and femoral artery preperations, and 80 mmHg for the muscular branch of the femoral artery (24). Isometric tension was monitored continuously before and after experimental interventions and is expressed as millinewtons per millimeter vessel length. Experiments were initiated after a 30- to 60-min stabilization interval.

**Measurement of Internal Diameter in Isolated Gracilis Muscle Arteries**

The gracilis anticus muscles were isolated, removed, and placed into a dish containing ice-cold Krebs buffer. The muscles were pinned to the Silastic bottom of the dish and allowed to equilibrate for 15 min. Segments of the first-order arterioles were isolated as previously described (29). Individual arteriolar segments (1–2 mm in length) were transferred to a water-jacketed vessel chamber (18 ml volume; Instrument Shop, New York Medical College, Valhalla, NY) containing Krebs buffer at room temperature and two glass micropipettes filled with Krebs’ buffer. After the vessel was mounted on the proximal micropipette and secured with a ligature, the lumen was flushed to remove residual blood and the other end of the arteriole was mounted on the distal micropipette (29).

Silicone tubing was used to connect the proximal micropipette to a pressure servocontroller (model CH/200/Q, Living Systems Instrumentation, Burlington, VT) and the distal pipette to a stopcock. Continuous nonrecirculating superfusion with Krebs’ buffer (5 ml/min), bubbled with 95% O\(_2\)-5% CO\(_2\), was then started while the lumen was perfused. After several minutes of perfusion, the distal stopcock was closed and the intraluminal pressure was allowed to increase to 80 mmHg, unless noted otherwise. The vessel length was adjusted to remove buckling, and the temperature of the superfusion buffer was increased to 37°C. The vessel chamber was mounted on the stage of a microscope (Olympus model BX40) fitted with a video camera (Avision, Newbury, NH) leading to a video caliper (Texas A&M, College Station, TX), monitor (Avision), and recorder. This setup permitted continuous measurement of internal diameter, which was directly available both as digital readout and on analog signals.

Drugs were added directly to the vessel chamber, followed by continuous infusion (SAGE Pumps, model 341B, Boston, MA) into the superfusion buffer. This way, we were able to quickly achieve and maintain the desired concentration of drugs in the chamber, without interrupting tissue superfusion.

**Experimental Design**

Protocol to investigate the effect of heme oxygenase inhibitor on isometric tension in vascular rings. The effect of the heme oxygenase inhibitor CrMP (31) on isometric tension development was examined in rings of descending thoracic aorta, femoral artery, and muscular branch of the femoral artery. Isometric tension was monitored before and after cumulative addition to the bathing buffer of CrMP to establish concentrations ranging from 0.1 to 100 mmol/l. The effect of CrMP on isometric tension also was studied in rings of aorta and femoral artery bathed in buffer containing phenylephrine (0.1 mmol/l).

Protocols to investigate the effect of heme oxygenase inhibitors on the internal diameter of gracilis muscle arterioles. Experiments were initiated after the microvessel prepara-
tions were allowed to stabilize for 60 min. After the stabilization period, the nitric oxide synthase inhibitor L-NAME (1 mmol/l) was included in the superfusion buffer to avoid potential interactions between the nitric oxide and carbon monoxide systems (1, 20). The internal diameter of gracilis muscle arterioles pressurized to 80 mmHg decreased spontaneously from 131.0 ± 2.1 to 87.9 ± 2.3 µm (n = 82, P < 0.05) during the stabilization period; the addition of L-NAME caused a further decrease to 70.0 ± 2.1 µm (n = 82, P < 0.05). The following protocols were executed 45 min after L-NAME pretreatment.

Protocol 1 was designed to examine the concentration-related effects of various metalloporphyrins differing in their heme oxygenase inhibitory potency on the internal diameter of gracilis arterioles pressurized to 80 mmHg. Two potent heme oxygenase inhibitors, CrMP (31) and CoPP (7), and a weak inhibitor, MgPP (7), were used. Arteriolar responses to cumulative increments in concentration of metalloporphyrins (0.175–102 µmol/l) were examined.

Protocol 2 was designed to determine the time course of the changes in internal diameter elicited by CrMP in arterioles pressurized to 80 mmHg. After a control period, 15 µmol/l CrMP or matched vehicle only was administered, and the internal diameter was monitored until an apparent asymptote was approached. In additional experiments, the time-related effects of CrMP on the internal diameter of preparations superfused with media containing exogenous carbon monoxide were examined. Carbon monoxide infusion (100 µmol/l) was started, and 5 min later 15 µmol/l CrMP was administered. After 4 min, the carbon monoxide infusion was stopped while the CrMP infusion was continued until a stable arteriolar diameter was attained.

Protocol 3 investigated whether the effect of CrMP on the diameter of pressurized (80 mmHg) arterioles can be reversed by the administration of exogenous carbon monoxide. CrMP (15 µmol/l) was added to the superfusion buffer. Once the CrMP-induced effect had reached a plateau, carbon monoxide was infused to set the concentration of the gas in the superfusion buffer at 10, 50, and 100 µmol/l in each of three consecutive 5-min periods. In additional experiments, the effect of exogenous carbon monoxide (10–100 µmol/l) on arteriolar diameter was studied in preparations superfused with media not containing CrMP.

Protocol 4 examined the effect of CrMP on the internal diameter of pressurized and unpressurized gracilis muscle arterioles. Arteriolar diameter was monitored before and for 10 min after inclusion of CrMP (15 µmol/l) into the buffer-superfusing preparations in which intraluminal pressure had been set at 0, 40, 60, or 80 mmHg.

Protocol 5 was designed to compare the effect of CrMP (15 µmol/l) on arterial diameter of pressurized preparations (80 mmHg) superfused with media containing and not containing ODQ (10 µmol/l), an inhibitor of soluble guanylate cyclase (13).

Protocol 6 investigated whether the effect of the heme oxygenase inhibitor CrMP on the diameter of pressurized arterioles (80 mmHg) can be reversed by endothelin receptor blockade. CrMP (15 µmol/l) was added to the superfusion media. Once the CrMP-induced effect had reached a plateau, the endothelin receptor antagonist L-754,142 (34) (100 µmol/l) was administered and the internal diameter of the arterioles was monitored for 10 min. In other experiments, 10 nmol/l ET-1 was added to superfusion media. Once the response to ET-1 had reached a plateau, 100 µmol/l L-754,142 was administered, and the internal diameter was monitored until a stable arteriolar diameter was achieved.

Protocol to examine the effect of CrMP on the pressure-diameter relationship in gracilis muscle arterioles. Unpressurized arteriolar preparations were superfused with Krebs’ buffer containing L-NAME (1 mmol/l). After the equilibration period, CrMP (15 µmol/l) was added to the superfusion buffer. Ten minutes later, the intravascular pressure was increased in 20-mmHg steps until it reached 100 mmHg. The pressure was maintained for ~5–10 min at each pressure step to allow the vessel to reach a steady-state diameter.

Statistics

Data are expressed as means ± SE. Data were analyzed by one- or two-way ANOVA for repeated measures. If differences were noted, orthogonal contrasts or the Newman-Keuls modified t-test was used to make specific comparisons. The null hypothesis was rejected when the probability value was <0.05.

RESULTS

As shown in Fig. 1, a protein band with the molecular mass of heme oxygenase-2 was identified by Western blot analysis of proteins in homogenates of pooled descending thoracic aortas (1,427 ± 349 density units; n = 3) and gracilis muscle arterioles (910 ± 190 density units; n = 3). A protein band with the molecular mass of heme oxygenase-1 was not apparent in immunoblots of any vascular homogenate.

As shown in Fig. 2, CrMP at 10 and 100 µmol/l increased (P < 0.05) isometric tension in muscle branch (of the femoral artery) rings (n = 5) by twofold over the basal tension. In contrast, changes in isometric tension produced by CrMP (0.1–100 µmol/l) in rings of descend-
ing thoracic aorta (n = 5) and femoral artery (n = 5) were minimal, amounting to <10% of the basal tension. Likewise, CrMP (0.1–100 µmol/l) did not elicit isometric tension development in aortic (n = 4) or femoral (n = 4) artery rings in which pretreatment with phenylephrine (0.1 µmol/l) had increased baseline isometric tension from 5.4 ± 1.2 to 8.2 ± 0.9 mN/mm and from 1.1 ± 0.4 to 1.6 ± 0.5 mN/mm, respectively.

The effect of various metalloporphyrins on the diameter of gracilis arterioles pressurized to 80 mmHg is shown in Fig. 3. The heme oxygenase inhibitor CrMP elicited a concentration-dependent decrease in internal diameter (n = 4; P < 0.05). CoPP, another heme oxygenase inhibitor, also decreased arteriolar diameter in a concentration-dependent manner (n = 4, P < 0.05). However, MgPP, a weak inhibitor of heme oxygenase, had little or no effect on arteriolar diameter (n = 4). As shown in Fig. 4, in preparations (n = 7) in which arteriolar diameter had been reduced by administration of CrMP (15 µmol/l), coadministration of carbon monoxide (10–100 µmol/l) resulted in concentration-dependent reversal of the vasoconstriction elicited by the inhibitor of heme oxygenase. In contrast, exogenous carbon monoxide did not affect the internal diameter of pressurized arterioles superfused with media not containing CrMP (n = 6; Fig. 4).

The time course of the effect of CrMP (15 µmol/l) on the internal diameter of gracilis muscle arterioles pressurized to 80 mmHg is illustrated in Fig. 5. When given alone, CrMP caused a rapid and sustained reduction in arteriolar diameter from 72.3 ± 7.1 to 9.7 ± 6.5 µm (n = 6, P < 0.05; Fig. 5, top). Figure 5, bottom, shows the effects of CrMP in the presence and absence of exogenous carbon monoxide (100 µmol/l; n = 10). Carbon monoxide infusion had little or no effect on arteriolar diameter. However, during carbon monoxide infusion CrMP did not induce vasoconstriction. When the carbon monoxide infusion was stopped, the powerful vasoconstrictor effect of CrMP was uncovered and resulted in a rapid decrease in arteriolar diameter (from 60.0 ± 7.6 to 14.5 ± 6.1 µm; P < 0.05).

Figure 6 depicts the effect of CrMP (15 µmol/l) on the internal diameter of pressurized and unpressurized gracilis muscle arterioles. CrMP did not affect the diameter of unpressurized arterioles (n = 5) but decreased (P < 0.05) the diameter of arterioles pressurized to 40, (n = 6) 60 (n = 6), and 80 (n = 6) mmHg. Importantly, the higher the level of intravascular pres-
sure, the greater was the reduction of arteriolar diameter induced by CrMP. In arterioles pressurized to 80 mmHg, CrMP (15 µmol/l) elicited reductions of arteriolar diameter both in preparations superfused with media containing (from 66.3 ± 2.9 to 27.7 ± 6.0 µm; n = 3; P < 0.05) and not containing (from 56.3 ± 12.4 to 32.1 ± 4.8 µm; n = 4; P < 0.05) the guanylate cyclase inhibitor ODQ.

The reduction in diameter produced by CrMP in preparations pressurized to 80 mmHg (from 68.7 ± 11.2 to 7.3 ± 7.3 µm; n = 3; P < 0.05) was unmodified (5.8 ± 5.1 µm) by the endothelin receptor antagonist L-754,142 (100 µmol/l). In contrast, in control experiments, the decrease in diameter produced by ET-1 (10 nmol/l) (from 70.0 ± 13.2 to 5.7 ± 5.5 µm; n = 3; P < 0.05) was fully reversed (77.3 ± 7.8 µm; P < 0.05) by L-754,142.

Pressure-diameter relationships in gracilis muscle arterioles superfused with Krebs buffer containing (n = 6) and not containing (n = 6) CrMP (15 µmol/l) are shown in Fig. 7. In preparations not treated with CrMP, steady-state arteriolar diameter increased (P < 0.05) in response to elevations of intravascular pressure over the range 0–40 mmHg, but further elevations of pressure to 100 mmHg caused the diameter to decrease (P < 0.05) toward a value slightly below that of unpressurized vessels. In contrast, in preparations pretreated with CrMP, the arteriolar diameter decreased (P < 0.05) as a function of the intravascular pressure over the range 20–100 mmHg, reaching at 100 mmHg a value one-tenth that of vessels in the unpressurized state. Over the range 20–100 mmHg, the steady-state internal diameter of arterioles superfused with CrMP-containing buffer was decreased (P < 0.05) relative to the diameter of control arterioles at comparable pressure values.

The internal diameter of gracilis muscle arterioles did not change significantly with the various drug-vehicle administrations used in the study. For example, drug-vehicle administration-induced changes in arteriolar diameter were −4.0 ± 2.3 µm (n = 3; P > 0.05) for the CrMP-vehicle, −2.2 ± 3.5 µm (n = 3; P > 0.05) for the CoPP-vehicle, and −1.0 ± 0.7 µm (n = 5; P > 0.05) for carbon monoxide-vehicle administration.

DISCUSSION

CrMP is a competitive inhibitor of heme oxygenase (31), which decreases carbon monoxide formation in vascular tissues (6). In this study, CrMP was used to examine the participation of endogenous carbon monoxide in the regulation of vascular tone. We found that CrMP administration results in contraction of vascular smooth muscle, as evidenced by its ability to cause rapid and sustained reduction of internal diameter in pressurized gracilis muscle arterioles and elicit development of isometric tension in the muscular branch of the

Fig. 7. Effect of intraluminal pressure on ID of gracilis muscle arterioles superfused with buffer containing CrMP (15 µmol/l). Results are means ± SE. *P < 0.05 relative to corresponding values in preparations superfused with buffer not containing CrMP.
femoral artery. Contraction of vascular smooth muscle in response to CrMP may be characteristic of arterioles and small arteries, because, in our study, CrMP did not stimulate contraction of rings of thoracic aorta or of femoral artery. Because heme oxygenase-2 protein content was comparable in aorta and gracilis muscle arterioles, the dissimilar responsiveness of small and large arterial vessels to CrMP cannot be attributed to differences in prevailing heme oxygenase expression.

If the vasoconstrictor effect of CrMP is linked to inhibition of heme oxygenase, other heme oxygenase inhibitors also should cause arteriolar constriction. CrMP and CoPP have comparable heme oxygenase inhibitory potency. Our study shows that CrMP and CoPP are similarly effective in their ability to constrict gracilis muscle arterioles. However, MgPP, a weak inhibitor of heme oxygenase (7), had little or no vasoconstrictor effect even at the highest concentration tested. These data suggest that the ability of CrMP to produce vasoconstriction is closely linked to its ability to inhibit heme oxygenase.

The possibility that the effect of CrMP on vascular contractility is not solely a functional manifestation of heme oxygenase inhibition also merits consideration. In this regard, some metalloporphyrins were reported to promote photosensitization and to inhibit nitric oxide synthase and soluble guanylate cyclase (11, 14, 31). A confounding influence of such metalloporphyrins actions on the interpretation of our studies using CrMP is unlikely for several reasons. First, CrMP, as opposed to tin- and zinc-based metalloporphyrins, lacks photosensitizing activity (31). Second, the constrictor effect of CrMP is independent of nitric oxide, because all the experiments were conducted in preparations exposed to concentrations of N-NAME that produce maximal inhibition of nitric oxide synthesis. Third, the constrictor effect of CrMP in gracilis muscle arterioles is independent of soluble guanylate cyclase, because it was similar in the absence and the presence of the guanylate cyclase inhibitor ODQ.

In the present study, exogenous carbon monoxide elicited robust dilatatory responses in preparations pretreated with CrMP, reversing the constrictor effect of the heme oxygenase inhibitor. Exogenous carbon monoxide also was effective in preventing the constrictor effect of CrMP in gracilis muscle arterioles. These observations promote the idea that the vasoconstrictor effect of CrMP is due to the withdrawal of a tonic vasorelaxing mechanism mediated by endogenous carbon monoxide. However, exogenous carbon monoxide did not produce arteriolar dilation in preparations not exposed previously to CrMP. One possible explanation for the ineffectiveness of exogenous carbon monoxide as a vasodilator in pressurized preparations not exposed to an inhibitor to heme oxygenase is that, in such a setting, the vasodilatory mechanism mediated by endogenous carbon monoxide is maximally active.

Carbon monoxide was shown to interfere with the vascular production of endothelin (3, 22). Hence, inhibition of endogenous carbon monoxide production may increase endothelin release, which, in turn, may contribute to the vasoconstrictor effect of heme oxygenase inhibitors (5). Our present study shows that L-754,142, a nonpeptidyl endothelin receptor antagonist with high affinity for all types of endothelin receptors (34), does not affect CrMP-induced vasoconstriction. These data suggest that the vasoconstrictor effect of CrMP can be independent of endothelin release.

According to the current study, the response of gracilis arterioles to CrMP varies in relation to the level of intravascular pressure. That CrMP elicits constriction of pressurized but not of unpressurized gracilis muscle arterioles is most interesting, calling attention to the possibility that the reduction of arteriolar diameter produced by the heme oxygenase inhibitor is the consequence of an intensification in myogenic tone. If so, the inability of CrMP to stimulate contraction of rings of aorta and femoral artery may be explained by the lack of myogenic tone in these large arteries (25).

The idea that inhibition of heme oxygenase facilitates myogenic behavior in small arterial vessels is supported by the results of our experiments comparing the pressure-diameter relationship of arterioles superfused with buffer containing and not containing CrMP. As previously reported by other investigators (29), in preparations not exposed to CrMP, arteriolar diameter increased in response to elevations of intravascular pressure over the range 0–40 mmHg, but further elevations of pressure to 100 mmHg prompted vascular constriction so that arteriolar diameter decreased to slightly below the diameter in the unpressurized state. In contrast, in preparations exposed to CrMP, increases of intravascular pressure over the range 0–100 mmHg elicited progressive reductions of arteriolar diameter so that at 100 mmHg the diameter rarely exceeded 10% of that in the unpressurized state. Hence, according to these data, the heme oxygenase inhibitor CrMP appears to amplify the pressure-sensitive mechanism of vascular constriction responsible for that component of vascular tone that is myogenic in nature. A corollary of this conclusion is that carbon monoxide manufactured by arterial vessels may serve as an inhibitory modulator of myogenic tone.

The current study suggests a major tonic regulatory influence of the endogenous heme-heme oxygenase-carbon monoxide system on gracilis muscle arterioles. This conclusion implies that carbon monoxide is produced tonically by intact arterial vessels. Pertaining to this point, a recent study documented basal production of carbon monoxide in rat aorta (23). An additional consideration is that our experiments were conducted in the presence of the nitric oxide synthase inhibitor N-NAME. Because nitric oxide has been shown to interfere with heme synthesis (28), inhibition of nitric oxide synthesis after exposure to high concentrations of N-NAME might create a setting that maximizes the functional impact and the activity of the vascular heme-heme oxygenase-carbon monoxide system.

In summary, the present study demonstrates that the heme oxygenase inhibitor CrMP causes a rapid and sustained reduction in the internal diameter of gracilis muscle arterioles and stimulates tension development
in small but not in large arteries. CoPP, another potent inhibitor of heme oxygenases, also caused arteriolar constriction, whereas the weak heme oxygenase inhibitor MgPP did not. Arteriolar constrictor responses to CrMP were demonstrable in pressurized preparations only, were blunted in vessels exposed to exogenous carbon monoxide, and were unaffected by endothelin receptor blockade. Most importantly, exposure of gracilis arterioles to CrMP greatly amplified the constric tor response to step-wise increases in vascular pressure. These findings suggest that the vasoconstrictor effect of heme oxygenase inhibitors is linked to magnification of myogenic tone due to the withdrawal of a modulating mechanism mediated by endogenous carbon monoxide. A corollary of this conclusion is that production of carbon monoxide by vascular heme oxygenase serves as a vasodilatory mechanism that contributes to the regulation of basal tone in resistance vessels, independently of the influences of the central nervous system and of circulating hormones.

Perspectives

Our present findings documenting an inhibitory influence of carbon monoxide produced by arterial vessels on myogenic behavior of arterioles have major implications in terms of circulatory homeostasis. A myogenic mechanism(s) responsive to changes in intravascular pressure contributes importantly to the regulation of blood flow in various tissues. There is also evidence that myogenic mechanisms of vasomotor control are facilitated in hypertension. Accordingly, the vascular heme-heme oxygenase-carbon monoxide system functioning as an inhibitory regulator of myogenic tone may impact importantly on mechanisms controlling tissue perfusion and blood pressure.

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