Effects of carbon monoxide on trout and lamprey vessels

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Submitted 17 June 2008; accepted in final form 4 November 2008

CO is produced endogenously via the catabolism of heme by heme oxygenase (HO) enzymes, which also generates biliverdin and Fe2+. Heme oxygenase-2 (HO-2) is a constitutively expressed isoform, while heme oxygenase-1 (HO-1) is inducible (39). A third isoform, HO-3, has been described in multiple tissues, and while it bears structural similarity to HO-2, its physiological functions are unclear (42). HO-1 and HO-2 have been detected in mammalian vascular smooth muscle cells and CO generated from smooth muscle is thought to be a regulator of vascular tone (54). The vasodilatory effects of CO are predominantly via the activation of soluble guanylyl cyclase and calcium-activated potassium (KCa) channels (54).

Even though it is widely accepted that CO is a vasodilator of mammalian blood vessels (15), this is not always the case. For example, CO has been demonstrated to dilate cerebral arterioles in piglets (35) but is ineffective at dilating cerebral vessels in dogs or rabbits (4). This demonstrates heterogeneity of the effects of CO within similar vascular tissues of species from the same vertebrate class.

CO production by and regulation within fish vascular smooth muscle is of interest for several reasons. First, it has been reported that CO plays a more significant role in the regulation of mammalian vascular tone in the absence of endothelial NO (4, 33). Fish do not appear to possess endothelial nitric oxide synthase (30, 46, 50), with the possible exception being retinal tissue (25), and thus a greater role for CO might be anticipated. Second, environmental toxins and stress have been demonstrated to alter HO activity or induce HO-1 in both elasmobranch (17) and teleost (2, 7, 34, 43) fishes. Third, organic solvents, pesticides, and trace metals have a demonstrated ability to alter fish HO function (2, 7, 48, 51) and are far more common environmental toxicants in water than in air (28, 48, 59). Thus, aquatic vertebrates subjected to such pollutants may have diminished or altered CO-mediated vasoregulation.

With this in mind, we investigated the role of CO in fish vascular smooth muscle by examining the effects of a water-soluble CO-releasing molecule (CORM-3) on efferent branchial (EBA) and celiacomesenteric (CMA) arteries of the rainbow trout and dorsal aorta of the sea lamprey. The potential mechanisms involved in the pharmacological action mediated by CO was also examined in the context of the interplay between CO and the other two “gasotransmitters”, NO and H2S.

MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee.

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**Animals**

Sea lamprey (*Petromyzon marinus*, 130–450 g) were trapped in streams feeding into the Great Lakes during the spring-summer spawning season and airdaired to the University of Notre Dame, where they were maintained in 500-liter rectangular tanks with aerated, flowing well water (15°C) and exposed to a 12:12-h light-dark photoperiod. They were not fed. Lampreys were anesthetized in benzoic acid (1:5,000, wt/vol), and the dorsal aortas were dissected out and placed in HEPES buffer at 4°C until use.

Rainbow trout (*Oncorhynchus mykiss*, kamloops strain, 0.3–0.8 kg) of either sex were used for studies on isolated vessels. They were purchased from a local hatchery (Harriett Hills Trout Farm, Harrietta, MI), kept in circulating 2,000-liter tanks containing through-flowing well water at 12–15°C, aerated with room air, and exposed to 12:12-h light-dark cycles. The fish were fed a maintenance diet of commercial trout pellets (Purina, St. Louis, MO). Trout were stunned by a blow to the head, and the EBA and CMA were removed and placed in HEPES buffer at 4°C until use. All procedures followed National Institutes of Health guidelines.

**Myography**

Vessels were cut into approximately one-half centimeter-long segments and mounted on 280-μM-diameter stainless-steel wire hooks and suspended in 5-ml water-jacketed smooth muscle baths filled with the appropriate buffer at 14°C and aerated with room air. The bottom hooks were stationary; the upper ones were connected to Grass model FT03C force-displacement transducers (Grass Instruments, West Warwick, RI). Tension was measured on a Grass Model 7E or 7F polygraph (Grass Instruments, West Warwick, RI). Polygraph sensitivity was set to detect changes as small as 5 mg. Data were archived on a PC computer at 1 Hz using SoftWire (Measurement Computing, Middleboro, MA). The chart recorders were calibrated prior to each experiment.

Length-tension relationships have been derived from KCl-contracted vessels from both species in other studies (unpublished) in this laboratory. These, plus general vessel size and physical characteristics were taken into account to apply a reasonable baseline (resting) tension (500–700 mg for all vessels) for 0.5–1 h prior to experimentation. The vessels were then contracted with 80 mM KCl, washed twice, and resting tension re-established for a minimum of 30 min before further experimentation. This procedure has been used to achieve optimum in vitro vessel activity (Olson KR, unpublished observations). Unless otherwise noted, vessels were used for only one experiment.

In the lamprey dorsal aorta, two concentrations, 200 and 500 μM, of CORM-3, [CORM hereafter; tricarbonylchloroglycinato ruthenium(II), Ru(CO)3Cl(NH2CH2CO2)], structure shown in Fig. 1D, inset], which when placed in buffer liberates 1 mol CO/mol CORM-3 (6, 20, 44), were examined on otherwise unstimulated dorsal aortas and vessels when placed in buffer liberates 1 mol CO/mol CORM-3 (6, 20, 44), and vessels precontracted with NE in this study were treated with 1 μM NE. All vessels were exposed to only one ZnPP concentration. After the NE contraction plateaued, 300 μM Na2S (which generates H2S in solution) was added to the bath. Since 300 μM Na2S produces a triphasic response in the trout EBA (10), with the third dilatory phase predominating in NE-precontracted vessels, only the magnitude of the prolonged relaxation was examined. To examine any possible interaction of CO with NO, vessels were incubated with 30 μM ZnPP, contracted with 1 μM NE, and then exposed to 100 μM SNP, a known NO donating agent and vasodilator in trout (46).

**Polarographic Measurement of Sulfide Binding by Zinc Protoporphyrin**

The above experiments showed that ZnPP inhibited Na2S relaxations (see also Fig. 6). To determine whether this was due to ZnPP inhibiting H2S-mediated increase in CO production, or a direct effect of ZnPP binding to or catalyzing degradation of H2S, we used a polarographic H2S sensor to measure the concentration of H2S gas in buffer in the presence and absence of ZnPP. This polarographic H2S sensor (9, 58) measures H2S gas in real time and has a resolution of 0.25 nM (58). The sensor was connected to an Apollo 4000 Free Radical Analyzer (WPI, Sarasota FL). In one experiment, sulfide binding by zinc protoporphyrin-IX (ZnPP) was determined in a closed 1-ml chamber at 14°C by adding increasing concentrations of ZnPP (1, 3, 10, 30 μM) to pH 7.8 HEPES buffer spiked to 300 μM Na2S. H2S concentration was converted to total sulfide based on pH (58). In a second experiment, in an attempt to determine binding efficiency and duration, 10 μM ZnPP was first added to the chamber followed by three consecutive additions of 5 μM Na2S and then two subsequent additions of 5 μM ZnPP.

**Data Analysis**

Data were analyzed using BIOPAC software (BIOPAC Systems, Goleta, CA). The effective concentration producing half-maximal response (EC50) was determined from dose-response curves for individual vessels using Table Curve (Jandel, Chicago, IL). Student’s t-tests or one-way ANOVA repeated-measures followed by Bonferroni’s t-test for multiple comparisons (SigmaStat, Jandel Corp) were used for comparisons between groups of vessels. Results are expressed as means ± SE. Significance was assumed at P ≤ 0.05.
HEPES Na

by 80 mM KCl. The threshold for CORM-induced (Fig. 3). The threshold for CORM-induced (H11011) with NE (H11011) approximately five-fold more efficacious in EBA precontracted (H11006) CORM, respectively. CORM-mediated relaxation was also than with KCl (Fig. 2; E C50, 352 ± 77 and 698 ± 21 μM CORM, respectively (data not shown). The maximal relaxation of unstimulated EBA and CMA was 27 ± 14% and 32 ± 17% of resting tension (500–700 mg), respectively. Cumulative dose-response curves for CORM-induced relaxation of EBA precontracted with NE or KCl are shown in Fig. 2 and 3. EBA were ~2-fold more sensitive (P < 0.05) to CORM when precontracted with NE than with KCl (Fig. 2B; EC50, 352 ± 77 and 698 ± 21 μM CORM, respectively). CORM-mediated relaxation was also approximately five-fold more efficacious in EBA precontracted with NE (~80% relaxation) than in KCl-contracted EBA (~15% relaxation; Fig. 3). The threshold for CORM-induced relaxation was ~30 μM. NE (1 μM)-induced contractions in control EBA were ~80% of the maximal contractions elicited by 80 mM KCl.

A bolus of 1 mM CORM applied to unstimulated (Fig. 4A) or NE-precontracted (Fig. 4B) vessels produced a biphasic response, a brief 1–2 min contraction (phase 1) followed by a prolonged relaxation (phase 2). The magnitudes of both phases were significantly larger in vessels precontracted with NE than in unstimulated vessels. Glybenclamide (10 μM) was the only antagonist to have an effect on the NE-induced contraction, reducing it by ~50% (Fig. 4C). NE-induced contractions were unaffected by apamin (50 nM), charybdotoxin (50 nM), or NS-2028 (10 μM). Incubation with apamin (50 nM), charybdotoxin (50 nM), or glybenclamide (10 μM) had no effect on either phase of the CORM-induced response of NE-precontracted EBA, while application of 10 μM NS-2028 resulted in nearly complete inhibition of both phases (Fig. 4D).

ZnPP (0.1–30 μM) produced a dose-dependent increase in baseline tension in trout EBA (Fig. 5A). However, the presence of ZNPP did not affect a subsequent NE contraction (Fig. 5B).

Na2S (300 μM) elicited a triphasic relaxation-contraction-relaxation in NE-precontracted EBA (Fig. 6A; cf. Ref 10). Pretreatment with ≥3 μM ZnPP significantly reduced the magnitude of the Na2S relaxation in apparent dose-dependent relationship (Fig. 6, B and C). ZnPP (30 μM) did not affect the ability of SNP (100 μM) to relax NE-contracted EBA (Fig. 7, A and C), whereas in vessels treated with both NS-2028 (10 μM) and ZnPP, the SNP relaxation was abolished (Fig. 7, B and C).

Polarographic Measurement of Sulfide Binding by Zinc Protoporphyrin

ZnPP appeared to bind sulfide in a stable fashion (Fig. 8A). Na2S (300 μM) produced a large electrode current followed by a fall, indicating sulfide oxidation and/or escape from the chamber. One and three micromoles of ZnPP had little effect on the sulfide current, but 10 and 30 μM ZnPP produced incremental reductions in the electrode current. However, within a minute or two after ZnPP addition, the electrode current became stable, indicating that ZnPP does not catalyze H2S degradation. A second experiment to clarify the stoichiometry of sulfide-ZnPP binding was performed using lower ZnPP and Na2S concentrations. Prior addition of 10 μM ZnPP reduced the 5 μM sulfide current by ~80% but had little to no effect on the current increase generated by two subsequent 5 μM Na2S spikes (Fig. 8B). Two subsequent additions of 5 μM ZnPP produced reductions in electrode current approximately

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**Chemicals**

HEPES buffer contained (in mM): 145 NaCl, 3 KCl, 0.57 MgSO4·7H2O, 2 CaCl2·2H2O, 5 glucose, 3 HEPES acid, and 7 HEPES Na+ salt, pH 7.8. All buffers were stored in the refrigerator and used within 72 h of preparation. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). CORM-3 was synthesized and handled as previously described (6).

**RESULTS**

**Effect of CORM on Lamprey Vessels**

CORM at 200 and 500 μM relaxed lamprey dorsal aortas precontracted with NE (1 μM) and U-46619 (100 nM) but had only a small transient dilatory effect on otherwise unstimulated vessels (Fig. 1). The extent of relaxation mediated by CORM on U-46619-contracted vessels was significantly greater than the one observed in NE-contracted vessels. However, the force generated by U-46619 was significantly less than that produced by NE, and the absolute magnitude of the 200 and 500 μM CORM-mediated relaxation was similar between the groups.

**Effect of CORM on Trout Vessels**

CORM relaxed unstimulated trout EBA and CMA with similar EC50, 393 ± 111 and 464 ± 93 μM, respectively (data not shown). The maximal relaxation of unstimulated EBA and CMA was 27 ± 14% and 32 ± 17% of resting tension (500–700 mg), respectively. Cumulative dose-response curves for CORM-induced relaxation of EBA precontracted with NE or KCl are shown in Fig. 2 and 3. EBA were ~2-fold more sensitive (P < 0.05) to CORM when precontracted with NE than with KCl (Fig. 2B; EC50, 352 ± 77 and 698 ± 21 μM CORM, respectively). CORM-mediated relaxation was also approximately five-fold more efficacious in EBA precontracted with NE (~80% relaxation) than in KCl-contracted EBA (~15% relaxation; Fig. 3). The threshold for CORM-induced relaxation was ~30 μM. NE (1 μM)-induced contractions in control EBA were ~80% of the maximal contractions elicited by 80 mM KCl.

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equivalent to the 5 μM Na₂S-induced increases in current. These results suggest that in a cell-free environment, ZnPP binds sulfide in an approximate 1:1 ratio.

**DISCUSSION**

The importance of CO as a physiologically relevant signaling molecule in a variety of mammalian systems has been well documented (61). Here, we show that CO produces vasorelaxation in vessels from lamprey and trout. Otherwise, unstimulated trout vessels appear to respond only slightly to CO and CO-induced relaxation in precontracted trout vessels is partially dependent on activation of guanylyl cyclase (GC). The decreased efficacy of CO in KCl-depolarized vessels also suggests that a portion of the CO response involves either potassium or chloride channel activation, but small-conductance or large-conductance KCa channels do not appear to be involved. To our knowledge, this is the first report of CO-mediated relaxation of vascular smooth muscle from nonmammalian vertebrates. Furthermore, these studies suggest that CO vasoactivity has been well conserved throughout vertebrate evolution.

**CORM-3-Induced Relaxation and Mechanisms of Action**

The vasorelaxation produced in lamprey and trout vessels by CORM is consistent with CO-mediated relaxation of mammalian blood vessels (6, 20, 61). Both GC and cGMP-independent activation of big-conductance calcium-activated potassium channels (BKCa channels) mediate the endothelium-independent aspect of this response in mammals (20, 54). In the present study, we found that activation of GC also appears to be involved in CO relaxation of trout EBA, but evidence for a contribution of potassium channels to this response is more tenuous.

GC and cGMP: CO binds ferrous iron of heme proteins, which generally inhibits their function (61). The notable exception to this is GC (61), and the basis of CO activation of soluble GC has been described (36). In this study, NS-2028, a specific inhibitor of soluble GC in mammalian tissue (45) almost completely inhibited CORM-mediated relaxation of trout EBA (Fig. 4D) and completely inhibited the vasodilatory effect of SNP in trout EBA (Fig. 7). Because SNP releases another soluble GC activator, NO, it seems reasonable to conclude that NS-2028 is also an effective inhibitor of trout CG and that much, if not all, of the vasodilatory effect of CO in trout is mediated through cGMP formation.

The brief vasoconstriction that we observed shortly after the addition of CORM to trout EBA (Fig. 4) has not, to our knowledge, been observed in mammalian vessels. This constriction is significantly greater in prestimulated vessels, which suggests that an increased level of cytosolic Ca²⁺, and possibly Ca²⁺ cycling, during cell activation may enhance the contractile response. We think it is unlikely that this contractile response is mediated by anything other than CO because the degassed inactive CORM (iCORM) was not vasoactive in EBA, nor has it been found to be vasoactive in mammalian vessels (20). The failure to observe a similar constriction in mammalian vessels, however, suggests that this is more likely a unique response of trout vessels to CO. Interestingly, both the constriction and relaxation were inhibited by NS-2028. This suggests that the constriction is also cGMP dependent. The CO-mediated constriction could be due to the presence of a sensitive cGMP-dependent Cl⁻ channel (41, 40), but this remains to be determined.
Ion channels. Trout EBA precontracted with a ligand such as NE were approximately twofold more sensitive to the CO-releasing agent than vessels precontracted with KCl (Fig. 2). In addition, CORM was fivefold more potent in NE- than in KCl-contracted vessels (Fig. 3). This suggests that either a portion of the CO-induced vasodilation is mediated by ion channels that are rendered ineffective when the cell is depolarized or that the Ca\(^{2+}\)/H\(^{+}\) influx associated with KCl depolarization cannot be overcome by CO.

The two most likely candidates for CO-activated channels are K\(^{+}\) and/or Cl\(^{-}\) channels. Although one study has suggested that CO activates K\(_{ATP}\) channels (20), most (54 –56, 60) have focused more on CO binding and activation of large-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channels. Previous studies have demonstrated the apparent presence of K\(_{Ca}\) channels in isolated retinal ganglion cells from trout (26, 27), and even charybdotoxin-sensitive gill epithelial cells from sea bass, *Dicentrarchus labrax* (16). However, these channels have yet to be characterized in trout vascular smooth muscle. The ineffectiveness of glibenclamide, apamin, and charybdotoxin in our experiments (Fig. 4D) suggest that either 1) K\(_{Ca}\) and K\(_{ATP}\) channels are not present in trout EBA, or 2) K\(_{Ca}\) and K\(_{ATP}\) channels are not a molecular target of CO in these fish. It should be noted that our experience with glibenclamide as an inhibitor of K\(_{ATP}\) in trout tissues has been generally inconclusive (Olson KR, unpublished observations), which leads us to question whether these channels are even functional in trout vessels.

The inhibitory effect of glibenclamide on the NE-induced contraction of trout EBA (Fig. 5) may shed some light on another potential mechanism of cell activation in trout vessels. In addition to its inhibitory effect on mammalian K\(_{ATP}\) channels, glibenclamide is also an inhibitor of cystic fibrosis transmembrane conductance regulator (CFTR) Cl\(^{-}\) channels and of swelling- and Ca\(^{2+}\)-activated Cl\(^{-}\) channels (49, 62). Furthermore, NE-induced contraction in mammalian vessels is par-
tially dependent on an increase in Cl⁻ permeability and subsequent Cl⁻ efflux (37). Collectively, these studies suggest that Cl⁻ channels are also involved in NE-mediated contraction of trout EBA.

Endogenous CO Production

Mammalian blood vessels possess a complete enzymatic pathway for de novo heme biosynthesis, and it appears that local heme production is sufficient to provide substantial substrate for CO production (29). The nonspecific heme-oxygenase (HO) inhibitor zinc protoporphyrin-IX (ZnPP) elicited a small, yet dose-dependent increase in resting trout EBA tension (Fig. 6A). Although we cannot rule out a potential stimulatory effect of ZnPP, these studies suggest that there is a tonic level of CO on vessel tonus. Since HO activity has been demonstrated in both bony (1, 2, 7, 51, 52) and cartilaginous (17) fishes, this activity is not unexpected.

Interactions of CO With Other Vasoactive Gases, NO, and H₂S

In addition to its direct effects, CO may also exert activity indirectly through interactions with mechanisms mediated by nitric oxide (NO) and hydrogen sulfide (H₂S). For example, NO forms a pentacoordinate complex with the GC heme, whereas CO forms a hexacoordinate complex of CO with the GC heme, resulting in decreased potency (36). Thus, depending on the relative degree of GC activation by NO, CO may either exacerbate or ameliorate the NO-induced dilation.

Fig. 6. Effects of ZnPP on the H₂S-induced relaxation of trout efferent branchial arteries. A: typical trace showing the triphasic response to H₂S (300 μM Na₂S) in norepinephrine- (NE; 1 μM) precontracted EBA. B: typical trace of the same protocol after treatment with ZnPP (30 μM). C: overall relaxation after 30 min of H₂S exposure represented as a percent relaxation of the NE-induced precontraction. Control, n = 24; all other, n = 16. Scale, 30 min and 1 g. *Significantly different from the control group. **Significantly different from all other treatment groups.

Fig. 7. The effects of sodium nitroprusside (SNP, 100 μM) on norepinephrine (NE, 1 μM)-precontracted trout efferent branchial arteries. A: typical trace of SNP-induced relaxation of NE-precontracted vessels pretreated with ZnPP (30 μM). B: typical trace of SNP-induced relaxation of NE-precontracted vessels pretreated with ZnPP (30 μM) and NS-2028 (10 μM). C: effects of SNP (100 μM) on NE (1 μM)-precontracted vessels that were otherwise untreated (control, C), incubated with 30 μM ZnPP, or incubated with both 10 μM NS-2028 and 30 μM ZnPP. Scale 15 min and 1 gram of tension; *Significantly different from both other groups.
direct CO-mediated inhibition of NO synthesis has also been reported (32). Inhibition of the CO/HO pathway by H2S and of H2S synthesis by CO/HO has been proposed in aortic smooth muscle (31), whereas the CO/HO pathway may be upregulated by H2S in pulmonary vessels (47).

There is considerable debate on the presence of a NO system in fish, although recent evidence supports the presence of nitricergic perivascular nerves and absence of an endothelial source of NO production (12). Certainly, there is little evidence to support endothelial NO production in trout EBA (46). Thus CO-NO interactions are not likely to occur in isolated trout vessels. On the other hand, our studies suggest that there may be an interaction between CO and H2S because ZnPP had a substantial inhibitory effect on the third phase of H2S relaxation of trout EBA (Fig. 6).

To determine whether the inhibitory effect of ZnPP was indirect, due to either ZnPP binding with H2S or catalytically degrading H2S, we measured the effect of ZnPP on H2S in a cell-free solution in real time using a polarographic H2S sensor. As shown in Fig. 8, ZnPP appears to bind to H2S in a 1:1 ratio but does not appear to catalytically degrade it. Because we found that the vasorelaxation produced by 300 μmoles/l H2S was substantially inhibited by only 30 μmoles/l ZnPP, which would be expected to reduce the H2S concentration to 270 μmoles/l, which is still vasoactive (10), it is possible that at least a portion of the complex triphasic H2S response in trout EBA is mediated by sulfide-induced activation of the HO/CO pathway.

Along these lines, Christodoulides et al. (5) demonstrated that while ZnPP did not bind to endogenous NO, it still inhibited soluble GC. Our results, however, do not suggest that ZnPP inhibits soluble GC, as ZnPP-treated vessels relaxed in a similar fashion as controls when exposed to the NO-donor SNP (Fig. 7C).

With the absence of endothelial NO, the interaction of H2S and CO is perhaps even more intriguing in fish. Both H2S (10) and CO (Fig. 6A) appear to serve as endogenously produced gases involved in the modulation of vascular smooth muscle tone in trout. While the trout EBA response to H2S is complex (10), this study demonstrates that the activity of heme oxygenase is necessary for the predominant third dilatory phase of the H2S-mediated response (Fig. 7).

Evolutionary conservation of CO as a gasotransmitter. It is interesting that all three “gasotransmitters”, NO, CO, and H2S, were originally thought to be merely toxic molecules to living organisms, and now, all three are recognized in mammals for their wide range of biological activities. The present study also suggests that important biological activities of all three gasotransmitters are relatively ancient and common traits in vertebrates.

The dilatory response to exogenous CO is similar in conductance vessels from sea lamprey (Fig. 1), trout (Figs. 2–4), and in multiple mammalian models (see Refs. 22 and 61). To date, CO is the only one of the three gasotransmitters that is both produced in the vasculature and, as these initial studies suggest, appears to have identical vasoactive properties across the vertebrate phylum. While NO dilates most vertebrate blood vessels, it constricts cyclostome vessels and a few vessels in elasmobranchs (14). Vessel responses to H2S have both species- and intravascular-related variability and range from monophasic relaxation to monophasic vasoconstriction or complex multiphasic responses (11). Clearly, further examination of CO responses in other vertebrate vessels, and especially in small-resistance vessels in fish where tissue perfusion is regulated, is needed to confirm this observation.

Environmental Impact of CO in Fish

CO production by and regulation within fish vascular smooth muscle is of interest for several reasons. First, it has been reported that CO plays a more significant role in the regulation of mammalian vascular tone in the absence of endothelial NO (4, 33). Fish do not appear to possess endothelial nitric oxide synthase (30, 46, 50), with the possible exception being retinal tissue (25), and thus a greater role for CO might be anticipated. Second, environmental toxins and stress have been demonstrated to alter HO activity or induce HO-1 in both elasmobranch (17) and teleost (2, 7, 34, 43) fishes. Third, organic solvents, pesticides, and trace metals have the ability to alter fish HO function (2, 7, 48, 51) and are far more common environmental toxicants in water than in air (28, 48, 59). Thus, aquatic vertebrates subjected to such pollutants may have diminished or altered CO-mediated vasoregulation.

Perspectives and Significance

CO, like the other gasotransmitters NO and H2S, appears to be a phylogenetically ancient vascular signaling molecule. However, unlike NO and H2S, the effects of CO appear to be well conserved across vertebrate phylogeny, at least at this preliminary stage of investigation. Although it remains to be determined how CO production is regulated, as well as its overall impact in the piscine cardiovascular system, it is clear that the environmental and physiological diversity of fishes provides unique and ample opportunities to explore the forces that forged the exploitation of this regulatory mechanism. The
physiological impact of exogenous gases also deserves consideration given the potential ability of these gases to readily cross the respiratory epithelium. Finally, these studies point out the need for a greater awareness of the environmental impact of an ever-increasing number of molecules with adverse affects on heme oxygenase and a demonstrated propensity for them to accumulate in aquatic environments.

ACKNOWLEDGMENTS
The authors are indebted to Dr. W. Swink, U.S. Geological Survey Biological Research Station for providing lampry. This work was supported in part by NSF Grant IOS 0641436 (to K. R. Olson) and a SISTAR grant (Student Independent Study and Teaching Research) from Saint Mary’s College (R. A. Dombkowski). The authors also thank Prof. Brian Mann from the University of Sheffield (UK) for the synthesis CORM-3.

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