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Forum Review

Vascular Actions of Hydrogen Sulfide in Nonmammalian Vertebrates

KENNETH R. OLSON

ABSTRACT

Hydrogen sulfide (H_2S) vasoactivity has been observed in isolated vessels from all vertebrate classes, and its effects, which include constriction, dilation, and multiphasic responses, are both species- and vessel-specific. H_2S is synthesized by mammalian and fish vessels, and because plasma H_2S titers are also vasoactive *in vitro*, it is likely that H_2S is a tonic effector of cardiovascular homeostasis in many vertebrates. Mechanisms of H_2S vasoactivity in nonmammalian vertebrates have been limited to the trout where the triphasic relaxation–contraction–relaxation includes endothelium-dependent and -independent components, ATP-dependent K^+ channels, and extracellular and intracellular Ca^{2+}, all independent of cyclic GMP production. The observation that at least some H_2S constrictory activity has been observed in all vertebrates except sharks suggests that H_2S may have been an ancestral pressor gasotransmitter. However, the ability of H_2S to serve as either (or both) an endothelium-independent constrictor or dilator, which is relatively unique among vasoregulatory molecules, is a feature that seems to have been exploited, for unknown reasons, by nearly all vertebrates. Aquatic vertebrates appear particularly vulnerable to H_2S because of their intrinsically low blood pressure and the potential for increased H_2S exposure from the environment. *Antioxid. Redox Signal.* 7, 804–812.

INTRODUCTION

Comparative physiology, roughly defined as the study of any animal that is not human (or at least not a classical vertebrate model thereof), has considerable utility in understanding the physiology of hydrogen sulfide (H_2S). From a phylogenetic perspective, comparative physiology provides an evolutionary window on the forces and constraints that shaped organ systems and their functions. From a methodological approach, the Krogh Principle, *i.e.*, "For a large number of problems there will be some animal of choice . . . on which it can be most conveniently studied," embodies practicality in unraveling complex and at times seemingly unrelated phenomena. From a practical standpoint, our knowledge of the biology of some 300 million extant species inhabiting this planet is presently based on a sample size of <0.001%.

Perhaps nowhere is the utility of the comparative approach more apparent than in cardiovascular physiology. The closed, myogenically driven vertebrate cardiovascular system initially evolved in essentially a gravity-free, osmotically interactive environment whose gaseous composition undoubtedly differed, both qualitatively and quantitatively, from that presently experienced by present-day (especially terrestrial) vertebrates. However, despite both historical and ecological differences, there is a striking continuity in form and function of nearly all vertebrate vascular effector mechanisms examined to date, including the sympathetic (26), renin–angiotensin (28), and kallikrein–kinin (4, 28) systems, natriuretic peptides (20, 35), vasotocin–vasopressin (39), and endothelin (14, 37). In fact, even the urotensins, which were originally thought to be unique to aquatic vertebrates, are now established mammalian vasoregulators (5).

H_2S has recently joined nitric oxide (NO) and carbon monoxide (CO) as the third vasoregulatory gasotransmitter (38; see also reviews in this *Forum*), and this has added another dimension of regulation (and level of complexity) to cardiovascular integration in mammals. The comparative physiology of gasotransmitters is only beginning to be examined. The role

Indiana University School of Medicine, South Bend Center For Medical Education, University of Notre Dame, Notre Dame, IN.

of NO as an endothelium-derived vasorelaxing factor (EDRF) has been clearly established in amphibians, reptiles, birds, and mammals (21). Whether or not NO is an EDRF in fish remains controversial. Although classical NO agonists and antagonists have been shown to predictably affect perfusion of organs such as the trout heart (23), they have been ineffective on isolated vessels (10, 11, 18, 29) and it is possible that nonendothelial (*i.e.,* neuronal) vascular NO production, which has recently been shown in the eel (16), could account for the observed responses in perfused tissues. CO-mediated vasoactivity has not, to my knowledge, been addressed in nonmammalian vertebrates and, with the exception of ongoing work in my laboratory, neither has H_2S. This raises several questions. Can modern vertebrates provide information on the origin of vascular gasotransmitters? Was there a unique signal or circumstance that triggered the implementation of gasotransmitters? Were some (or all) gasotransmitters integral components of the primordial cardiovascular system? Can the vascular responses of nonmammalian vertebrates provide additional clues to their activity in mammalian vessels? And from a more pragmatic standpoint relevant to aquatic vertebrates, does environmental H_2S exposure affect cardioregulatory mechanisms and, if so, what is the impact of industry and agriculture? This review encompasses the current work from my laboratory on the cardiovascular actions of H_2S in nonmammalian vertebrates. Obviously, it is only an initial, and very myopic, attempt to address these questions.

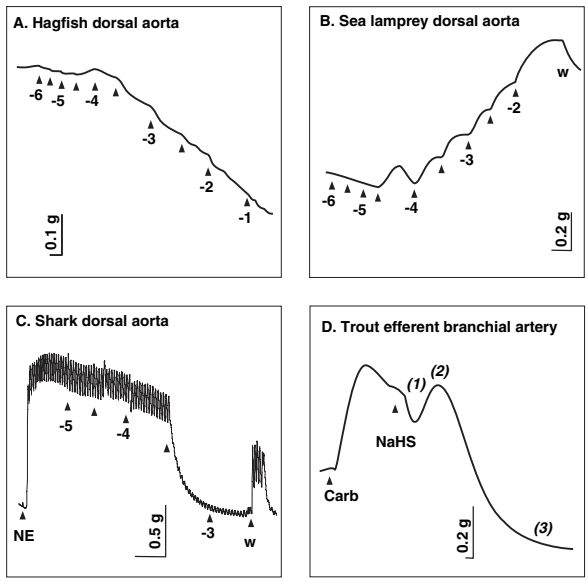

FIG. 1. Variety of H_2S responses in aquatic vertebrate systemic arteries. In otherwise unstimulated vessels, H_2S produces a slight contraction (at 3×10^{-5} *M* H_2S) and then a dose-dependent, monophasic relaxation of hagfish dorsal aorta (**A**) and a dose-dependent constriction of lamprey dorsal aorta (**B**). In norepinephrine (NE; 10^{-6} *M*) prestimulated, spontaneously contracting shark dorsal aortas, H_2S produces a threshold-dependent monophasic relaxation (**C**), whereas in carbachol (Carb, 10^{-5} *M*)-contracted trout efferent branchial arteries, 3×10^{-4} *M* H_2S produces a triphasic relaxation (*1*), contraction (*2*), contraction (*3*) (**D**). In many vessels (*e.g.,* B and C) the effect of high H_2S concentrations can be reversed upon washout (w). H_2S concentration is shown in figures as log M with arrowheads. Unlabeled arrowheads indicate incremental log M concentrations. Vertical scale indicates tension in grams; horizontal line represents 5 min. Redrawn from 6 and 7 with permission.

THE PHYLOGENY OF H_2S VASOACTIVITY

There are seven vertebrate classes. The Agnatha (hagfish and lamprey), Chondrichthyes (sharks, skates, and rays), and Osteichthyes (bony fish) are all aquatic and comprise nearly half (48.1%) of all known vertebrate species. Amphibia have both aquatic and terrestrial species, whereas the integument of Reptilia, Aves, and Mammalia isolates them from the environment and they are all essentially terrestrial. Hagfish are perhaps the most "primitive," and as the only osmoconforming vertebrate, intracellular and extracellular milieu is isoosmotic with the seawater environment. Thus, the ionic strength of hagfish tissues is nearly three times that of other vertebrates (28). Although elasmobranchs are also isoosmotic with seawater, much of this is attributable to organic osmolytes (urea and trimethylamine oxide), and ionic activities are only 50% greater than those of higher vertebrates. All other vertebrates, from bony fish to mammals (including lampreys), are osmoregulators, and the ionic strength of their body fluids is surprisingly uniform (28).

We (6, 7) have observed H_2S vasoactivity in isolated vessels of at least one species from each vertebrate class. This suggests that H_2S is both a phylogenetically ancient gasotransmitter and a ubiquitous vasoregulator. However, unlike mammals where only vasodilatory responses to H_2S have been reported (15, 43, 44; although see below), in nonmammalian vertebrates H_2S may be constrictory, dilatory, or both. These various responses are shown in Figs. 1 and 2; Fig. 3 presents a preliminary cladogram of H_2S responses in vertebrate vessels.

Perhaps the most phylogenetically ancient and prevalent response of vertebrate vessels to H_2S is vasoconstriction, and this has been observed in all vertebrate classes except Chondrichthyes. In the Pacific hagfish (*Eptatretus stouti*) dorsal aorta (equivalent to the aorta of terrestrial vertebrates), 3×10^{-5} *M* H_2S produces a slight contraction (Fig. 1A), whereas in the sea lamprey (*Petromyzon marinus* L.) dorsal aorta, H_2S produces a monophasic, dose-dependent, constriction (Fig. 1B) with a threshold of ~10^{-5} *M*. The responses in both hagfish and lamprey vessels are unaffected by precontracting the vessel with another agonist. Dose-dependent, monophasic, prestimulation-independent contractions are also found in the bulbus arteriosus (pre-gill vessel) of the steelhead or rainbow trout (both *Oncorhynchus mykiss*, threshold > 10^{-5}), aorta of the marine toad (*Bufo marinus* L., threshold > 10^{-4} *M*), aorta and pulmonary artery (Fig. 2B) of the American alligator (*Alligator mississippiensis*; threshold ~ 10^{-4} *M* and 10^{-6} *M*, respectively), and aorta of the Pekin duck (*Anas platyrhynchos domesticus*; threshold > 10^{-4} *M*; Fig. 2C). Surprisingly, we also observed a monophasic, dose-dependent contraction in the otherwise unstimulated aorta of the rat (*Rattus rattus*; threshold > 10^{-4} *M*; Fig. 2D). This is strikingly different from the dose-dependent relaxation commonly observed

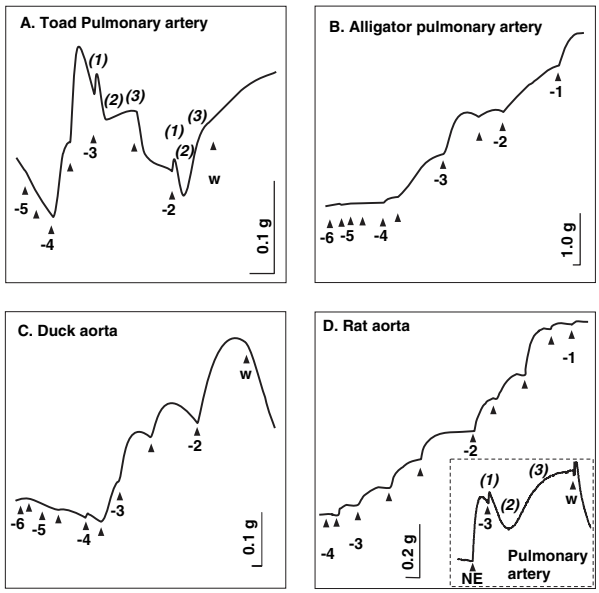

FIG. 2. Variety of H_2S responses in arteries of terrestrial vertebrates. H_2S produces an apparent triphasic contraction–relaxation–contraction in toad aorta (**A**), and the phases appear to have distinct sensitivity to H_2S concentration. H_2S produces a dose-dependent, monophasic constriction of otherwise unstimulated alligator pulmonary artery (**B**) and aortas of duck (**C**) and rat (**D**). In norepinephrine (NE; 1×10^{-7} *M*)-precontracted rat pulmonary arteries (inset in D), 1×10^{-3} *M* H_2S produces a triphasic contraction (*1*), relaxation (*2*), contraction (*3*). Other symbols and abbreviations are as in Fig. 1. Redrawn from 7 with permission.

in precontracted aortas (see below) and, to our knowledge, is the first report of H_2S contractions in any mammalian vessel. However, it is also the only study of H_2S effects in unstimulated vessels from mammals.

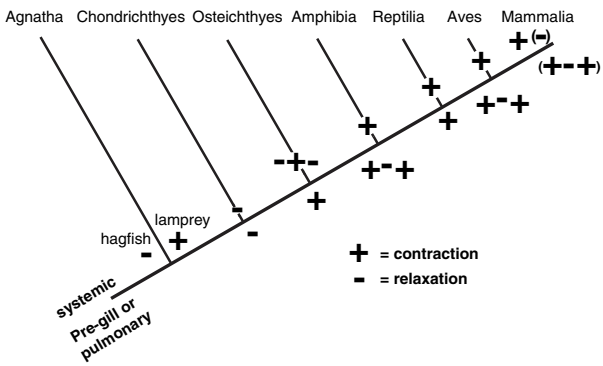

FIG. 3. Cladogram illustrating the phylogenetic progression of the response of systemic (symbols above thick line) or either pre-gill or pulmonary (symbols below line) vessels to H_2S. + represents contraction, − relaxation. Groups of symbols represent multiphasic responses. H_2S has essentially similar effects in otherwise unstimulated vessels, or ligand-precontracted vessels in all vertebrates except mammals. In the rat, H_2S contracts otherwise unstimulated aortas (+), whereas it relaxes precontracted aortas (−) and has multiphasic effects on pulmonary arteries (+−+).

Multiphasic responses to H_2S that embody both dilatory and constrictory components have been observed in vessels from four vertebrate classes, Osteichthyes, Amphibia, Aves, and Mammalia, and only in pulmonary vessels in the latter three. In the efferent branchial (EBA) and celiacomesenteric arteries (post-gill, systemic conductance arteries) and anterior cardinal vein (systemic vein) from rainbow trout, H_2S produces a triphasic, relaxation–contraction–relaxation (Fig. 1D) (see also 6). This response is independent of preexisting tone, and distinct EC_{50} values have been obtained for the latter two phases indicating that they are distinct processes. Specific properties of these two responses have been examined in some detail, and they will be described in the following section. Conversely, H_2S produces an apparent contraction–relaxation–contraction in pulmonary arteries of the marine toad (Fig. 2A), duck (Fig. 2C), and rat (inset, Fig 2D). The details of this response in the toad have not yet been thoroughly examined, although it is evident from Fig. 2A that the initial contraction is prevalent at lower H_2S concentrations (10^{-4} *M*), whereas at higher H_2S concentrations (~10^{-3} *M*) the second-phase relaxation predominates, and at still higher H_2S concentrations (~10^{-2} *M*) the third-phase contraction becomes most pronounced. The inability of the vessels to recover from the highest dose of H_2S, however, may be indicative of irreversible pathological changes. In the duck, both second (relaxation) and third (contraction) phases are dose-dependent with EC_{50} values of 5×10^{-5} *M* and 2×10^{-3} *M*, respectively. A triphasic contraction–relaxation–contraction in response to 10^{-3} *M* H_2S is also found in rat pulmonary arteries precontracted with norepinephrine (inset, Fig. 2D). This response can be reversed upon washout of H_2S, but has not yet been further characterized.

A monophasic, dose-dependent relaxation has been observed only in the dorsal aorta of the hagfish (*Eptatretus stouti*, Agnatha; threshold ~ 10^{-4} *M*) and in Mammalia (15, 43, 44). Relaxation in hagfish aorta is independent of preexisting stimulation (Fig. 1A), whereas in otherwise unstimulated rat aortas, H_2S paradoxically vasoconstricts (Fig. 2D). A single, threshold concentration of H_2S (3×10^{-4} *M*) relaxes otherwise unstimulated dorsal aorta, ventral aorta, and afferent branchial arteries (the latter two are pre-gill vessels) of the sandbar shark, *Carcharhinus milberti* (Chondrichthyes); subsequent higher H_2S concentrations are ineffective. If the response in the shark is dose-dependent, then it is complete between 10^{-4} and 10^{-3} *M* H_2S; this has not yet been examined. When stimulated with an agonist, such as norepinephrine, shark vessels often exhibit spontaneous contractions (30). Under these conditions, the same threshold concentration of H_2S nearly completely inhibits both the spontaneous contractions and overall tone of dorsal aortas (Fig. 1C) and afferent branchial arteries (pre-gill), but does not appreciably affect the ventral aorta. A threefold increase in H_2S concentration (to 10^{-3} *M*) does not augment the relaxation, but the spontaneous contractions and tone return if H_2S is removed from the bath. Monophasic, dose-dependent relaxation of the precontracted rat aorta has been demonstrated repeatedly by others (15, 43, 44) and observed in our laboratory (Dombkowski *et al.,* unpublished observations).

The physiological relevance of vascular responses produced by higher H_2S concentrations remains to be determined. Plasma H_2S in the rat ranges between 4.5×10^{-5} *M* and 3×10^{-4} *M*

(40, 41, 44), and those in the trout are ~4×10^{-5} *M* (6). This may represent the minimum H_2S concentration experienced by vascular smooth muscle as both rat (15, 40, 44, 45) and trout (see below) vessels possess the enzymatic capacity for intravascular H_2S production, implying a gradient from tissue to plasma. Nevertheless, it seems quite possible that under certain circumstances, especially in aquatic environments where ambient H_2S levels may be high (1; also see below), plasma H_2S concentrations in the vascular wall could be in the millimolar range. Because the blood pressure of fish and amphibians is only 20–30 mm Hg, which is considerably lower than that of birds and mammals, H_2S may have had more utility as a vasoconstrictor in aquatic vertebrates and their ancestors.

CARDIOVASCULAR EFFECTS *IN VIVO*

H_2S produces both vasoconstriction and dilation in a number of different trout vessels (6; also see below). In isolated EBA, both the dose-dependent H_2S relaxation and constriction curves are relatively steep, over half of the response occurring within a 10-fold change in concentration (Fig. 4). In addition, the EC_{50} values for relaxation and contraction are close to each other [6.7×10^{-5} versus 1.1×10^{-3} *M,* respectively (6)]. With trout plasma H_2S around 4×10^{-5} *M* (6), it is quite likely that H_2S is tonically active *in vivo*. Furthermore, because trout vessels also synthesize H_2S (see below), intravascular H_2S concentration may exceed plasma levels, and with only a 16-fold difference between dilatory and constrictory EC_{50} values, the transition between H_2S as a dilator and H_2S as a constrictor may be a physiologically relevant and regulated process. Thus, the *in vivo* activity of H_2S in the trout cardiovascular system may be multifaceted.

Given the multiphasic responses of trout vessels *in vitro,* it is no surprise that *in vivo* H_2S produces both depressor and pressor responses. Injection of 4×10^{-5} mol/kg sodium hydrogen sulfide (NaHS) into unanesthetized rainbow trout increases arterial (dorsal aortic) blood pressure by nearly 70% (Fig. 5A). This dose is only three times the NaHS dose (1.4×10^{-5} mol/kg) that decreases rat arterial blood pressure by nearly 30% (44). Because the respiratory and systemic circulations are in series in fish with the heart proximal to the former, arterial blood pressure is simultaneously, but reciprocally, affected by gill and systemic resistance. With only a single measurement of dorsal aortic pressure, it is not possible to determine if H_2S injection causes gill dilation, systemic constriction, or both. However, because ventral aortic (pre-gill) pressure in trout is only ~37 mm Hg (42), which is similar to dorsal aortic pressure following H_2S injection (Fig. 5), if gill dilation were the sole contributor to the H_2S response, then gill resistance would have to fall to zero, which is impossible. Thus, it is quite probable that H_2S generated from injection of 4×10^{-5} mol/kg NaHS produces systemic vasoconstriction in trout. In another fish, 10×10^{-5} mol/kg NaHS elicited a triphasic response consisting of a transient fall, then rise, in pressure that was then followed by a prolonged depression (Fig. 5B). This is surprisingly similar to the response of isolated arteries (Fig. 1D), but it remains to be determined if it is due to uniform responses of systemic resistance vessels or asynchronous changes in gill and systemic resistance. The cardiovascular actions of H_2S in other nonmammalian vertebrates have not been reported.

MECHANISMS OF H_2S VASOACTIVITY

In trout, H_2S produces a triphasic relaxation–contraction–relaxation in both otherwise unstimulated and precontracted (Fig. 1D) EBA (6). The more robust second and third phases have different EC_{50} values (Fig. 4), suggesting that they are

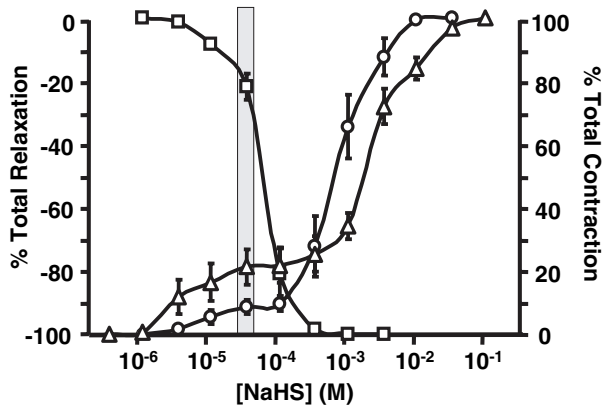

FIG. 4. H_2S dose-response characteristics of phase-2 constriction (ascending lines) and phase-3 relaxation (descending line) in trout EBA to H_2S. Phase-2 responses were examined in both precontracted (circles; 10^{-5} *M* carbachol) and otherwise unstimulated (triangles) vessels, and phase-1 responses (squares) were obtained from carbachol-contracted vessels; *n* = 7 fish for all experiments. Shaded box shows plasma H_2S concentration. Redrawn from 6 with permission.

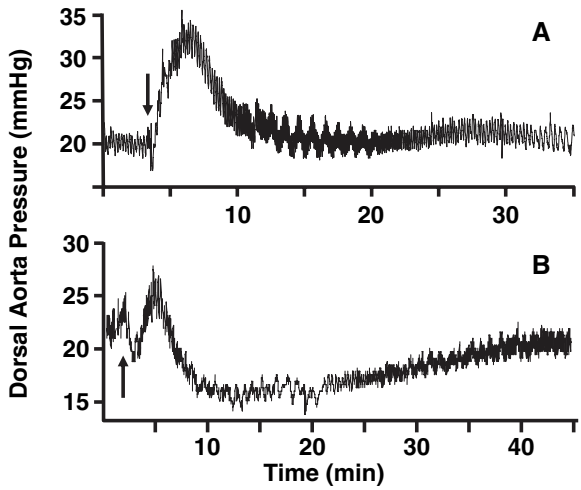

FIG. 5. Effect of bolus H_2S injection (arrows) on dorsal aortic (systemic) blood pressure in unanesthetized trout. (**A**) Pressor response to 4×10^{-5} mol/kg body weight NaHS. (**B**) Long-term depressor response to 10×10^{-5} mol/kg body weight NaHS follows transient depressor–pressor responses.

distinct events and allowing us to tailor the H_2S dose to focus on the mechanisms involved in either relaxation or contraction. The first phase relaxation is less consistent and somewhat more difficult to evaluate. To date, we have examined H_2S responses in two strains of the same species of *O. mykiss*, the steelhead (Skamania strain) and rainbow (Kamloops strain), and the results, although at times disparate, have begun to identify the processes involved. To my knowledge, the mechanisms of H_2S vasoactivity have not been examined in any other nonmammalian vertebrate.

H_2S is less efficacious in phase-3 relaxation of steelhead EBA precontracted with 30 m*M* K^+ than it is in vessels precontracted with either norepinephrine or carbachol (Fig. 6), suggesting involvement of K^+ channels in the H_2S effect. Indeed, the mammalian ATP-dependent K^+ (K_{ATP}) channel inhibitor, glibenclamide, inhibits both phase-1 and -3 H_2S relaxation and augments phase-2 contraction of otherwise unstimulated EBA (Fig. 7). However, it should be pointed out that we (Smith and Olson, unpublished observations) were previously unable to find any evidence for K_{ATP} channels, even after a thorough search, and it remains to be determined if the glibenclamide effects on the H_2S response are nonspecific. In mammals, nonspecific effects of glibenclamide include inhibition of a variety of chloride channels (19, 32), inward rectifying K^+ channels (36), and cyclic AMP-dependent protein kinase (27); any one of these effects could be manifest in the trout. Phase-1 relaxation may be endothelium-mediated (Fig. 7) as it can be blocked by a cocktail of inhibitors of EDRFs, esculetin (lipoxygenase), clotrimazole (cytochrome P-450), and indomethacin (cyclooxygenase), and indirect evidence suggests that much of this effect is due to inhibition of prostaglandin synthesis (6). The involvement of other processes in H_2S relaxation is equivocal. Phase-3 relaxation in steelhead EBA is blocked by soluble guanylate cyclase inhibitors, ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) and NS-2028. However, cyclic GMP production by isolated rainbow trout EBA is actually decreased by H_2S (6), and the effects of ODQ (and perhaps NS-2028) may have been nonspecific, as has been shown in the rat (43). NO is not produced by the endothelium in trout EBA (29) and, unlike the situation in rats (15), NO does not contribute to H_2S relaxation in trout vessels.

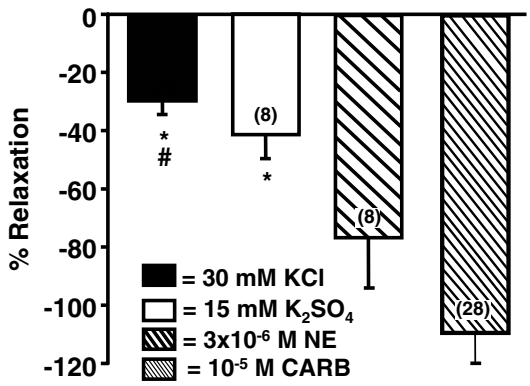

FIG. 6. H_2S (1.1×10^{-4} *M*) relaxation (phase 3) of steelhead EBA precontracted by either potassium depolarization (KCl or K_2SO_4) or the ligands, norepinephrine (NE) or carbachol (CARB). *Significantly different from CARB; #significantly different from NE. *n* is indicated in bars. Redrawn from 6 with permission.

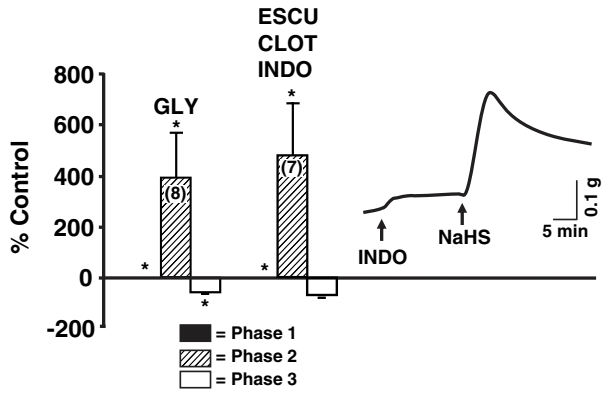

FIG. 7. The effect of glibenclamide (GLY) or a tripartite cocktail of esculetin (ESCU), clotrimazole (CLOT), and indomethacin (INDO; all 10^{-5} *M*) on the response of steelhead EBA branchial arteries to 3.7×10^{-4} *M* NaHS. Treatment effects on each of the three phases (solid, striped, and open bars) are expressed as a percentage of the NaHS (3.7×10^{-4} *M*) response in the same vessel before inhibitor treatment; <100% indicates no change. *Significantly different from control NaHS response ($p < 0.05$); *n* is indicated in bars. Trace at right shows response of an individual vessel to INDO and 3.7×10^{-4} *M* NaHS; note predominance of phase-2 contraction (upward pen deflection).

Phase-2 contraction of steelhead EBA involves activation of light chain kinase (LCK) as it can be partially inhibited by the LCK inhibitor, ML-9 (6). The involvement of extracellular and intracellular Ca^{2+} stores in the contraction is unclear. In initial studies, we (6) showed that the L-type Ca^{2+} inhibitor, methoxyverapamil (D-600), had no effect on NaHS contractions in steelhead EBA, whereas the L-type Ca^{2+} inhibitor, nifedipine, reduced contractions in rainbow trout EBA by 20% and removal of extracellular Ca^{2+} inhibited rainbow trout responses by 50%. In subsequent studies (Dombkowski and Olson, unpublished observations), we found that both D-600 and removal of extracellular Ca^{2+} significantly increased the NaHS response in steelhead EBA ($n = 7$ and 8, respectively), and nifedipine ($n = 8$) had no significant effect (although the average contraction increased 18%). Clearly, steelhead EBA do not require extracellular Ca^{2+} for phase-2 contraction. Why steelhead and rainbow trout EBA respond differently remains to be determined. Figure 8 summarizes potential pathways of H_2S relaxation and contraction in trout arteries.

H_2S PRODUCTION IN NONMAMMALIAN VESSELS

Homogenates of rat vessels have been shown to produce H_2S, predominantly through the enzymatic activity of cystathionine γ-lyase (CSE) (15, 40, 44, 45). We (Dombkowski, Schul-

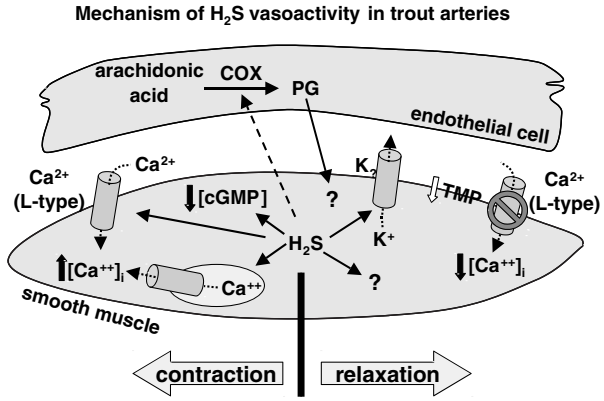

FIG. 8. Summary of potential mechanisms leading to H_2S-mediated relaxation or contraction of steelhead and rainbow trout EBA. Relaxation may be mediated in part by K^+-channel activation, which may hyperpolarize transmembrane potential (TMP) and lower intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). It is unclear if K_{ATP} channels are utilized. H_2S stimulation of endothelial cyclooxygenase (COX) produces a vasodilatory prostaglandin (PG) contributing to the relaxation. Other pathways in smooth muscle cells may also be involved. H_2S-mediated contraction of smooth muscle increases $[Ca^{2+}]_i$ from intracellular stores, and in rainbow trout, via L-type Ca^{2+} channels. H_2S also lowers intracellular cyclic GMP, which may contribute to the contraction.

man, Doellman, and Olson, manuscript in preparation) recently measured H_2S production by homogenized vessels from steelhead trout (Fig. 9). In the presence of 10 m*M* L-cystine and 2 m*M* pyridoxal 5′-phosphate (45), muscular conductance arteries (efferent branchial and celiacomesenteric) produced ~9×10^{-9} nmol/wet tissue weight per minute, whereas the less muscular dorsal aorta generated H_2S at only one-third this rate. H_2S production by anterior cardinal veins was nearly 50% greater than arterial production. Although our methods differ from those used in previous studies on rats in that we used ion-selective electrodes as opposed to wick-diffusion and methylene-blue colorimetry, it is worth noting that, with the exception of the aorta, H_2S production by trout vessels is equivalent to, or greater than, that reported for rat vessels in spite of the fact that the trout vessels were incubated at 22°C and rat vessel homogenates were incubated at 37°C.

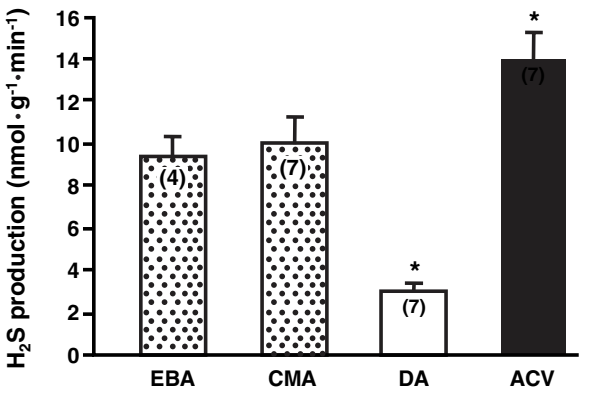

FIG. 9. H_2S production by trout vessel homogenates at 22°C and in the presence of 10 m*M* L-cystine and 2 m*M* pyridoxal 5′-phosphate. EBA, efferent branchial artery; CMA, celiacomesenteric artery; DA, dorsal aorta; ACV, anterior cardinal vein. *Significantly ($p < 0.05$) different from either EBA or CMA; *n* is in bars. Dombkowski, Schulman, Doellman, and Olson, unpublished observations.

H_2S production in trout vessels is partially (50%) inhibited by CSE inhibitors DL-propargylglycine (20 m*M*) or β-cyano-L-alanine (βCAN; 5 m*M*). Thus, the involvement of CSE in vascular H_2S production appears to have a phylogenetic precedent. The cystathionine β-synthase (CBS) inhibitor aminooxyacetate (AOAA) does not affect H_2S production by rat thoracic aortas or portal veins at concentrations up to 1 m*M* (15), and there is little evidence to support intravascular H_2S generation via CBS in mammals. However, we also observed that 3 m*M* AOAA did inhibit H_2S production by trout EBA. This concentration of inhibitor is greater than that used in mammalian studies, and it remains to be determined if there are nonspecific effects of AOAA at these higher concentrations.

There is little information on regulation of H_2S production by trout vessels. It is well known that NO, or NO mimetics, potentiate the relaxant effects of H_2S in rat vessels (15, 39, 41). Given the lack of a vascular NO system in trout (29), one would not expect such a synergism, and indeed, this appears to be the case. We (Dombkowski, Schulman, Doellman and Olson, manuscript in preparation) have found that H_2S production by steelhead vessels is unaffected by the exogenous NO donor, sodium nitroprusside (SNP), even at SNP concentrations (10^{-5} *M*) sufficient to relax these vessels (34) and increase vascular cyclic GMP production nearly 100-fold (6). Trout vessels do, however, produce prostanoid EDRFs (29), and it will be interesting to determine if they provide an alternative mechanism with which to regulate H_2S production.

H_2S availability

A number of questions remain regarding the impact of the environment on H_2S chemistry and potential for vasoactivity, especially in ectothermic and aquatic vertebrates. Dissolved H_2S exists in the equilibrium:

$$H_2S \overset{k_a}{\rightleftarrows} H^+ + HS^- \overset{k_b}{\rightleftarrows} H^+ + S^{2-} \quad (1)$$

With the pK_b nearly 12 (2), S^{2-} is essentially nil in biological systems and can be ignored. However, the pK_a for H_2S dissociation is well within the pH of physiological fluids, and the fraction of dissolved sulfide as H_2S can vary considerably. Furthermore, the pK_a is temperature-dependent according to the relationship:

$$pK_a = 3.122 + 1{,}132/T \quad (2)$$

where *T* is degrees Kelvin (2). The temperature range over which the pK_a was determined was 10–25°C (2). Extrapolating this to mammalian body temperature (37°C) and pH (7.4),

the predicted pK_a for H_2S is 6.77, and 19% of the total sulfide (H_2S plus HS^-) will exist as H_2S.

The H_2S concentration in ectothermic vertebrates becomes complex as both the pK_a for H_2S and the plasma pH change with temperature. It is evident from Eq. 2 (2) that the pK_a will change from 7.27 at 0°C to 6.74 at 40°C. Over this temperature range, plasma pH can be as high as 8.4 at 0°C and as low as 7.3 at 40°C (31). Assuming all other factors (H_2S production, metabolism, etc.) remain constant, the percentage of total sulfide existing as H_2S can vary from 50% at 0°C to 22% at 40°C. It remains to be determined if plasma H_2S concentration is regulated by adjustments in total plasma sulfide, or if tissue sensitivity or other factors are adjusted to compensate for variations in plasma H_2S concentration. It will also be interesting to determine if regulation occurs at the species and/or individual level in order to compensate for variation in habitat, or even diurnal fluctuations.

Water-breathing vertebrates, most of which are fish, face an even greater challenge imposed by their environment because total sulfide, ambient pH, and dissolved oxygen content can change abruptly, diurnally, or seasonally. Total sulfide in sediment can exceed millimolar levels, and it can vary locally and regionally (see 1 for an excellent review). Agitation of this sediment, acidification, and/or hypoxia can potentially expose fish to high H_2S concentrations, and because only the gas appears to readily traverse epithelial tissues (1), plasma titers and sulfide balance may be affected. This can be further exacerbated by industrial and agricultural H_2S production (1). The following paragraphs illustrate a few of these points.

Unlike a terrestrial environment where pH is irrelevant, pH in an aquatic environment will determine the proportion of H_2S relative to total sulfide, and this may substantially affect transepithelial H_2S flux. Equation 2 permits an estimation of the fraction of ambient sulfide present as H_2S in the aquatic environment. For the Lake Magdi tilapia (*Oreochromis alcalicus grahami*) that inhabits water with a pH in excess of 10 and temperature exceeding 40°C, (17), ambient H_2S concentration will be essentially nil (<0.05% of total). On the other end of the spectrum, many fish can survive in pH 5.0 water (8, 9), and some, such as the tetras, can survive pH 3.5 indefinitely (8). Irrespective of temperature, when ambient pH is 5 or lower, essentially 100% of the total sulfide exists as H_2S (99.9% at 0°C and 98.2% at 40°C). Even at a pH of 6, cold and temperate water fish will experience >90% of total sulfide as H_2S. With increasing acidification of lakes and streams from acid rain (12, 13, and references therein), the bioavailability of H_2S will only increase and so will the potential for toxicity.

In well-mixed, oxygenated water, H_2S is thought to be rapidly oxidized and probably of little consequence. However, because the oxygen capacitance coefficient of water is only 1/30th that of air and diffusion is 200,000 times slower (3), aquatic hypoxia is not uncommon and could potentially foster elevated H_2S. Aquatic hypoxia can result from rapid mixing of the sediment, and from tidal, diurnal, seasonal, or continual (below 50–100 m depth) decreased photosynthesis relative to oxygen demand (3, 26, 33). Fish such as the carp forage in the sediment and, being extremely anoxia-tolerant (24), could face high ambient H_2S titers, perhaps accounting for their higher H_2S tolerance (33). Other vertebrates, such as amphibia or reptiles (turtles), spend time buried in the sediment and could potentially experience elevated and prolonged H_2S levels. The fact that many of the effects of H_2S mimic those of hypoxia makes it difficult to identify their respective impact.

Another function of H_2S?

Carbon dioxide (CO_2) is the only known volatile buffer; could H_2S be the second? H_2S has several attributes that suggest it may be a physiologically important buffer: (a) the pK_a for H_2S is close to the pH of blood and intracellular fluids; (b) the association/dissociation products are a gas and ions, respectively; (c) plasma and tissue sulfide levels are 1,000-fold greater than plasma H^+ concentration; and (d) tissue production can be regulated. Although plasma sulfide levels are considerably lower than those of bicarbonate, the sulfide buffer may still have utility. The pK_a for sulfide is closer to blood pH than that of bicarbonate (6.1), making it a more efficient buffer. Sulfide production can be physiologically regulated (22, 40, 41, 44, 45), unlike CO_2 which is obligated to follow oxidative metabolism. Spontaneous hydration of CO_2 is slow enough to require the enzymatic assist of carbonic anhydrase, which may not be the case for sulfide. Finally, as only one of the reactants is a gas, there is the capacity for independent regulation of H_2S and HS^- concentrations.

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ABBREVIATIONS

AOAA, aminooxyacetate; CBS, cystathionine β-synthase; CO, carbon monoxide; CO_2, carbon dioxide; CSE, cystathionine γ-lyase; D-600, methoxyverapamil; EBA, efferent branchial arteries; EC_{50}, effective concentration producing half-maximal response; EDRF, endothelium-derived relaxing factor; H_2S, hydrogen sulfide; K_{ATP} channel, ATP-dependent K^+ channel; LCK, light chain kinase; NaHS, sodium hydrogen sulfide; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one); SNP, sodium nitroprusside.

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Address reprint requests to:
K.R. Olson, Ph.D.
SBCME, B-19 Haggar Hall
University of Notre Dame
Notre Dame, IN 46556

E-mail: olson.1@nd.edu

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