Hypoxic vasoconstriction of cyclostome systemic vessels: the antecedent of hypoxic pulmonary vasoconstriction?

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Olson, Kenneth R., Michael J. Russell, and Malcolm E. Forster. Hypoxic vasoconstriction of cyclostome systemic vessels: the antecedent of hypoxic pulmonary vasoconstriction? Am J Physiol Regulatory Integrative Comp Physiol 280: R198–R206, 2001.—Hypoxic vasoconstriction (HV) is an intrinsic response of mammalian pulmonary vascular smooth muscle (VSM). In the present study, HV was examined by myography of vessel rings from three primitive vertebrates: New Zealand hagfish (NZH), Pacific hagfish (PH), and sea lamprey (SL). Hypoxia dilated pre-gill arteries (ventral aorta, afferent branchial) from all species, whereas it contracted systemic arteries [dorsal aorta (DA), efferent branchial, celiaomesenteric]. DA HV was reproducible over several days, and it could be sustained in NZH for 8 h without adverse effects. Tension was proportional to PO2, and half-maximal HV was obtained at PO2 (mmHg) of 4.7 ± 0.2 (NZH), 0.8 ± 0.1 (PH), and 10.7 ± 1.9 (SL). HV did not require preconditioning (preexisting contractile stimulus) and was unaffected by elevated extracellular potassium (200 mM NZH; 80 mM SL); removal of the endothelium (NZH); or inhibitors of cyclooxygenase, lipoxygenase, cytochrome P-450 or antagonists of α-adrenergic, muscarinic, nicotinic, purinergic, or serotoninergic receptors. These results show that HV is an intrinsic feature of systemic VSM in cyclostomes and suggest that HV has been in the repertoire of VSM responses, since the origin of vertebrates. The exceptionally hardy HV in cyclostome DA may provide a useful model with which to examine both the phylogeny and mechanisms of this response.

vascular smooth muscle; hagfish; lamprey

HYPOXIA HAS PROFOUND but dichotomous effects in mammalian vascular smooth muscle (VSM). In systemic vessels, hypoxia produces vasodilation and provides a local means of matching blood flow with metabolic demand. Hypoxic vasodilation is believed to be accomplished directly at the level of the smooth muscle, probably through potassium channels (K+ATP) that close when the cellular ADP-to-ATP ratio increases (19, 25). This hyperpolarizes and relaxes the cell, and the resulting vasodilation restores oxygen delivery. Hypoxia produces a paradoxical vasoconstriction in the pulmonary circulation. Hypoxic pulmonary vasoconstriction (HPV) is mediated by both endothelial and direct smooth muscle effects (reviewed in Refs. 5, 12, 24, 25), and it matches perfusion to ventilation by decreasing blood flow to underventilated alveoli. Intrinsic hypoxic vasoconstriction (HV) of pulmonary VSM may also be mediated by potassium channels, although the exact mechanism of stimulus-response coupling is unknown.

The effects of hypoxia on nonmammalian vertebrates have received less attention, although there appears to be a trend for vasodilation of the systemic and vasoconstriction of the respiratory circuits. In bony fish, such as the rainbow trout, Oncorhynchus mykiss, hypoxia relaxes systemic conductance arteries in vivo (18) and it constricts gill vessels in both trout (21) and cod, Gadus morhua (20). It is not clear whether these effects in fish are direct or paracrine mediated.

As part of our ongoing study of the phylogeny of VSM control in fish, we examined the hypoxic response of vessels from the most primitive fish, the New Zealand and Pacific hagfish (Eptatretus cirrhatus and E. stoutii, respectively) and the sea lamprey (Petromyzon marinus). Surprisingly, a profound HV was observed in post-gill, but not pre-gill, systemic vessels from both cyclostomes. These responses are examined in detail in the present study. Our findings show that there is a number of similarities between HV in systemic vessels of cyclostomes and pulmonary vessels of mammals. This indicates that HV has a long lineage in vertebrate phylogeny. Our results also suggest that the cyclostomes may be useful models with which to examine the mechanism(s) of hypoxia-induced vasoconstriction.

MATERIALS AND METHODS

Animals

New Zealand hagfish (Eptatretus cirrhatus, 800–2,100 g) were collected off Motunau Beach, New Zealand, and were transferred to the University of Canterbury in Christchurch where they were held in aquariums containing running seawater (16°C). They were held at least 1 wk before experimentation and were not fed during this period. Pacific hagfish (Eptatretus stoutii, 80–150 g) were captured off the coast of
California near San Diego and maintained in saltwater aquariums at Scripps Oceanographic Institute until transfer to the University Notre Dame. At Notre Dame, they were maintained in a 300-gallon recirculating aquarium containing aerated artificial seawater (12°C; Instant Ocean Aquarium Systems, Eastlake, OH). They were not fed. New Zealand hagfish were anesthetized in MS-222 (ethyl-maminobenzoate; 1:2,500, wt/vol) and benzocaine (ethyl-paminobenzoate; 1:2,500, wt/vol), and Pacific hagfish were anesthetized in benzocaine (1:5,000, wt/vol). Vessel segments were dissected out, rinsed with a modified hagfish HEPES-buffered saline (HHBS), and stored in fresh HHBS at 4°C until use.

Sea lamprey (Petromyzon marinus, 130–450 g) were captured by the US Geological Survey, Biological Resources Division, in Michigan during the spring-summer spawning migration and airlifted to Notre Dame. At Notre Dame, they were housed in 500-liter rectangular tanks in aerated, flowing well water (15°C) and exposed to a 12:12-h light-dark photoperiod. They were not fed. Lamprey were anesthetized in benzocaine (1:5,000, wt/vol), and the vessels were dissected out and placed in lamprey HEPES-buffered saline (LHBS) at 4°C.

VSM

The vascular anatomy of the three cyclostomes is basically similar, with the gills and systemic vessels in series. Blood is pumped from a single ventricle into a single (lamprey) or paired (hagfish) ventral aorta (VA), which then branches to form the afferent branchial arteries (ABA) supplying the gills. Postbranchial blood exits the dorsal aspect of the gills via the efferent branchial arteries (EBA), the latter subsequently anastomose to form a single dorsal aorta (DA) that travels the length of the fish just beneath the spine. A single celiacomesenteric artery (CM) branches from the DA and perfuses the splanchnic circulation.

DA, EBA and ABA, and VA from New Zealand hagfish; DA and VA from Pacific hagfish and lamprey; and CM from lamprey were cut transaxially into 3- to 4-mm-long rings. Rings were hung on 280-μm stainless steel hooks and suspended in 20-ml water-jacketed (15°C), smooth muscle chambers (13) containing the appropriate saline. In experiments with Pacific hagfish and lamprey, tension was measured with Grass FT03C force-displacement transducers (West Warwick, RI) and recorded on either a computer-interfaced Gould 8000 series or Grass model 8TC polygraph. Data were collected electronically using Labtech Notebook data-collection software (Laboratory Technologies, Andover, MA). In experiments with New Zealand hagfish, VA, ABA, EBA, and DA tension were measured with Ugo Basile (Comerio, Italia) isometric force transducers (model 7004) and the signals were amplified with Gould (Valley View, OH) transducer preamplifiers (model 13–4615–50). Signals were displayed and digitized on a Yokogawa LR4100E recorder (Yokogawa Electric, Tokyo, Japan). In experiments with some of the New Zealand hagfish ABA and EBA, a myograph and myo-interface 410A (JP Trading, Aarhus, Denmark) were used. In all instances, polygraph sensitivities were set to detect changes at least as small as 5 mg.

Optimal resting tension for the different types of vessels used in this study was determined in preliminary experiments by measuring the magnitude of 80 mM (lamprey) or 150 mM (hagfish) KCl contractions over a range of resting tension from 0 to 1.5 g. This resting tension, 300–500 mg for EBA and ABA, or 500–750 mg for DA and VA, was subsequently applied to all experimental vessels at least 1 h before experimentation. During this hour, vessels were precontracted with either potassium chloride (KCl; 80 mM), the acetylcholine analog carbachol (carbachol, 10−5 M), or epinephrine (Epi; 10−5 M) and washed three times or bubbled with 100% nitrogen gas (N2) for 15–20 min and returned to room air. Baseline tension was then reestablished for at least 30 min before further experimentation.

In all experiments, hypoxia was administered by aerating the muscle chambers with 100% N2 gas and normoxia was restored by aeration with room air. The effects of hypoxia on resting (unstimulated) or precontracted vessels was examined, and various combinations of enzyme inhibitors and receptor antagonists were used to determine if paracrine or endothelium-derived mediators contributed to the hypoxic response. Agonists and drugs were applied 1 h (New Zealand hagfish) or 15 min (Pacific hagfish and lamprey) before hypoxic exposure. The effect of temperature on the hypoxic response was examined in the lamprey by first exposing the vessels to hypoxia at 15°C and then increasing the temperature to 22°C over 1 h and repeating the hypoxia.

In another series of experiments, the endothelium was removed from New Zealand hagfish DA by impaling the vessel on a wooden stick and gently twisting the stick against the lumen. Removal of endothelium was verified by scanning electron microscopy (see below).

The relationship between Po2 and smooth muscle tension was examined in DA from all three species. Vessels were aerated with step-wise reductions in Po2 at intervals sufficiently long to allow tension to plateau (~20 min for Pacific hagfish and lamprey and 45 min to 1 h for New Zealand hagfish). In a few experiments, the Po2 was stepped down to zero then back up to room air (using the same incremental steps), and 1–2 h later the whole process was repeated. Po2 was regulated by mixing room air with 100% N2 (Po2 from 150 to 7 mmHg) or 1% O2-99% N2 with 100% N2 (Po2 from 7 to 0 mmHg) using a Wosthoff gas-mixing pump. During these experiments, the muscle chambers were gassed vigorously and they were covered with Plexiglas or parafilm lids to minimize oxygenation from the atmosphere. In preliminary experiments, a calibrated Po2 electrode (Cameron Instruments, Port Aransas, TX, or Microelectrodes, Londonderry, NH) was placed in one of the muscle chambers to verify the Po2 and to monitor the rate of change in Po2 when the gas mixtures were changed. With this method, a steady-state Po2 was attained within 60 s after the gas mixture was changed.

Scanning Electron Microscopy

Preparation of fish vessels for scanning electron microscopy (SEM) has been described previously (14). Briefly, the vessels were fixed in 5% glutaraldehyde while suspended under tension in the myograph chamber. They were then removed from the chamber, rinsed twice for 1 h in distilled water, dehydrated in a graded series of distilled water and tert-butyl alcohol, and freeze-dried. When dry, they were cut longitudinally, affixed lumen side up on the SEM specimen stub, coated with gold, and examined with a Leica, model S 440 SEM.

Chemicals

The composition of LHBS was as follows (in g/l): 8.74 NaCl, 0.22 KCl, 0.29 CaCl2·2 H2O, 0.14 MgSO4·7H2O, 0.72 HEPES acid form, 1.8 HEPES sodium salt, 0.9 glucose, pH 7.8. The composition of HHBS was as follows (in g/l): 27.70 NaCl, 0.60 KCl, 0.75 CaCl2·2 H2O, 0.75 MgSO4·7H2O, 0.72 HEPES acid form, 1.82 HEPES sodium salt, 1.00 glucose, pH 7.8. All other chemicals were purchased from Sigma (St. Louis, MO).
Calculations

At the end of an experiment, the vessel was blotted on paper toweling and weighed, and vessel tension was normalized to wet weight, i.e., milligrams tension per milligram wet weight. Because the hypoxic responses of individual vessels were reproducible, each vessel served as its own control and treatment effects were statistically examined by paired t-test or repeated-measures tests. Student’s t-test and ANOVA were used for comparisons between vessels. The fiducial limit of significance was P $\leq$ 0.05.

RESULTS

Figure 1 shows the response of pre- and post-gill vessels to hypoxia. The pre-gill vessels, VA, and ABA in the New Zealand hagfish transiently contracted then relaxed back to or below initial resting tension. The maximum contraction of VA was $1.2 \pm 0.4$ mg/mg wet wt ($n = 10$ fish) and of ABA was $5.1 \pm 1.5$ mg/mg wet wt ($n = 9$ fish). Hypoxia relaxed VA from Pacific hagfish, whereas lamprey VA slightly contracted or were unaffected.

Hypoxia produced a profound contraction in all post-gill vessels examined, i.e., EBA in the hagfish and DA in all three species (Table 1). Hypoxia was also as potent a constricting stimulus to lamprey celiacomesenteric arteries as it was to the DA (Table 1). The magnitude of DA hypoxic contraction normalized to vessel weight was greatest in the lamprey and lowest in the New Zealand hagfish. Contraction was further increased in lamprey DA by warming the vessels to 22°C. At 22°C, lamprey DA developed nearly twice as much tension per unit vessel weight as comparably sized rat aorta contracted by 80 mM KCl (Table 1).

HV in the New Zealand hagfish had two components, an initial fast-onset contraction that lasted between 10 and 30 min followed by a slower steady increase in tension. In many vessels, the slow phase lasted for 2 h or more and it could account for as much as 30–50% of the total tension.

DA from New Zealand hagfish were responsive to hypoxia for extended periods. Contraction of vessels ($n = 4$ fish) exposed to hypoxia by aeration with N$_2$ continuously for 8 h remained within 70% of maximum (not shown). On return to normoxia, the vessels relaxed back to resting tension and a subsequent N$_2$ exposure produced a contraction of similar magnitude to the first. The effects of extended hypoxia were not examined in the Pacific hagfish or lamprey, although lamprey DA maintained a steady-state contraction when hypoxia was applied for 1 h.

Repeated exposure to hypoxia did not affect the magnitude of vasoconstriction by hagfish or lamprey DA. Figure 2 shows the effect of two consecutive hypoxic treatments, the second applied within 20 min (lamprey) or 3 h (hagfish) of the first (longer in hagfish because of the slow relaxation after first exposure). In some lamprey DA, as many as seven hypoxic treatments were applied in a single day with no diminution in response. Hypoxic contractions from all fish were also reproducible when the DA was maintained in the myographs for 3–4 days (not shown).

Table 1. Magnitude of hypoxic contraction in post gill vessels

<table>
<thead>
<tr>
<th></th>
<th>Posterior Dorsal Aorta</th>
<th>Anterior Dorsal Aorta</th>
<th>Efferent Branchial Artery</th>
<th>Celiacomesenteric Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand hagfish</td>
<td>25.9 ± 1.2 (89)</td>
<td>23.3 ± 1.9 (3)</td>
<td>23.8 ± 6.7 (7)</td>
<td>—</td>
</tr>
<tr>
<td>Pacific hagfish</td>
<td>97.4 ± 27.8 (5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sea lamprey</td>
<td>125.2 ± 14.2 (18)</td>
<td>144.8 ± 28.3 (6)</td>
<td>—</td>
<td>176.3 ± 38.2 (4)</td>
</tr>
<tr>
<td>15°C</td>
<td>213.4 ± 83.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>22°C</td>
<td>464.0 ± 142.9 (5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rat (80 mM KCl)</td>
<td>239.7 ± 21.9 (7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SE of mean active (total minus resting) tension (mg/mg wet wt) ($n =$ no. of rings from 3–20 animals, except for sea lamprey at 15 and 22°C, where $n =$ no. of fish). Rat values are from abdominal aorta. ($n =$ no. of rats); Russell and Olson, unpublished observations.
HV of the DA (and vasodilation of the VA) appeared to occur independent of a preexisting tonus of the vessel or the nature of the agonist used to apply this tonus. The transient HV and subsequent vasodilation of the New Zealand hagfish VA were unaffected by a preexisting contraction produced by elevated KCl (Fig. 3) or $10^{-6}$ M carbachol (not shown). Similarly, elevated KCl did not affect the HV in DA from New Zealand hagfish (Figs. 2 and 3), Pacific hagfish (not shown), or lamprey (Fig. 2). Figure 3 also shows that 150 mM KCl produced the maximal response in both VA and DA of the New Zealand hagfish; an additional 50-mM increase in KCl had minimal effect on the initial contraction (pilot studies with these vessels showed that 150 mM KCl produced the maximal contraction). Precontraction of New Zealand hagfish DA with carbachol ($10^{-6}$ M) did not affect the magnitude of HV (not shown), whereas epinephrine ($10^{-6}$ M) precontraction of lamprey DA actually enhanced the magnitude of HV (Fig. 4). Furthermore, the epinephrine enhancement effect persisted when the entire protocol (hypoxia, epinephrine during normoxia, hypoxia during epinephrine background) was repeated (Fig. 4).

HV of DA from all three species of fish was reversibly dependent on oxygen tension, and the magnitude of contraction could be titrated with $P_{O_2}$. Figure 5 shows the response of an individual lamprey DA to a stepwise decrease, then increase, in $P_{O_2}$, the dose-response curves for all three species are shown in Fig. 6. In a few vessels, this process was repeated a second time and the dose-response curves were virtually identical (not shown). The $P_{O_2}$ at which half-maximal contraction.
was obtained (P50) was highest (10.7 mmHg) in the lamprey and lowest (0.8 mmHg) in the Pacific hagfish. The P50 for the New Zealand hagfish was 4.7; however, the slow response time of DA from this fish made construction of the dose-response curves a little more difficult. P50 values from the three species were all significantly different (P < 0.05) from each other. Some hysteresis was observed when PO2 was returned to ambient, and the P50 values obtained during the return from 100% N2 back to room air were slightly lower than those obtained when PO2 was lowered. The hysteresis was most notable in the New Zealand hagfish, probably due to a large extent to the slow response time of these vessels. The P50 values reported are only those obtained when PO2 was lowered from ambient to 0% O2.

The endothelium from New Zealand hagfish DA was effectively removed by abrasion with a wooden stick (Fig. 7). This procedure reduced the contractility of the vessels to all agonists by 15–35% (not shown); however, it did not significantly affect the ratio of tension produced by a hypoxic contraction to that produced by carbachol (Table 2).

HV of the New Zealand hagfish DA was unaffected by inhibitors of cyclooxygenase (indomethacin; 10−5 M), lipooxygenase (esculetin; 10−5 M), and cytochrome P-450 (clotrimazole; 10−5 M) or by antagonists of α-adrenergic (phenolamine; 10−5 M), muscarinic (atropine; 10−4 M), nicotinic (hexamethonium; 10−4 M), purinergic (aminophylline; 10−5 M), and serotonergic (methysergide maleate; 10−5 M) receptors (Table 3). Similarly, HV of the lamprey DA was not affected by pretreatment with indomethacin, esculetin, clotrimazole, phenolamine (all 10−5 M), or the purinergic A1 receptor antagonist 8-phenyltheophylline (10−4 M; Table 3).

DISCUSSION

The present experiments showed that hypoxia produced a profound and sustained contraction of post-gill, but not pre-gill, vessels in two species of hagfish and the sea lamprey. This HV was reproducible over multiple hypoxic episodes and could be repeated for several days. It occurred independent of any preexisting tone, and it was not diminished by precontraction of the vessel with either elevated KCl or a ligand. The magnitude of HV could be titrated with PO2, and there were species-specific differences in the P50. HV was endothelium independent, and it was unaffected by a variety of enzyme inhibitors or receptor antagonists known to influence HV in the mammalian lung. These results indicate that HV is an intrinsic and vessel-specific property of VSM in the cyclostomes, and it is, therefore, a phylogenetically ancient response.

Comparison of Hypoxic Responses in Cyclostome and Mammalian Vessels

HV in cyclostomes has a number of similarities to HPV observed in mammals, yet there are also differences. Both HV and HPV are reproducible on repeated hypoxia, both contractions are sustained, both are (at least in part) intrinsic to VSM, the magnitude of their response is PO2 dependent, and both responses are specific for certain vessels. HV is unlike HPV in that HV does not require preconditioning, it is present in systemic vessels, it may be independent of potassium channels, and it has no endothelium-dependent component. The similarities between HV and HPV suggest that both responses share a common basic mechanism, whereas the differences between them appear to be more likely due to evolutionary embellishment of the latter.

Similarities Between Cyclostome HV and Mammalian HPV

Reproducibility. The ability to produce virtually identical contractions on repeated hypoxic exposure, not only permits use of each vessel as its own control, but it also indicates that HV is dependent on a substrate or signal that is quantitatively monitored and probably not rapidly depleted from the cell. This was evident in the New Zealand hagfish. After 8 h of hypoxia and a brief period of normoxia, the contraction produced by a second hypoxic treatment was identical...
to the first. Similarly, repeated hypoxic episodes over one or several days did not diminish HV in the lamprey. To our knowledge, there has not been as extensive an examination of the reproducibility of mammalian HPV; however, most studies have shown that the response can be repeated several times with no diminution of response (8, 9).

**Sustainability.** In our experiments, HV was not a transient response and it could be sustained from 1 (lamprey) to 8 h (New Zealand hagfish). In mammals, the sustainability of HV is variable. In perfused rat lungs, HPV is stable (see Ref. 24), whereas in isolated vessels such as the rat pulmonary artery, the contraction has an initial transient peak and then a relaxation followed by a sustained contraction of at least 1 h (2). Some, if not much, of the variability in pulmonary vessels is undoubtedly due to the confounding effects of endothelium-derived factors (see below) that either mask the direct smooth muscle response or produce additional contractions. However, the rat pulmonary vein contracts strongly to hypoxia and then slowly falls over the ensuing 30 min (29). This response is endothelium independent, and the inability to maintain tension appears to be an intrinsic characteristic of venous smooth muscle.

**Endothelium independence.** The lack of multiphasic HV in cyclostomes may be attributable, at least in part, to the absence of both an endothelial contribution to the response and intrinsic oxygen-sensitive nerves in the vascular wall. Although the magnitude of HV in New Zealand hagfish was reduced by removing the endothelium, the ratio of tension produced by HV and carbachol was unaffected (Table 2). It is probable that the decrease in tension after de-endothelialization was due to slight mechanical damage to the vessel, and it is unlikely that both hypoxia and carbachol stimulate contracting factors from the intact endothelium. The lack of effect of inhibition of cyclooxygenase and lipoxygenase and blockade of α-adrenergic, muscarinic, nicotinic, purinergic, and serotonergic receptors (Table 3) indicate that neither the endothelium nor intrinsic vascular nerve plexes contribute to HV. Furthermore, although endothelin-1 is a potent agonist in both hagfish and lamprey vessels, it is unlikely that endogenous endothelin contributes to HV because, in addition to the lack of endothelium dependence, the recovery time after an endothelin-1 stimulus is considerably longer than the relaxation observed on return to normoxia (unpublished observation).

The hypoxic response in mammalian pulmonary arteries shows surprising variability not only between species, but even in the same species. Much of the

<table>
<thead>
<tr>
<th>Table 2. Effect of removal of endothelium on hypoxic vasoconstriction by the New Zealand hagfish dorsal aorta</th>
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<tbody>
<tr>
<td>With Endothelium</td>
</tr>
<tr>
<td>N₂/carbachol contraction</td>
</tr>
</tbody>
</table>

 Values are means ± SE (n = 4 vessels from 3 fish) in ratio of a hypoxic contraction relative to that produced by 10⁻⁶ M carbachol (times 100%). The values are not significantly different.
Table 3. Effect of various inhibitors on hypoxic vasoconstriction of New Zealand hagfish and lamprey dorsal aortas

<table>
<thead>
<tr>
<th>% Control</th>
<th>Indomethacin, Esculetin, Cotrimazole</th>
<th>Phenolamine</th>
<th>Atropine, Hexamethonium</th>
<th>Aminophylline (Hagfish), 8-Phenylotheophylline (lamprey)</th>
<th>Methysergide Maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hagfish</td>
<td>99.6 ± 9.5(8)</td>
<td>97.0 ± 3.0(7)</td>
<td>94.9 ± 8.7(7)</td>
<td>101.6 ± 11.2(6)</td>
<td>116.8 ± 15.9(4)</td>
</tr>
<tr>
<td>Lamprey</td>
<td>96.7 ± 5.8(4)</td>
<td>101.2 ± 10.3(5)</td>
<td>10-4 M</td>
<td>100.6 ± 7.7(4)</td>
<td>10-5 M</td>
</tr>
<tr>
<td>Concentration</td>
<td>10-5 M</td>
<td>10-5 M</td>
<td>10-4 M</td>
<td>10-5 M</td>
<td>10-5 M</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = no. of fish) in percent tension produced during a hypoxic contraction in inhibitor-treated vessels relative to the hypoxic contraction in the same vessels before addition of inhibitors. Inhibitors in same column were applied simultaneously. There were no significant treatment effects.

Interspecies variation may be attributable to differences in endothelial cell involvement through increased or decreased production of both relaxing or constricting factors (5, 24). Interpretation of endothelial cell involvement in HPV appears to also be confounded by vagaries in methodology, and the best example of this is the biphasic transient constriction (phase 1), followed by brief relaxation, and then a prolonged constriction (phase 2) of the rat pulmonary artery. Bennie et al. (2) and Jin et al. (7) claim that phase 1 is endothelium dependent and phase 2 is endothelium independent, Ward and Aaronson (24) and Zhang et al. (28) claim that phase 2 is endothelium dependent and phase 1 is endothelium independent, whereas Leach et al. (9) provided evidence that phase 1 is partially endothelium dependent and phase 2 is completely dependent on the endothelium. Other factors, such as the strain of rat (27) or the degree of wall tension applied to the vessel before hypoxia (15), can also affect HPV and they further complicate a mechanistic examination of the intrinsic smooth muscle response. Perhaps the best generalization of HPV is that it is an intrinsic feature of VSM and that a variety of other factors, including the endothelium, modulates rather than mediates the vasoconstrictor response (5). Because HV in cyclostomes does not appear to be encumbered by these modulators, it therefore affords a more straightforward approach to mechanistic analyses.

Dependence on $P_{O_2}$. The magnitude of both HV (Figs. 5 and 6) and HPV (10, 29) is related to $P_{O_2}$ in a dose-dependent, rather than an all-or-none, manner. Thus the degree of hypoxia is coupled with tension, thereby affording proportional control of the vascular response in both vertebrates. The physiological significance of this is discussed below.

Vessel specificity. In mammals, HV is a response unique to pulmonary vessels; systemic arteries exposed to hypoxia respond either with an immediate relaxation or with a transient contraction, followed by relaxation (2, 9). Although we have not made a thorough survey of the hypoxic responses of different vessels from hagfish and lamprey, it is evident that HV is also vessel specific in these fish as well. HV was a consistent response of all systemic arteries examined (Table 1), whereas an appreciable sustained HV was not observed in any pre-gill vessel (VA and ABA) studied. There are further similarities between the dilatory responses of the mammalian aorta and the New Zealand hagfish VA and ABA in that hypoxic vasodilation is preceded by a transient contraction (see Fig. 1 this study and Fig. 1 in Ref. 2 and Fig. 1 in Ref. 9). Thus a repertoire of hypoxic responses appears to have been in place in the early vertebrates, and perhaps only the vessel specificity has been relocated commensurate with its physiological value to the animal.

Differences Between Cyclostome HV and Mammalian HPV

Preconditioning. One of the most notable differences between HV and HPV is the need for preconditioning in many isolated mammalian pulmonary vessels (24) and the independence from it in the cyclostome systemic arteries. Robust HV can be produced in cyclostome DA, EBA, and CM in the absence of any other stimulus (Figs. 1, 2, 4), whereas many pulmonary vessels must be partially contracted by either a ligand or depolarizing KCl (9, 24). Thus the processes contributing to HV in cyclostomes can be examined in the absence of other stimuli.

Although preconditioning is not necessary for cyclostome HV, the underlying mechanism(s) for it may also be present in lamprey DA. The process of preconditioning in mammalian pulmonary vessels is believed to be due to a hypoxia-induced stimulation of Ca$^{2+}$ sequestration by the smooth muscle sarcoplasmic reticulum, which on subsequent release, fortifies the contraction (22). The magnitude of HV in lamprey DA in the presence of epinephrine was also consistently greater than the contraction produced by hypoxia alone (Fig. 4), indicative of a significant effect of epinephrine on lamprey HV. However, HV in both lamprey and hagfish DA was unaffected by KCl (Fig. 2) preconditioning. It remains to be determined if the epinephrine-stimulated preconditioning in lamprey DA is also Ca$^{2+}$ mediated and, if so, whether epinephrine and KCl mobilize Ca$^{2+}$ from distinct stores.

Involvement of Potassium Channels

There is substantial evidence that closure of potassium (K$^+$) channels and the subsequent cell depolarization contribute to (or are) the primary factor initiating HPV (5, 12, 19, 25). Although our studies were...
not designed to specifically address this issue, they do raise an important question regarding the contribution of K⁺ channels in cyclostome HV: can K⁺ channels contribute to HV in the apparent absence of a transmembrane potassium gradient?

Robertson (17) showed that intracellular potassium concentration ([K⁺]ᵢ) in skeletal muscle from a related hagfish, Myxine glutinosa, is 142 mM. If it can be assumed that [K⁺]ᵢ in smooth muscle of the New Zealand hagfish is not substantially different from Robertson’s value for skeletal muscle, then it seems unlikely that there is any outward K⁺ gradient across the cell and that closure of K⁺ channels while the smooth muscle cells are bathed in 200 mM K⁺ could not produce cell depolarization. However, it is evident from Figs. 2 and 3 that HV in New Zealand hagfish DA is unaffected by 200 mM KCl in the bathing medium. Similarly, 80 mM extracellular [K⁺], which should also effectively abrogate a transmembrane K⁺ gradient across lamprey DA, had no effect on HV (Fig. 2). Clearly, additional information on transmembrane potential and ion balance is needed, but these preliminary studies suggest that HV occurs without involvement of K⁺ channels.

**Relationship Between HV and Oxygen Transport**

There appears to be an evolutionary progression in both the sensitivity of VSM to hypoxia and the oxygen loading of hemoglobin. As shown in Fig. 8, the PO₂-dependent vasoconstriction of cyclostomes, a reptile (turtle), and two mammals is shifted toward higher PO₂ values as the vertebrates evolved higher metabolic rates. Similarly, the oxy-hemoglobin saturation curve is also right shifted. Thus, for a given species, the point of intersection of the vascular and hemoglobin curves is surprisingly close to their respective P₅₀ values. The physiological implication of this is to couple vascular reactivity with oxygen availability, i.e., the vasoconstriction becomes more intense as oxygen transport capacity of the blood falls. The evolutionary implication of this is that the oxygen-sensing mechanisms intrinsic in the VSM coevolved with the decreasing affinity of hemoglobin for oxygen.

The point of intersection of the vascular response and hemoglobin saturation curves, i.e., the degree of vascular response relative to the percent oxygen unloaded from hemoglobin also appears somewhat species dependent. This may imply that the vascular response to hypoxia becomes more important in moderate hypoxia as this function becomes sequestered in the pulmonary circulation or it may be correlated with the hypoxia tolerance of the animal. Regarding the latter, the vascular response in the hypoxia-tolerant Pacific hagfish (11) only begins to occur when the blood is relatively oxygen depleted (<30% saturated), whereas the New Zealand hagfish and the lamprey are progressively less hypoxia tolerant (11, 6) and the intersection points have increased to 35 and 50%, respectively. In the turtle, dog, and cat, more than one-half of the vascular response has occurred before the blood is half depleted of oxygen. Unfortunately, there is very little direct information on the dose-dependent responses of isolated vessels to hypoxia (the vascular responses in turtle, dog, and cat, curve Cp in Fig. 8, were obtained from in vivo or perfused preparations). Because these experimental conditions are not the same as those employed in isolated vessel experiments, the relationship between hypoxia tolerance, HV, and oxy-hemoglobin interaction remains to be determined.

**Physiological Significance of HV in Cyclostomes**

The importance of HPV in mammals to minimize lung perfusion in the fetus and to prevent ventilation/perfusion mismatching in the adult is intuitively obvious and it has been verified experimentally. The physiological purpose of HV in systemic vessels of cyclostomes is neither obvious nor has it been investigated. It is possible that HV decreases perfusion of systemic tissues, thereby sustaining vital organs such as the heart and brain, analogous to the diving reflex in mammals. Alternatively, HV in large vessels may partially offset hypoxic vasodilation of systemic resistance vessels and protect against hypotensive circulatory collapse. Clearly, additional studies of the responses of resistance vessels to hypoxia and the effects of hypoxia on cardiovascular function in vivo are necessary to explore this possibility.
Hypoxic Vasoconstriction in Cyclostomes

We found that systemic arteries from both orders of the class agnatha, the myxiniformes and petromyzoniformes, contract when exposed to hypoxia, whereas prebranchial arteries relax. As these two orders are living representatives of the earliest vertebrates, it is likely that HV is a phylogenetically ancient attribute of the cardiovascular system, and physiological benefits have been obtained by limiting its expression to specific vessels. Furthermore, our experiments indicate that this HV is an intrinsic response of VSM, because it is endothelium independent and unaffected by a variety of enzyme inhibitors or receptor antagonists known to mediate secondary contractile responses in vessels of higher vertebrates. We feel that this ancient response is a fundamental process that has been modified in two ways during the course of evolution; first it has become nearly exclusively associated with respiratory organs in higher vertebrates and, second, it has been embellished with secondary regulatory mechanisms, such as those derived from the endothelium or of neuronal origin. Furthermore, the phylogenetic progression in oxygen sensitivity of both VSM and respiratory pigments suggests that these systems are interdependent and that they have been simultaneously integrated into homeostatic processes. A comparative physiological approach may well provide key answers to the coevolution of HV and hemoglobins and to the basic mechanism of hypoxia-contraction coupling in VSM.

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