A prostaglandin, not NO, mediates endothelium-dependent dilation in ventral aorta of shark (*Squalus acanthias*)

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Evans, David H., and Mark P. Gunderson. A prostaglandin, not NO, mediates endothelium-dependent dilation in ventral aorta of shark (*Squalus acanthias*). *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R1050–R1057, 1998.—In mammals, the vascular endothelium releases a variety of paracrine factors, including the vasodilatory prostaglandin (PG)I_2 and nitric oxide (NO), which is generally accepted as the major endothelium-derived relaxing factor (EDRF) in mammals. Current evidence for the vascular NO-EDRF system in fishes is contradictory. In addition, the role of PGs in the control of fish vascular tension is also unclear. We have utilized isolated rings of the ventral aorta of the spiny dogfish shark to examine the ability of various components of the NO system to dilate this vessel. Neither the NO precursor L-arginine, the NO donor sodium nitroprusside, nor NO itself dilated the rings. The Ca^{2+} ionophore A-23187 did produce an endothelium-dependent dilation that was not inhibited by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester but was inhibited by the cyclooxygenase inhibitor indomethacin, suggesting that PGs are involved. PGE_1 and carbaprostacyclin, but not PGI_2, produced concentration-dependent dilation, and intact aortic rings secreted five times as much PGI_2 as PGE in both the unstimulated state and after stimulation with A-23187. Our data suggest strongly that a PG, most probably PGI_2, is the EDRF in the ventral aorta of this shark species.

gill hemodynamics; smooth muscle; vasodilation; nitric oxide; endothelium-derived relaxing factor

ROBERT FURCHGOTT DISCOVERED the importance of the endothelium in controlling vertebrate vascular resistance by chance (reviewed in Ref. 16), and in the ensuing years it has become apparent that the intima of blood vessels plays a pivotal role in the control of vascular homeostasis (e.g., Refs. 20 and 47). The classical delineation of an endothelium-derived relaxing factor (EDRF) in mammals is via the differentiation of the effect of acetylcholine (ACh) on vessels with an intact endothelium (dilation) vs. vessels with the endothelium removed (constriction). The gas nitric oxide (NO) is generally considered to be the primary EDRF in mammals (e.g., Ref. 20), in part because inhibition of prostaglandin (PG) synthesis generally does not inhibit the endothelium-dependent dilation produced by ACh (e.g., Refs. 17 and 38). It is well established, however, that various PGs are vasoactive, in particular the vasodilatory prostacyclin (PGI_2; e.g., Refs. 27 and 46), but the paracrine role of PGI_2 as an EDRF, at least in mammals, is generally considered to be minimal (e.g., Ref. 20).

Physiological evidence for a role of endothelium-derived factors (specifically, NO and PGs) in hemodynamic control in fishes is sparse and somewhat contradictory. Three species of fishes [the rainbow trout, *Oncorhynchus mykiss* (=*Salmo gairdneri*); Japanese eel, *Anguilla japonica*; and lingcod, *Ophiodon elongatus*] responded to injection or infusion of ACh with vasoconstriction and/or increased blood pressure (3, 14, 22), contrary to the hypotension that is usually found in intact mammals. In the trout, perfusion of isolated gills with ACh produced an increase in branchial resistance (45, 52). ACh constricted the isolated ventral aorta (26, 37) and coronary artery (44) of the trout, even with an intact endothelium (26, 37), suggesting the absence of a classical EDRF in this species. Application of NO directly to the trout aorta did not produce dilation (26), supporting this hypothesis; however, the NO precursor L-arginine did dilate the perfused trout coronary system in situ, and infusion of two NO synthesis inhibitors [N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine] contracted this preparation (31). In addition, direct application of NO donors [nitroglycerine and sodium nitroprusside (SNP)] to trout aortas or coronary arteries lacking endothelium produced dilation (37, 44), and injection of SNP into intact *O. mykiss* produced a significant fall in ventral and dorsal aorta blood pressure and gill resistance (23, 36). Thus it is not clear if the complete NO signaling axis (Fig. 1) is present in the vasculature in the trout or any other fish, but the data suggest strongly that at least the second messenger system for NO (cGMP) may be present.

Application of the calcium ionophore A-23187 produced dilation of the trout ventral aortic vascular smooth muscle (VSM), which was blocked by the addition of cyclooxygenase (COX) inhibitors (meclofenamate or indomethacin) but not an inhibitor of NO synthase (N^G-monomethyl-L-arginine acetate), suggesting that PGs, not NO, may be the EDRF in this species (25, 37). The fact that direct application of either PGI_2 or PGE_1 dilated the trout aorta (26) and coronary artery (15) supports this hypothesis. However, an earlier study (40) using isolated, saline-perfused heads of the teleosts *Conger conger*, *Anguilla anguilla*, *Scorpaena porcus*, and *Solea solea* found that perfusion with PGI_2 produced an increase in vascular resistance, presumably by constriction of branchial arteries. In fact, single branchial arches from *C. conger* and *A. anguilla* showed the same increase in vascular resistance after perfusion with PGI_2. Moreover, the isolated ventral aortic strip from *C. conger* also constricted when PGI_2 was applied. Interestingly, the total vascular resistance of the perfused heads of two elasmobranchs, *Scyliorhinus stellaris* and *Torpedo marmorata*, declined when PGI_2 was added to the perfusate, and the isolated ventral aortic strip from *S. stellaris* relaxed when this PG was

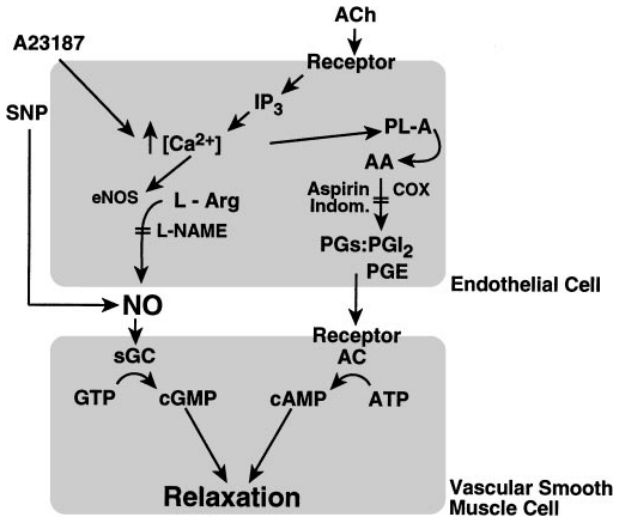

Fig. 1. Nitric oxide (NO) and prostaglandin (PG) signaling pathways involved in endothelium-dependent responses of vascular smooth muscle cells in mammals. ACh, acetylcholine; SNP, sodium nitroprusside; eNOS, endothelial NO synthase; L-Arg, L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; Indom, indomethacin; sGC, soluble guanylyl cyclase; IP_3, inositol trisphosphate; PL-A, phospholipase A; AA, arachidonic acid; COX, cyclooxygenase; PGI_2, prostacyclin; AC, adenylyl cyclase.

applied (40), suggesting fundamental differences in the PG-mediated, endothelial control of vascular tension in teleosts versus elasmobranchs.

The extant data suggest that the VSM of fishes may be sensitive to NO and PGs, but it is unclear if fish endothelial cells produce these messengers or which messenger may function as the primary EDRF. Our previous studies have characterized α-adrenergic (constrictory) and β-adrenergic (dilatory) receptors in the branchial vasculature or ventral aorta of the spiny dogfish shark, *Squalus acanthias* (9); dilatory, C-type natriuretic peptide receptors (NPR-B) (12); and constrictory, endothelin $(ET)_B$-type receptors (11) as well as both constrictory (A_1) and dilatory (A_2) adenosine receptors (6) in the ventral aortic VSM of the same species. The present work was undertaken to determine if an EDRF is present in the ventral aortic VSM of this species and whether either or both NO and PGs function as the EDRF. In fishes, control of prebranchial hemodynamics is of major importance, because the gill epithelium is the site of gas exchange, osmoregulation, nitrogen excretion, and acid-base regulation in these aquatic vertebrates (e.g., Ref. 7). The relative role of the ventral aorta versus resistance vessels (e.g., afferent branchial and filamental arteries) in controlling gill perfusion is unknown, but initial input pressures must play some role, and at least NPRs are expressed both in the ventral aorta (12) and gill (5) of *S. acanthias*.

MATERIALS AND METHODS

Adult spiny dogfish sharks (*S. acanthias*, ~2–5 kg) were trapped in gill nets in Frenchman Bay, ME. Experimental animals were maintained for at least 24 h in floating live cars before death by pithing through the snout to destroy both the brain and spinal cord. Tissue rings from the ventral aorta (between the 2nd and 3rd afferent branchial arteries) were prepared and mounted in elasmobranch Ringer solution (ERS) in organ baths as described previously (13), except that the solution was aerated with 1% CO_2-99% O_2. Tension was recorded via Gould-Statham strain transducers, the output of which was recorded either by a Gilson Duograph or a Biopac MP100WS system, using AcqKnowledge III software, connected to a Macintosh Powerbook model 140. Initial tension was set at 500 mg for a 30- to 60-min equilibration period and returned to that value when the tension was stable. Our preliminary experiments had determined that this tension produced the maximal response to ACh in these rings. Specific compounds were added cumulatively to the 10-ml experimental bath in increments totaling <4% of the initial volume. When appropriate, the endothelium was removed before mounting by gentle abrasion of the intima with a short length of roughened polyethylene (PE-90) tubing. These rings are termed "rubbed"; those with the endothelium untouched are termed "intact." To examine the efficacy of endothelium removal, intact and rubbed rings were preserved in 10% Formalin in shark Ringer (13). The rings were then transferred to 75% ethanol, dehydrated through a series of ethanol baths to 100% ethanol, embedded in paraffin, sectioned at 7 μm, and stained with a modified Harris trichrome stain (19).

On the basis of the putative signaling axes depicted in Fig. 1, protocols were established to determine the presence and nature of the EDRF in this tissue. To determine if ACh elicits an endothelium-dependent response, intact and rubbed rings were mounted and exposed to a cumulative addition of ACh over the range of 10^{-9} to 10^{-4} M. To test for the presence of the NO signaling pathway, we exposed precontracted (10^{-4} M ACh), intact rings to the NO precursor L-arginine (10^{-4} M). In another series of experiments, we exposed intact (but not precontracted) rings to the NO donor SNP (10^{-4} M). As a control, after exposure to SNP, these rings were exposed to 10^{-7} M porcine C-type natriuretic peptide (pCNP), which we previously have shown to be dilatory in intact (not precontracted) rings (12). In a third experiment, we exposed rubbed, precontracted rings to NO itself. As a control, we exposed rat thoracic aorta rings (1-*g* tension in Tyrode solution; 37°C) to the same concentration of NO.

Because none of these treatments (except pCNP) produced relaxations of the shark aortic rings, we tested for the presence of any EDRF by exposing precontracted, intact versus rubbed rings to the calcium ionophore A-23187, which has been shown to produce endothelium-dependent dilations in vascular tissue from the trout (25, 37). Presumably, A-23187 can produce dilation by activating either an NO signaling pathway and/or a parallel one for the PG signaling system, both of which can be stimulated by an increase in endothelial cell cytoplasmic Ca^{2+} (Fig. 1). Because A-23187 was dilatory only when the endothelium was intact (see RESULTS), we exposed (intact, precontracted) rings to 10^{-5} M A-23187 in the presence of either 10^{-4} M L-NAME or 10^{-5} M indomethacin in an attempt to differentiate between the two putative pathways. Paired rings were exposed to the inhibitors or appropriate vehicle for 30 min before the A-23187 was added. Inhibition of the A-23187 dilation of intact rings by indomethacin but not L-NAME (see RESULTS) suggested that only the PG pathway was effective, so we exposed (paired, precontracted, rubbed) rings to either PGI_2 or PGE_1, both of which have been shown to produce dilation in trout vessels (15, 26). Because only PGE_1 produced significant relaxation (see RESULTS), we exposed rings (precontracted, rubbed) to cumulative addition of PGE_1 to determine if the relaxation was concentration dependent. Because PGI_2 is unstable at neutral or acidic pH (e.g., Refs. 4 and 27), we also exposed precontracted, rubbed rings to the cummulative addition of carbaprostacyclin, a stable analog of PGI_2 (4, 50).

To determine if the aortic rings could actually secrete PGs in response to A-23187 application, intact pairs of rings were incubated in 1 ml ERS (maintained at 12°C) in polyethylene microfuge tubes for 30 min with and without 10^{-5} M A-23187. At the end of the experiment, the individual rings were removed and weighed and the microfuge tubes were immediately frozen in liquid N_2 and stored at −70°C. Commercial enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) were used to measure 6-ketoprostaglandin $F_{1\partial}$ (for PGI_2) and bicyclo-PGE_2 (for total PGEs). Production is expressed as picograms PG per milligrams tissue per minute.

ACh (Sigma, St. Louis, MO), L-arginine (Sigma), SNP (Sigma), and L-NAME (Research Biochemicals International, Natick, MA) were dissolved in sufficient distilled water to make 10^{-4} or 10^{-2} M stock solutions and stored at 4°C. In specific experiments, samples were diluted in the 10-ml experimental bath to provide the necessary concentration. A-23187 (Sigma) was dissolved in DMSO to make a 5×10^{-3} M stock solution, which was also stored at 4°C. Maximum final DMSO concentration in the experimental bath was 0.2%. pCNP (Peninsula Labs, Belmont, CA) was dissolved in 0.1 N acetic acid, dispensed into microfuge tubes, and lyophilized and stored at −70°C until samples were dissolved in distilled water and added to the experimental bath. A saturated NO solution was prepared by bubbling NO into O_2-free distilled water in a glass flask, which was sealed with a serum bottle stopper. The solution was stored at 4°C before use. For each experiment, 200 μl of this solution was removed from the flask with a tuberculin syringe and added to the tissue bath (12°C). This volume was calculated (from appropriate solubility-temperature tables; see Ref. 54) as sufficient to produce a putative NO concentration of 4.2×10^{-6} M in the 10-ml experimental bath. Indomethacin (Sigma) was dissolved in 100 mM $NaHCO_3$-ethanol (3:1) to make a 10^{-3} M solution and stored at room temperature before use. One hundred microliters of this solution was added to the 10-ml experimental bath to produce 10^{-5} M indomethacin, and control experiments found no vasoactivity with 100 μl of carrier only. PGI_2 (Sigma) was dissolved in ERS to a concentration of 10^{-3} M (not bubbled with 99% O_2-1% CO_2 to keep the pH >8.0), dispensed into microfuge tubes, and also kept at −70°C until use. Ten microliters of this solution was added to the 10 ml experimental bath to produce a final PGI_2 concentration of 10^{-6} M. PGE_1 (Sigma) and carbaprostacyclin (Cayman Chemical) were dissolved in DMSO to a concentration of 10^{-3} M, sampled into microfuge tubes, and kept at −70°C until use. Ten microliters of the PGE_1 solution was added to the 10-ml experimental bath to produce a final PGE_1 concentration of 10^{-6} M and final DMSO concentration of 0.1%. The concentration-response curves for PGE_1 and carbaprostacyclin were generated by the cumulative addition of a dilution of the original 10^{-3} M solution to a final concentration of 3×10^{-6} or 10^{-5} M, producing a final DMSO concentration of 0.3 or 1%, respectively. Our previous studies found that even 3% DMSO is not vasoactive in this preparation (6).

All data are expressed as means ± SE. P values for statistical differences were calculated using either paired or unpaired two-tailed Student's t-test, and $P \leq 0.05$ was taken as significant. Fifty percent effective concentrations (EC_{50}) were computed from nonlinear regression of the concentration-response curves. Regression and statistical analyses were carried out using Prism (GraphPad Software).

RESULTS

ACh produced a concentration-dependent contraction of the dogfish ventral aortic VSM, whether an endothelium was present or not (Fig. 2); the EC_{50}s of the responses are identical [7.85×10^{-8} M (intact) vs. 7.95×10^{-8} M (rubbed); $P = 0.94$]. The endothelial cells of the shark aorta are ovoid with punctate nuclei and overlay the elongate VSM cells (with spindle-shaped nuclei), and rubbing the intima with the roughened PE tubing effectively removed the endothelial cells (Fig. 3).

Exposure of intact rings to L-arginine, SNP, or NO itself did not produce dilation; in fact, all three produced significant contractions ($P = 0.006$, 0.02, and 0.001, respectively; Fig. 4). The NO concentration applied was nearly 1,000-fold higher than the EC_{50} of the NO-induced dilation of the perfused coronary of the guinea pig (21). To ensure that our NO solution was actually potentially vasoactive, we exposed isolated rat thoracic aortic rings to the same solutions (37°C) and demonstrated small but significant dilation (66 ± 27 mg, $n = 7$, $P = 0.05$; data not shown), demonstrating that the NO solution was actually vasoactive in a tissue that has already been shown to express the NO signaling system (e.g., Ref. 29). As we had found previously (12), pCNP produced significant dilation ($P = 0.009$; Fig. 4).

The calcium ionophore A-23187 produced an endothelium-dependent dilation (Fig. 5) that was significant at 3×10^{-6} M ($P = 0.004$) and 10^{-5} M ($P = 0.003$), suggesting that an increase in intracellular Ca^{2+} in endothelial cells does trigger the release of some sort of dilatory signal. Preincubation of intact rings with 10^{-6} M L-NAME did not change the resting tension of the rings ($P = 0.40$ compared with control; data not shown), nor did it inhibit the A-23187-dependent dilation (Fig. 6; $P = 0.77$). Preincubation with 10^{-6} M indomethacin also did not affect the resting tension when compared with the control ($P = 0.26$; data not shown), but it did inhibit the A-23187-induced dilation significantly (Fig. 7; $P = 0.004$). In fact, indomethacin treatment was followed by a significant contraction when A-23187 was applied ($P = 0.04$). PGI_2 (10^{-6} M) did not produce significant dilation of rubbed rings (−94.8 ± 48 mg, $n = 8$, $P = 0.09$), but PGE_1 did (−389 ± 121 mg, $n = 8$, $P = 0.01$), and the dilation was significantly different from

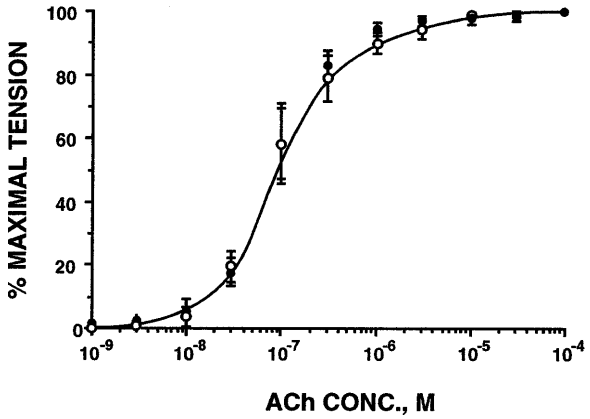

Fig. 2. ACh concentration (Conc)-response curve of isolated aortic rings from shark *Squalus acanthias*. ●, Intact rings; ○, rings rubbed to remove endothelial cells. Tension is expressed as %maximal tension at 10^{-4} M ACh. Data points are means ± SE; $n = 4$.

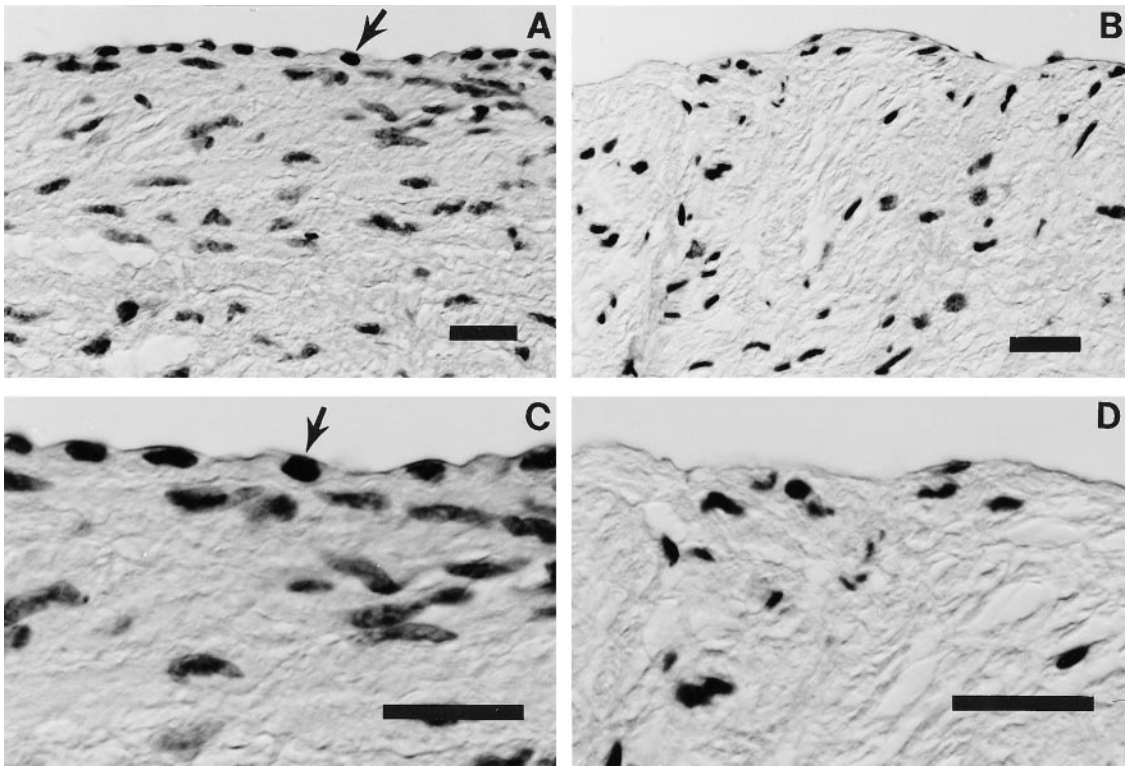

Fig. 3. *A* and *C*: intact shark aortic ring exhibits endothelial cells (arrows indicate same cell) lining tunica intima, with endothelial nuclei projecting into lumen. Vessel wall is composed of smooth muscle cells separated by connective tissue. Bar represents 10 μm. *B* and *D*: after rubbing of aortic rings, lumen is in direct contact with connective tissue and smooth muscle cells of tunica media. Few endothelial cells remain. Aortic tissue is from same animal as in *A* and *C*. Bar represents 10 μm.

the paired rings exposed to PGI_2 ($P = 0.01$). Moreover, the dilation produced by PGE_1 was concentration dependent, with an EC_{50} of 6.6×10^{-8} M (Fig. 8*A*). Because PGI_2 is unstable (4, 27), it is possible that the apparent lack of a response to PGI_2 was due to degradation. Moreover, PGE_1 can act as an agonist on the PGI_2 receptor (e.g., Ref. 4). Carbaprostacyclin, a stable PGI_2 analog, produced a concentration-dependent dilation of the shark rings ($EC_{50} = 7.5 \times 10^{-7}$ M; Fig. 8*B*). Intact rings released both PGE and PGI_2 when incubated in ERS, and 10^{-5} M A-23187 (which produced dilation; see Fig. 5) stimulated the release of both PGs. However, five times more PGI_2 than PGE was produced in both the control rings and after stimulation with A-23187 (Fig. 9).

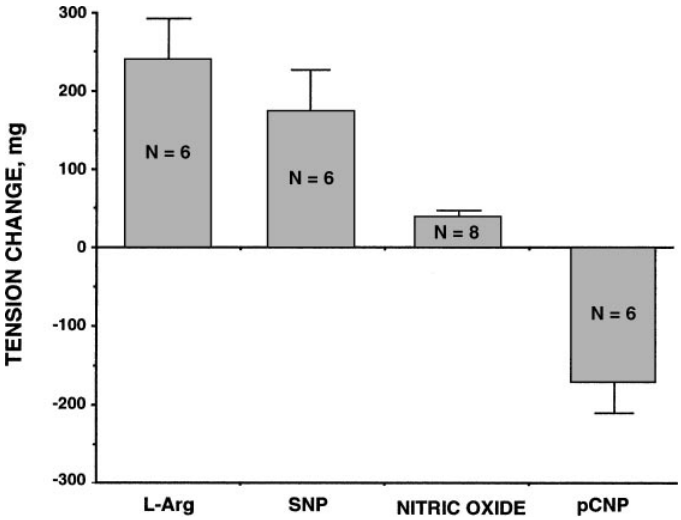

Fig. 4. Effect of L-Arg, SNP, NO, and porcine C-type natriuretic peptide (pCNP) on shark aortic rings. Only pCNP produced a significant decline in tension ($P = 0.009$). Contraction produced by L-Arg, SNP, and NO was significant and is unexplained. Error bars are SE.

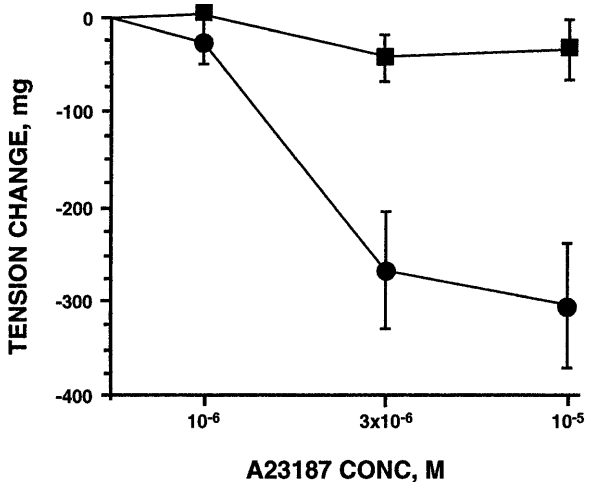

Fig. 5. Effect of Ca^{2+} ionophore A-23187 on intact (●) and rubbed (■) shark aortic rings. Only intact rings dilated when A-23187 was applied. Data points are means ± SE, $n = 7$–11.

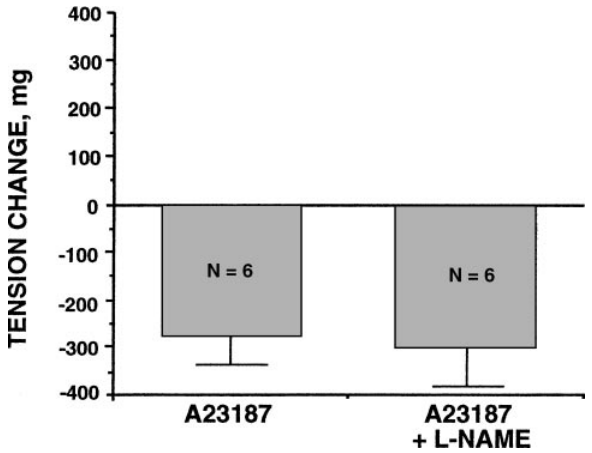

Fig. 6. Effect of preincubation with NOS inibitor L-NAME (10^{-4} M) on A-23187-induced (10^{-5} M) dilation of shark aortic rings. Error bars are SE.

DISCUSSION

Our finding that ACh contracted the isolated, intact, ventral aorta of the dogfish shark (Fig. 2) corroborates earlier studies using a variety of teleost fish species that demonstrated that ACh produces hypertension when injected in vivo, increases branchial resistance in perfused gills, and constricts the isolated ventral aorta and coronary artery of *O. mykiss* (see introduction). In addition, Farrell and Johansen (15) found that ACh constricted isolated coronary rings from *S. acanthias*, but the lowest concentration tested was 10^{-6} M, more than an order of magnitude higher than the EC_{50} found in our experiments with the aorta from the same species. Although the data suggesting cholinergic innervation of teleost systemic vasculature are equivocal (32), it is apparent that the branchial vasculature of teleosts has cholinergic innervation, with the major site in the efferent filamental artery in at least the cod (*Gadus morhua*) (33) and *O. mykiss* (45). Interestingly, there does not appear to be any cholinergic innervation of either systemic or branchial vasculature in the elasmobranchs (24, 28, 32), which makes one wonder about the function of the obvious cholinergic receptors that we and Farrell and Johansen (15) have described in the aorta and coronary vessels of *S. acanthias*.

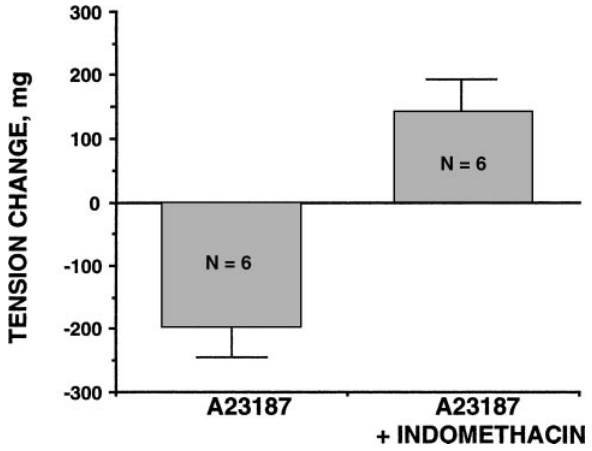

Fig. 7. Effect of preincubation with COX inhibitor indomethacin (10^{-5} M) on A-23187-induced (10^{-5} M) dilation of shark aortic rings. Treatment produced a significant contraction; see text for details. Error bars are SE.

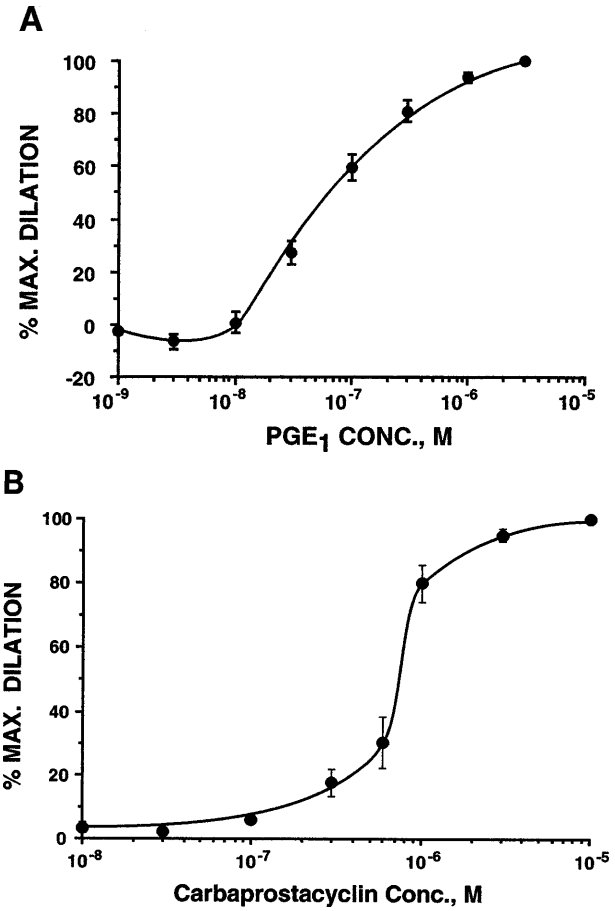

Fig. 8. *A*: PGE_1 concentration-response curve for shark aortic rings. Tension is expressed as %maximal tension at 3×10^{-6} M PGE_1. Data points are means ± SE, $n = 5$. *B*: carbaprostacyclin concentration-response curve for shark aortic rings. Tension is expressed as %maximal tension at 10^{-5} M carbaprostacyclin. Data points are means ± SE, $n = 8$.

Removal of the endothelium did not affect the ACh-stimulated contraction of the shark aorta (Fig. 3),

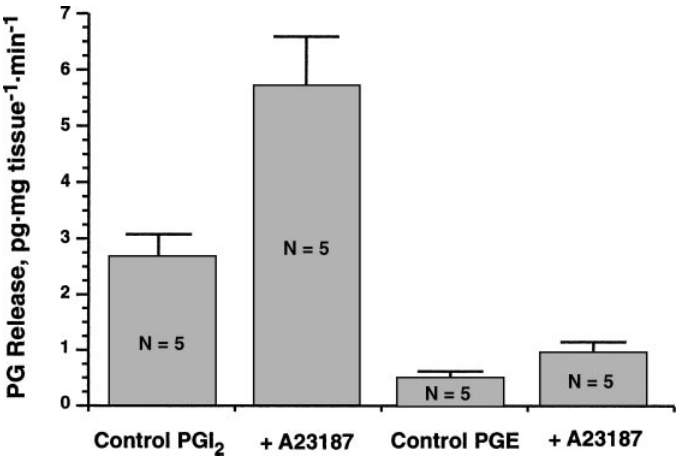

Fig. 9. Release of PGI_2 and PGEs by intact shark aortic rings and after stimulation with 10^{-5} M A-23187. Error bars are SE.

contrary to the increased contraction (e.g., Refs. 34 and 42) or reversal from dilation (e.g., Refs. 17 and 18) seen in mammalian vessels exposed to ACh after the endothelium is removed. Our data corroborate two studies of trout vessels that demonstrated ACh-induced contractions even when the endothelium was intact (25, 37). However, a recent study of perfused trout coronaries suggests that a dilation may occur in this preparation at low concentrations of ACh (10^{-8} and 10^{-7} M) that is offset by contractions at higher concentrations (31). Our data suggest that the shark aorta does not have cholinergic receptors on the endothelium, only on the VSM cells themselves. Our ACh concentration-response curve is the first published for any blood vessel of an elasmobranch and demonstrates a rather high sensitivity of the cholinergic receptor ($EC_{50} = 7.9 \times 10^{-8}$ M). Other studies in our laboratory have characterized the cholinergic receptor in *S. acanthias* VSM as the M_3 type (Refs. 8 and 10 and unpublished observations), consistent with what has been found in some mammalian VSM preparations (e.g., Refs. 34 and 42).

Thus our initial studies determined that at least one initial step in the EDRF signaling pathway (Fig. 1), endothelial cholinergic receptors, is apparently missing in the shark ventral aorta. Our earlier study (11) demonstrated that the contractile response of the shark aorta to ET-1 also is not endothelium dependent, suggesting that, unlike mammals (41), the endothelium of the shark aorta does not express ET receptors that mediate dilation via the NO signaling system. Therefore, at least two of the primary effectors in the classical EDRF system are missing in the shark ventral aorta. Whether receptors for some other hormones whose dilatory actions are mediated by the EDRF system in mammals (e.g., histamine, bradykinin, serotonin, and thrombin; e.g., Ref. 20) are present on the vascular endothelium of elasmobranchs is unknown, but histamine had no effect on aortic rings from *S. acanthias* (10^{-8} to 10^{-5} M; our unpublished data). Serotonin (10^{-9} to 10^{-5} M) was not vasoactive in the *S. acanthias* coronary ring, but it produced a concentration-dependent dilation of *O. mykiss* coronary rings (15). However, this effect apparently was not mediated by the endothelium, because removal with saponin did not alter the response. More studies are certainly warranted, because the bulk of current evidence suggests that a variety of receptors that mediate dilation secondarily through production of endothelial EDRFs in mammals may not be expressed in the endothelial cells of fishes.

The fact that addition of either the substrate for NOS, L-arginine; the NO donor, SNP; or NO itself to the shark aortic rings did not elicit dilation (Fig. 3) supports the conclusion that not only is the endothelial cell NO signaling pathway missing from the shark aorta but also the soluble guanylyl cyclase in the VSM that mediates the synthesis of cGMP, which produces dilation (Fig. 1) (our finding that all three substances actually produced contraction is interesting and unexplained). Our data contrast with our current understanding of at least one species of teleost fish, *O. mykiss*, that apparently expresses most if not all of the EDRF-NO system (see introduction and Refs. 23, 26, 31, 36, 37, 43, and 44).

Despite our inability to detect the major components of the NO signaling pathway, such as an endothelium-dependent ACh response or dilation secondary to the addition of either the substrate for NOS or NO itself (Figs. 2 and 4), we could elicit an endothelium-dependent response (dilation) to the calcium ionophore A-23187 (Fig. 5), suggesting that the endothelial cells could produce some sort of relaxing factor in response to an increase in intracellular Ca^{2+} (Fig. 1). Our data corroborate those of Olson and Villa (37) and Miller and Vanhoutte (26), who showed that A-23187 could dilate vessels in *O. mykiss* only if the endothelium is intact. In the shark aortic ring, this A-23187-induced dilation was not inhibited by even high concentrations (10^{-4} M) of the NOS inhibitor L-NAME (Fig. 6), which corroborates the data of Miller and Vanhoutte (26) on *O. mykiss* and our conclusion from Figs. 3–5 that the classic EDRF-NO signaling pathway is missing in the ventral aorta of *S. acanthias*.

Contrary to the lack of an effect of L-NAME, inhibition of COX with indomethacin (10^{-5} M) did inhibit the A-23187-induced dilation (Fig. 7), corroborating data on vessels in *O. mykiss* (25, 37). In their classic study, Furchgott and Zawadzki (17) found that neither indomethacin (4×10^{-5} M) nor aspirin (10^{-3} M; another inhibitor of COX) inhibited the endothelium-dependent dilation of the rabbit thoracic aorta, but the actual data were not given. Interestingly, these unpublished data are usually the only ones used to support the general statement that EDRF "is not a prostanoid as blockers of COX do not modify endothelium-dependent relaxation" (39). However, Miller and Vanhoutte (25) demonstrated that meclofenamate did not inhibit the A-23187- or ACh-induced dilation of the frog aorta or the ACh-induced dilation of the aorta of the cayman (a reptile), leading to their conclusion that the EDRF-NO control of vascular diameter evolved first in the amphibians (26). Our data suggest that in the ventral aorta of *S. acanthias*, the EDRF (released by A-23187 treatment) is a PG, extending the conclusions of Miller and Vanhoutte (25, 26) and Olson and Villa (37) that the EDRF in the trout is a PG, not NO. Our finding that indomethacin treatment actually reversed the A-23187-induced dilation to a significant contraction (Fig. 7) is of some interest. The A-23187 may have increased the intracellular Ca^{2+} concentration of the VSM cell itself, producing contraction when the EDRF release was inhibited by indomethacin. However, this seems unlikely because A-23187 did not contract rubbed rings (Fig. 5) when the endothelium had actually been removed. Another potential explanation is that the increased endothelial cell Ca^{2+} concentration activated the production of the vasoconstrictive peptide ET as well as a dilatory PG. We have already identified ET_B-type receptors, which mediate contraction, in the VSM of *S. acanthias* (11), and ET is released from mammalian endothelial cells when intracellular calcium rises (e.g., Refs. 20 and 53). Thus pretreatment with indomethacin could have inhib-

ited the PG production, and the constrictory action of ET was no longer overridden. If this is the case, we have underestimated the dilatory effects of PGs in the A-23187 experiments.

The fact that PGE_1, not PGI_2, could stimulate significant dilation and produced its effect in a concentration-dependent manner (Fig. 8*A*) may suggest that E-type PGs are the EDRFs in our system. Our data corroborate those of Miller and Vanhoutte (26), who found that PGE_1 was more dilatory than PGI_2 in the trout aorta. PGs are also dilatory in the *O. mykiss* coronary ring, but PGI_2 seems to be more effective than PGE_2 in this vessel (15). PGI_2 also dilated the perfused coronary arteries of this species (30), although the authors considered it a "weak vasodilator." Interestingly, PGI_2 has been shown prevously to be dilatory in two species of elasmobranchs but vasoconstrictive in four species of teleosts (see introduction and Ref. 40). This latter study suggests a fundamental difference in the vascular effects of at least PGI_2 in teleosts versus elasmobranchs, but it does contrast with the other studies that have shown that PGI_2 is dilatory in the trout aorta and coronary (15, 26). Both PGE_1 and/or PGE_2 also produced a fall in the dorsal aortic pressure of *O. mykiss* (*S. gairdneri*), *A. anguilla*, *Channa maculata*, and the hagfish (*Myxine glutinosa*), suggesting systemic rather than branchial dilation (2, 48, 49, 51). However, it should be pointed out that, given the series arrangement of heart, ventral aorta, branchial vessels, dorsal aorta, and systemic vessels in fishes, a fall in dorsal aortic pressure can be secondary to either systemic dilation or branchial constriction (e.g., Ref. 35). Thus it is possible that the data of Piomelli et al. (40) can be reconciled with these other studies that show a fall in dorsal aortic pressure.

PGI_2 is known to be chemically and metabolically unstable (e.g., Refs. 4 and 27), so it is possible that some disparate results may be due to metabolism of PGI_2. In addition, PGEs are known agonists of the PGI_{2n}(IP) receptor (e.g., Ref. 4), so it is possible that the apparent relative potency of PGE_1 versus PGI_2 in this and other studies may be due to differential metabolism versus receptor affinity of the two agonists. The fact that the stable PGI_2 analog carbaprostacyclin produced a concentration-dependent dilation in the shark aortic ring (Fig. 8*B*) suggests that the response to PGE_1 may be mediated via the IP receptor and that PGI_2 was ineffective because of degradation in the experimental ERS, which was maintained at pH ~7.8. The EC_{50} of the response to carbaprostacyclin was distinctly above that to PGE_1 (7.5×10^{-7} vs. 6.6×10^{-8} M), but carbaprostacyclin is generally only 3–10% as potent as PGI_2 in mammalian assays as well (e.g., Refs. 1 and 50).

Our hypothesis that PGI_2 is the actual effector in shark aorta is supported by our finding that the intact shark aortic ring secretes five times as much PGI_2 as PGE in the unstimulated state and in response to 10^{-5} M A-23187 (Fig. 9), which does produce an endothelium-dependent dilation (Fig. 5). Nevertheless, the actual cellular site of synthesis (endothelial vs. smooth muscle cell) was not determined in our study, so the endothelial synthesis of PGs in fishes is still not resolved.

In summary, our data support the conclusion that, although an EDRF is present in the ventral aorta of the spiny dogfish, *S. acanthias*, it is not linked to an endothelial cholinergic receptor, is not NO, and appears to be a PG, most likely PGI_2. These data corroborate earlier studies (e.g., Refs. 15, 25, 26, and 37) that suggested that there may be fundamental differences between endothelium-derived vascular control systems in mammals and fishes. It is clear that other fish and nonmammalian vertebrate species should be utilized to study the evolution of this important vascular control system.

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