JOURNAL OF EXPERIMENTAL ZOOLOGY 297A:45–51 (2003)

Characterization of the Effects of Vasoactive Substances on the Bulbus Arteriosus of the Eel, *Anguilla rostrata*

DAVID H. EVANS,*[1,2] ANN C. HARRIE,[1,3] AND MATTHEW S. KOZLOWSKI[1,2]
[1]*Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine 04672*
[2]*Department of Zoology, University of Florida, Gainesville, Florida 32611*
[3]*Department of Environmental Health Science, School of Public Health, University of South Carolina, Columbia, South Carolina 29208*

ABSTRACT The fish bulbus arteriosus (BA) smoothes cardiac output by expanding during cardiac systole and rebounding during diastole, thereby providing constant perfusion of the gills downstream. Published data have demonstrated innervation of the teleost BA and shown that the tension and compliance of the BA responded to vasoactive agonists, such as epinephrine and acetylcholine, suggesting that the BA was more than a mere ''windkessel.'' To examine vasoactivity in the BA more directly, we measured the responses of isolated tissue rings from the BA of the eel, *Anguilla rostrata* to a suite of putative vasoactive agonists, which had been shown to affect vascular smooth muscle in a variety of teleosts. The BA of the eel was insensitive to acetylcholine but constricted when endothelin (ET-1) was applied. Nitric oxide, sodium nitroprusside (SNP; NO donor), natriuretic peptides (NP), and prostaglandin E_1 (but not the prostacyclin agonist carbaprostacyclin) produced significant dilation in the BA. Since both ET-1 and sarafotoxin S6c produced concentration-dependent constriction, it appears that endothelin receptor B-type (ET_B) receptors (and possibly ET_A receptors) are present. The dilation produced by SNP was also concentration dependent, as were the dilations produced by porcine C-type natriuretic peptide, eel atrial natriuretic peptide (NP receptor agonists), Sulprostone and Butaprost (PGE receptor agonists). Our data demonstrate that the BA of eel is responsive to a variety of vasoactive agonists, suggesting that the BA is under neurohumoral control. The role of agonist-induced changes in BA tension in fish cardiovascular physiology remains to be determined, as do the specific receptor types involved. *J. Exp. Zool. 297A:45–51, 2003.* © 2003 Wiley-Liss, Inc.

INTRODUCTION

In the circulatory system of most teleosts, blood exiting the single cardiac ventricle enters the elastic bulbus arteriosus (BA) before flowing into the ventral aorta (e.g., Bushnell et al., '92; Olson, '97). The BA of the carp (*Cyprinus carpio)* is 32 times more distensible than the human thoracic aorta (Licht and Harris, '73), and the BA may store 25–100% of the cardiac output, depending upon levels of exercise (Priede, '76; Bushnell and Jones, '94). Expansion of the BA during ventricular systole reduces the pulsatility of the blood flow to the delicate branchial vasculature and reduces the hemodynamic afterload on the ventricular muscle. In addition, its rebound during ventricular diastole provides for a relatively constant perfusion of the gills through the ventral aorta (Bushnell et al., '92). Because the BA smoothes cardiac output, it has been termed a ''windkessel'' (von Skramlik, '35) or ''pressure chamber'' (Johansen and Martin, '65).

The teleost BA is composed of three layers: an outer adventitia containing nerve tracts, blood vessels, and collagenous connective tissue; a thick media layer comprised of occasional fibrocytes, variable numbers of smooth myocytes (arranged spirally or circumferentially), and elastic fibrils; and an intimal layer of endothelial cells (e.g. Licht and Harris, '73; Watson and Cobb, '79; Farrell and Jones, '92; Icardo et al., '99). The substantial

Grant sponsor: The National Science Foundation; Grant numbers: IBN–9604824 and IBN–0089943 to DHE and NSF REU BIR 9531348 to ACH.
*Correspondence to: David H. Evans, Department of Zoology, University of Florida, Gainesville, Florida 32611.
E-mail: devans@zoo.ufl.edu
Received 22 July 2002; Accepted 6 January 2003
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.a.10238

© 2003 WILEY-LISS, INC.

nerve tracts common in the adventitia send extensions to the adventitia-media boundary and are generally considered to be adrenergic fibers (Gannon and Burnstock, '69; Watson and Cobb, '79; Farrell and Jones, '92). Cholinergic fibers have not been described, but acetylcholine constricted spiral strips from the trout, *Oncorhynchus mykis* BA (Klaverkamp and Dyer, '74), and Farrell ('79) found that adrenergic and cholinergic agonists increased and decreased, respectively, the compliance of the BA of *O. mykiss*.

Other vasoactive agonists may play a role in BA function. Immunoreactive atrial natriuretic peptide (ANP) has been localized in the BA of a variety of freshwater fishes (Kim et al., '89) as well as the BA of the marine icefish *Champsocephalus gunnari* (Masini et al., '97), and a high density of receptors for natriuretic peptides has been described in the BA of the marine eel *Conger conger* (Cerra et al., '92). In addition, immunoreactive angiotensin II, bradykinin, and endothelin have been localized in the BA of *C. gunnari* (Masini et al., '97). These studies suggest that at least the teleost BA is controlled by a variety of neurotransmitters and paracrines and, therefore, may be more than a simple windkessel. To test this hypothesis, we have examined the responses of the BA from the American eel, *Anguilla rostrata*, to a suite of putative signaling agents that our previous studies have found to be vasoactive in ventral aorta in this species (Evans and Harrie, 2001).

MATERIALS AND METHODS

Freshwater (yellow) American eels (*Anguilla rostrata*; mixed sexes, 200–900g) were purchased from a commercial supplier and maintained in running seawater tanks (ca. 16°C) at the Mt. Desert Island Biological Laboratory, Salsbury Cove, ME for at least one week before use. They had been captured in the Union River (near Bangor, Maine) during the months of July and August, 1997, 1998, and 2001.

Eels were anesthetized in 0.1% MS–222 (Sigma Chemical) in sea water, double-pithed after cervical transection, and the BA was removed by dissection; cross-sectional rings were mounted in 10 ml of teleost Ringer's solution (mM/l: NaCl, 148; $NaHCO_3$, 14.9; NaH_2PO_4, 2.7; KCl, 2.6; $CaCl_2$, 1.26; $MgSO_4$, 1.24; Glucose, 5, bubbled with 1% CO_2 in air, pH=7.8) in organ baths and maintained at 12°C as described previously (Evans et al., '89; Evans and Gunderson, '98; Evans and Harrie, 2001). Initial tensions were maintained at ca. 500 mg for 30–60 minutes, because preliminary experiments determined that the rings were most responsive at this tension. Tension was recorded by WPI strain transducers connected through a Biopac MP100WS data acquisition system (using AcqKnowledge III software) to a Macintosh 8600 computer. After the rings reached a stable tension, putative agonists were added cumulatively to the experimental bath in increments totaling $<4\%$ of the initial volume.

Solutions of acetylcholine (ACh; Sigma Chemical, St. Louis, MO), human endothelin or sarafotoxin S6c (ET-1, SRX; American Peptide, Sunnyvale, CA), sodium nitroprusside (SNP; NO donor, Sigma), porcine C-type natriuretic pepdide (pCNP, Peninsula Laboratories), eel atrial natriuretic peptide (eANP; Peninsula Laboratories), carbaprostacyclin (CPR; stable prostacyclin agonist) and PGE_1 (Cayman Chemical, Ann Arbor, MI), were made as described previously (Evans et al., '96; Evans and Gunderson, '98). Butaprost and Sulprostone (Cayman Chemicals) were solubilized in DMSO. All solutions were stored at -20°C until diluted. Maximal DMSO concentrations never exceeded 0.01%, a concentration that had no effect on the rings (unpublished data). A saturated NO solution (in distilled water) was prepared as described previously (Evans and Gunderson, '98). Initial experiments tested the sensitivity of the BA rings to acetylcholine (10^{-4} M), followed by the sequential addition of the following: ET–1 (10^{-7} M), SNP (10^{-4} M), NO (4.2×10^{-6} M), eANP (10^{-7} M), CPR (10^{-6} M), and PGE_1 (10^{-6} M) to a given ring. Specific concentrations of all agonists were chosen because our earlier studies had determined that they produced significant responses in eel aortic rings (Evans and Harrie, 2001). Subsequent experiments were designed to determine the concentration dependence of the effects of some of the agonists, as well as to begin to characterize the specific receptor involved. Thus, the effect of ET-1 (agonist for both ET_A and ET_B receptors) was compared to that of SRX (ET_B-specific); the effect of eANP (natriuretic peptide receptor-A agonist) was compared to that of pCNP (NPR-B agonist); and Butaprost (EP_2 agonist) was compared to Sulprostone (EP_3 agonist). In these experiments, the effects of the putative agonists were compared using paired rings from the same BA. For dilatory concentration response curves, the rings were pre-constricted by 10^{-7} M ET before adding SNP, natriuretic peptides, or PGE agonists.

These protocols conformed to NIH Guidelines and were approved by the IACUC at MDIBL. All data are expressed as mean $\pm$ s.e. (N). In the initial experiments, tension changes (measured relative to the tension at the end of the previous experimental period) were compared to zero using Prism (GraphPad Software) and accepted as significant if $p < 0.05$ (Student's t-test, two-tailed). For the concentration-response experiments, the data were analyzed using a repeated measures ANOVA, with a post test for a linear trend and, when appropriate, a Tukey post test to examine differences between means at a specific concentration of putative agonists (e.g., 10^{-7} M ET–1 vs. 10^{-7} M SRX) using Prism. In all cases, $p < 0.05$ was accepted as significant.

RESULTS

The isolated ring of BA tissue of the eel was unresponsive to 10^{-4} M acetylcholine (N=8; data not shown), but responded to ET–1 with a very significant constriction (Figs. 1a and 2). Subsequent addition of the NO donor SNP produced a small, but not statistically significant, decline in tension; however, NO itself dilated the rings slightly, and significantly (Figs. 1a and 2). Eel ANP produced a very significant dilation of the ring, and PGE_1 (but not CPR), produced an additional significant dilation when added after the eANP (Figs. 1 and 2). The constriction produced by both ET–1 and SRX was concentration dependent (Fig. 3), and the effects of the two agonists were not significantly different. The dilation produced by increasing concentrations of SNP was significant and concentration dependent (Fig. 4), and both eANP and pCNP produced concentration-dependent dilation, with the effect of ANP only significantly greater at 10^{-7} M ($p < 0.001$; Fig. 5). The dilatory effects of both Sulprostone and Butaprost were concentration dependent, and were not significantly different from each other (Fig. 6).

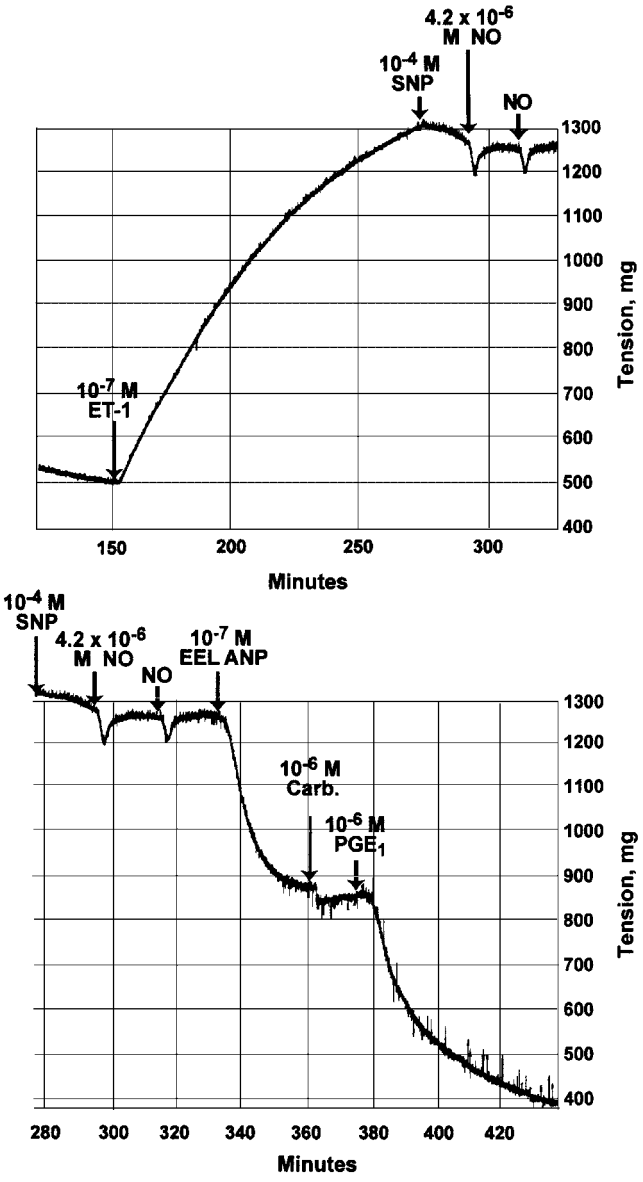

Fig. 1. Copy of the computer tracing of the response a single, representative preparation of tissue rings from the from bulbus arteriosus (BA) from the eel (*Anguilla rostrata*) to ET–1, SNP and NO, eANP, CPR, and PGE_1. Abbreviations: ET–1=endothelin 1; SNP=sodium nitroprusside (NO donor); NO=nitric oxide; eANP=eel atrial natriuretic peptide; CPR=carbaprostacyclin (stable prostacyclin agonist); PGE_1 =E-type prostaglandin.

DISCUSSION

It is clear that the bulbus arteriosus of *A. rostrata* is sensitive to a variety of agonists (Figs. 1–6) that produce constriction or dilation in other fish vascular smooth muscle preparations (see Introduction). The lack of a response to ACh in the eel BA, however, is surprising, considering the fact that ACh has been shown to contract strips of BA from the trout (Klaverkamp and Dyer, '74) as well as decrease the compliance of the BA in the trout (Farrell, '79). The BA of more teleost species should be tested for sensitivity to ACh, and examined for cholinergic innervation, since at least some data suggest that cholinergic receptors are present. The teleost heart itself receives ''vagosympathetic'' nerves containing both vagal and spinal autonomic fibers, but adrenergic

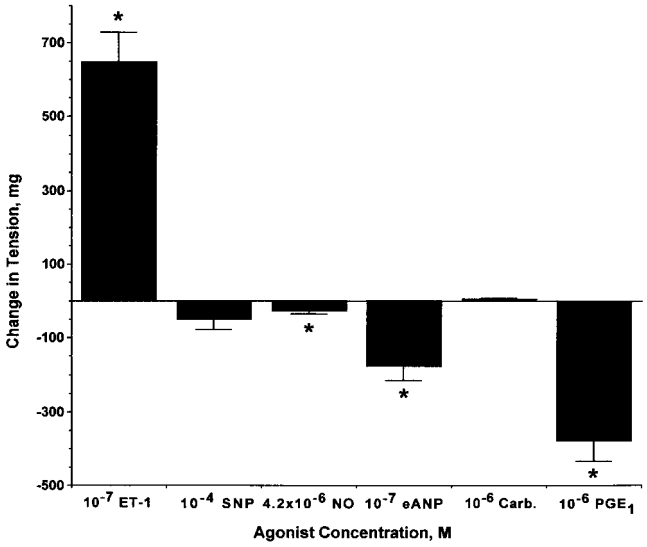

Fig. 2. Summary of the effect of the vasoactive agonists on the tension of the BA of the eel. Columns marked with an asterisk are significantly different from zero ($p<0.05$). N=8 for each agonist.

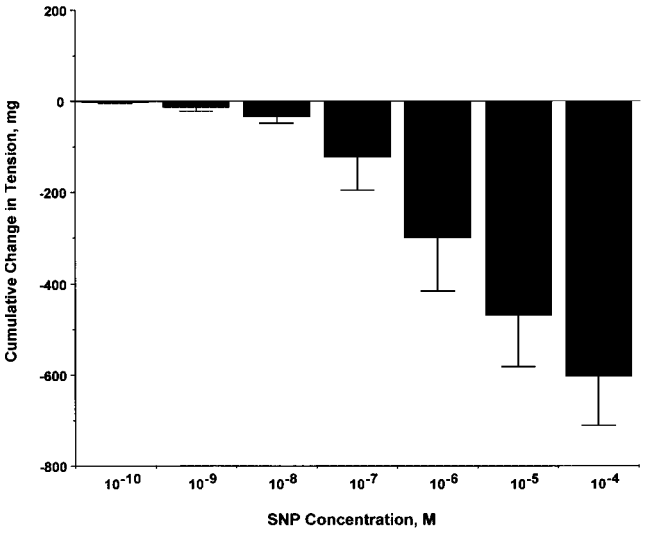

Fig. 4. The effect of concentration of the NO donor sodium nitroprusside (SNP) on the tension of the BA of the eel. The effect of concentration on dilation was linear ($p<0.0001$; N=8).

neurons appear predominant (Donald, '98). In fact "it has not been possible to determine the distribution of cholinergic nerves in the [teleost] heart" (Donald, '98). Nevertheless, vagal stimulation produced negative inotropic and chronotropic responses in the teleost *Platycephalus bassensis*, which could be inhibited by treatment with a muscarinic antagonist, suggesting involvement of cholinergic fibers (Donald and Campbell, '82).

ET–1 produced a substantial constriction of the BA (Figs. 1–3); this is not especially surprising since ET is considered the most powerful constrictive agent in the vertebrates (e.g., Miyauchi and Masaki, '99). We have now demonstrated a

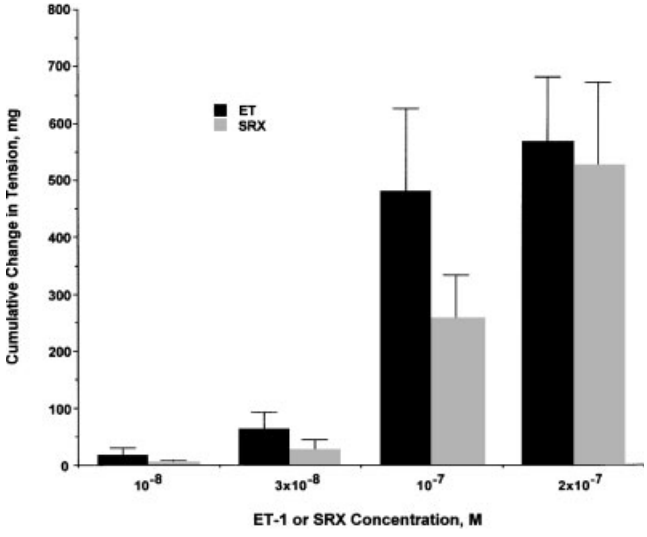

Fig. 3. The effect of concentration of either ET–1 or sarafotoxin S6c (SRX, ET_B-specific agonist) on the tension of the BA of the eel. The effect of concentration on contraction was linear ($p<0.0001$; N=8 for each agonist).

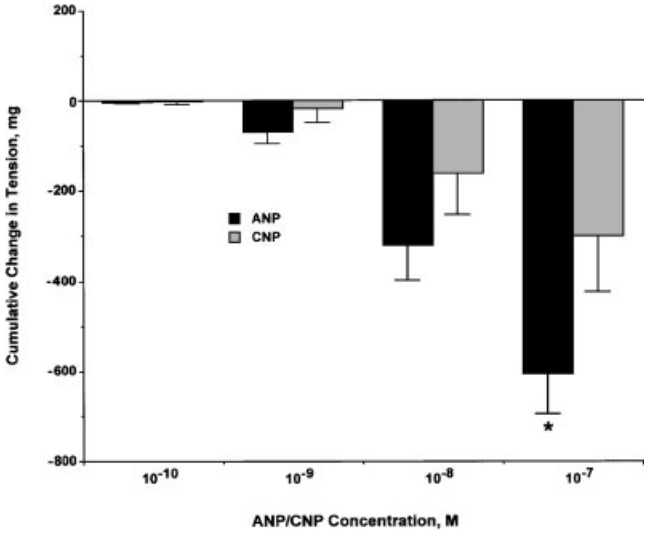

Fig. 5. The effect of concentration of two natriuretic peptides (eel ANP and porcine CNP) on the tension of the BA of the eel. The effect of concentration on dilation was linear ($p<0.0001$; N=8 for both agonists). The effect of ANP was greater than CNP only at 10^{-7} M)

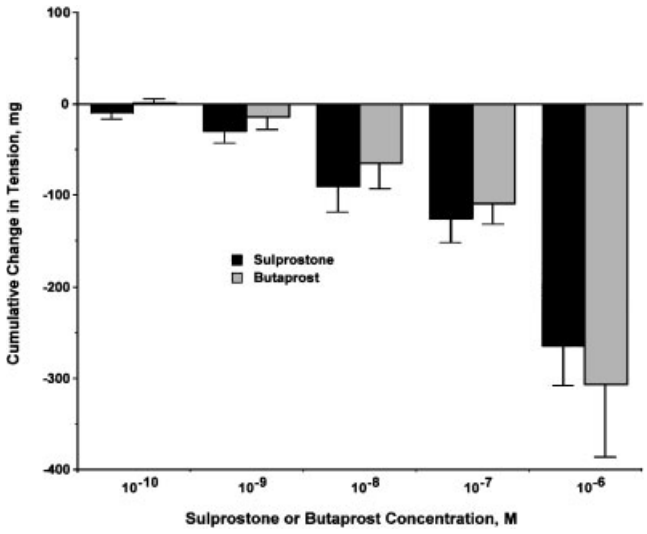

Fig. 6. The effect of concentration of two PGE agonists (Sulprostone and Butaprost) on the tension of the BA of the eel. The effect of concentration on dilation was linear ($p < 0.0001$; N=6–8 for both agonists).

response to mammalian ET in the aorta of the shark, *Squalus acanthias* (Evans et al., '96), as well as the posterior intestinal vein and anterior mesenteric artery of that species (Evans, 2001) and the ventral aorta of *A. rostrata*, *Myxine glutinosa* (hagfish), and *Petromyzon marinus* (lamprey) (Evans and Harrie, 2001). This suggests that a vascular ET signaling system is widespread in the early vertebrates. The fact that SRX is as effective as ET–1 in the eel BA (Fig. 3) suggests that ET_B-like receptors are involved, but the presence of ET_A-like receptors, in addition, cannot be ruled out by our data. In fact, our earlier data suggested that ET_A receptors mediate the ET-inducd constrictions in the ventral aorta of the same species, because SRX was ineffective on this tissue (Evans and Harrie, 2001). In contrast, aortic and venous vessels in the elasmobranch, *S. acanthias*, respond to SRX, suggesting the presence of an ET_B-like receptor (Evans et al., '96; Evans, 2001). Endothelin is generally thought to be a paracrine agent, produced by endothelial cells and stimulating underlying vascular smooth muscle (e.g., Miyauchi and Masaki, '99). Immunoreactive ET has been localized in the BA of at least one teleost, *Champsocephalus gunnari* (Masini et al., '97), so sources of ET to stimulate the BA may be local.

Since NO itself dilated the eel BA (Figs. 1 and 2) and the NO donor SNP produced a concentration-dependent dilation (Fig. 4), it appears that, like the ventral aorta (Evans and Harrie, 2001), the eel BA expresses the endothelium-derived relaxing system that appears to be nearly ubiquitous in the vertebrates (e.g., Fleming and Busse, '99). The source of the NO is unknown in the eel BA, but nitric oxide synthase (NOS; which mediates the production of NO from L-arginine) has been localized (via histochemical and immunohistochemical techniques) in the central and peripheral nervous system of a variety of teleost, elasmobranch, and even agnathan fishes (e.g., Schober et al., '94; Bruning et al., '96; Funakoshi et al., '97; Holmqvist and Ekstrom, '97; Karila et al., '97; Cioni et al., '98), and we have recently cloned a 336 bp fragment of a NOS from gill tissue from *S. acanthias* (Farmerie, Holland, and Evans, unpublished; GenBank Accession # AF232227), which is >90% identical with human and rat neuronal NOS. The site of expression of NOS in the eel BA remains to be determined.

Natriuretic peptides produced substantial relaxation in the BA of the eel (Figs. 1 and 2), and the dilation was concentration dependent for both eANP and pCNP (Fig. 5). Natriuretic peptide-induced dilation has been shown for the ventral aortae of *S. acanthias* (Evans et al., '93) and *M. glutinosa*, *P. marinus*, and *A. rostrata* (Evans and Harrie, 2001), and the response of the eel BA to both eANP and pCNP corroborates our data on the ventral aorta of this species, which also responded to both natriuretic peptides (Evans and Harrie, 2001). Since NPR-A receptors are relatively ANP specific and NPR-B receptors are very CNP specific (e.g., Takei, 2000; Hirose et al., 2001), our data suggest that NPR-B-like receptors are expressed in both the BA and ventral aorta of *A. rostrata*, but that NPR-A-like receptors also may be present. The BA itself may be the site of production of the natriuretic peptides (Kim et al., '89; Masini et al., '97), but the fish heart also produces a variety of natriuretic peptides (e.g., Takei, 2000).

The BA of the eel dilated substantially when PGE_1 was applied, but was insensitive to carbaprostacyclin, a stable PGI_2 agonist (Figs. 1 and 2). Thus, E-type prostaglandins should be considered a major dilatory agent in the BA, more stimulatory than NO on a molar basis. PGEs are considered to bind to four receptor subtypes (termed EP), and four EP receptors have been characterized and cloned (designated $EP_{1–4}$): EP_2 and EP_4 are thought to mediate vascular dilation, while EP_1 and EP_3 are thought to mediate constriction (e.g., Coleman et al., '94; Breyer et al., 2001). Thus, the

fact that we found that Butaprost (EP_2 -specific) produced concentration-dependent dilations of the eel BA (Fig. 6) is consistent with this classical designation of EP receptors. However, Sulprostone (EP_3-specific) also produced concentration-dependent dilation (Fig. 6). These data suggest either that the eel bulbus arteriosus EP receptor is more promiscuous to these agonists or that EP_3 receptors in this tissue can mediate dilation. Nevertheless, it is clear that prostaglandins can regulate BA tension.

In summary, our data demonstrate that the bulbus arteriosus of at least *Anguilla rostrata* is quite sensitive to a variety of vasoactive substances and suggests that the BA is much more than a ''windkessel.'' The role of agonist-induced changes in BA diameter in fish cardiovascular physiology remains to be determined, and determination of specific receptors involved awaits pharmacological and molecular examination.

ACKNOWLEDGEMENTS

Appreciation is expressed to Dr. Jeffrey Skimming, Dept. of Pediatrics, University of Florida School of Medicine, who supplied the NO gas to make the saturated NO solution.

LITERATURE CITED

Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. 2001. Prostanoid receptors: Subtypes and signaling. Annu Rev Pharmacol Toxicol 41:661–690.

Bruning G, Hattwig K, Mayer B. 1996. Nitric oxide synthase in the peripheral nervous system of the goldfish, *Carassius auratus*. Cell Tissue Res 284:87–98.

Bushnell PG, Jones DR. 1994. Cardiovascular and respiratory physiology of tuna and billfishes: adaptations for support of exceptionally high metabolic rates. Environ Biol Fishes 40:303–318.

Bushnell PG, Jones DR, Farrell AP. 1992. The Arterial System. In: Hoar WS, Randall DJ, Farrell AP, editors. Fish Physiology, Vol. 12A. San Diego: Academic Press. p 89–139.

Cerra MC, Canonaco M, Tota B. 1992. A quantitative autoradiographic study of ^{125}I atrial natriuretic factor in the heart of a teleost fish (*Conger conger*). J Exp Zool 263:215–9.

Cioni C, Francia N, Fabrizi C, Colasanti M, Venturini G. 1998. Partial biochemical characterization of nitric oxide synthase in the caudal spinal cord of the teleost *Oreochromis niloticus*. Neurosci Lett 253:68–70.

Coleman RA, Smith WL, Narumiya S. 1994. International Union of Pharmacology Classification of Prostanoid Receptors: Properties, distribution and structure of the receptors and their subtypes. Pharmacol Rev 46:205–229.

Donald J, Campbell G. 1982. A comparative study of the adrenergic innervation of the teleost heart. J Comp Physiol 147B:85–91.

Donald JA. 1998. Autonomic nervous system. In: Evans DH, editor. The Physiology of Fishes. Boca Raton, FL: CRC Press. p 407–439.

Evans DH. 2001. Vasoactive receptors in abdominal blood vessels of the dogfish shark, *Squalus acanthias*. Physiol Biochem Zool 74:120–126.

Evans DH, Gunderson M, Cegelis C. 1996. ET_B-type receptors mediate endothelin-stimulated contraction in the aortic vascular smooth muscle of the spiny dogfish shark, *Squalus acanthias*. J Comp Physiol 165:659–664.

Evans DH, Gunderson MP. 1998. A prostaglandin, not NO, mediates endothelium-dependent dilation in ventral aorta of shark (*Squalus acanthias*). Am J Physiol 274:R1050–7.

Evans DH, Harrie AC. 2001. Vasoactivity of the ventral aorta of the American eel (*Anguilla rostrata*), Atlantic hagfish (*Myxine glutinosa*), and sea lamprey (*Petromyzon marinus*). J Exp Zool 289:273–284.

Evans DH, More K, Robbins SL. 1989. Modes of ammonia transport across the gill epithelium of the marine teleost fish *Opsanus beta*. J Exp Biol 144:339–356.

Evans DH, Toop T, Donald J, Forrest JN Jr. 1993. C-type natriuretic peptides are potent dilators of shark vascular smooth muscle. J Exp Zool 265:84–87.

Farrell AP. 1979. The wind-kessel effect of the bulbus arteriosus in trout. J Exp Zool 209: 169–172.

Farrell AP, Jones DR. 1992. The Heart. In: Hoar WS, Randall DJ, Farrell AP, editors. Fish Physiology, Vol. 12A. San Diego: Academic Press. p 1–88.

Fleming I, Busse R. 1999. NO: the primary EDRF. J Mol Cell Cardiol 31:5–14.

Funakoshi K, Kadota T, Atobe Y, Goris RC, Kishida R. 1997. NADPH-diaphorase activity in the vagal afferent pathway of the dogfish, *Triakis scyllia*. Neurosci Lett 237:129–132.

Gannon BJ, Burnstock G. 1969. Excitatory adrenergic innervation of the fish heart. Comp Biochem Physiol 29:765–773.

Hirose S, Hagiwara H, Takei Y. 2001. Comparative molecular biology of natriuretic peptide receptors. Can J Physiol Pharmacol 79:665–672.

Holmqvist B, Ekstrom P. 1997. Subcellular localization of neuronal nitric oxide synthase in the brain of a teleost; an immunoelectron and confocal microscopical study. Brain Res 745: 67–82.

Icardo JM, Colvee E, Cerra MC, Tota B. 1999. Bulbus arteriosus of the Antarctic teleosts. II. The red-blooded *Trematomus bernacchii*. Anat Rec 256:116–126.

Johansen K, Martin AW. 1965. Comparative aspects of cardiovascular function in vertebrates. In: Hamilton WF, Dow P, editors. Handbook of Physiology; Circulation, Vol. 2. Washington, D.C.: Am Physiol Soc. p 2583–2614.

Karila P, Messenger J, Holmgren S. 1997. Nitric oxide synthase- and neuropeptide Y-containing subpopulations of sympathetic neurons in the coeliac ganglion of the Atlantic cod, *Gadus morhua*, revealed by immunohistochemistry and retrograde tracing from the stomach. J Auton Nerv Syst 66:35–45.

Kim SH, Cho KW, Koh GY, Seul KH, So JN, Ryu H. 1989. Phylogenetic study on the immunoreactive atrial natriuretic peptide in the heart. Gen Comp Endocrinol 74:127–131.

Klaverkamp JF, Dyer DC. 1974. Autonomic receptors in isolated rainbow trout vasculature. Eur J Pharmacol 28:25–34.

Licht JH, Harris WS. 1973. The structure, composition and elastic properties of the teleost bulbus arteriosus in the carp, *Cyprinus carpio*. Comp Biochem Physiol A 46:699–708.

Masini MA, Sturla M, Uva BM. 1997. Vasoactive peptides in the heart of *Champsocephalus gunnari*. Comp Biochem Physiol A Physiol 118:1083–1086.

Miyauchi T, Masaki T. 1999. Pathophysiology of endothelin in the cardiovascular system. Annu Rev Physiol 61:391–415.

Olson KR 1997. The Cardiovascular System. In: Evans DH, editor. The Physiology of Fishes, Boca Raton, FL: CRC Press. p 129–154.

Priede IG. 1976. Functional morphology of the bulbus arteriosus of rainbow trout (*Salmo gairdneri* Richardson). J Fish Biol 9:209–216.

Schober A, Malz CR, Schober W, Meyer DL. 1994. NADPH-diaphorase in the central nervous system of the larval lamprey (*Lampetra planeri*). J Comp Neurol 345:94–104.

Takei Y. 2000. Structural and functional evolution of the natriuretic peptide system in vertebrates. Int Rev Cytol 194:1–66.

von Skramlik E. 1935. Uber den kreislauf den fischen. Ergebn Biol 11:1–130.

Watson AD, Cobb JL. 1979. A comparative study on the innervation and the vascularization of the bulbus arteriosus in teleost fish. Cell Tissue Res 196:337–346.