Catecholaminergic regulation of venous function in the rainbow trout

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Zhang, Yutong, Leroy Weaver, J. r., Andrew Ibeawuchi, and Kenneth R. Olson. Catecholaminergic regulation of venous function in the rainbow trout. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1195–R1202, 1998.—The significance of the sympathetic nervous system (SNS) in regulating peripheral vascular resistance and cardiac function in fish has been well established, whereas its effect on venous function in vivo is unknown. Two protocols were employed in the present study to evaluate SNS effects on the venous system in intact, unanesthetized trout. In the first, trout were instrumented with pressure cannulas in the ventral (PVA) and dorsal (PDA) aortas and ductus Cuvier and placement of flow probe have been described in detail (17). Trout were anesthetized in benzo-
caine (ethyl-p-aminobenzoic acid, 1:6,000, wt/vol), and the dorsal aorta was cannulated with heat-tapered polyethylene tubing (PE-60). The gills were not irrigated during this <1 min procedure, but they were continuously irrigated with 10°C aerated water containing 1:24,000 (wt/vol) benzocaine during placement of remaining cannulas and the flow probe.

The pericardial cavity was exposed with a midline ventral incision, and both the right horn of the ductus Cuvier and the bulbus arteriosus were cannulated with 5-mm-long 0.51-mm-ID silicon tubing (Dow Corning veterinary grade; Konigsberg I Instruments, Pasadena, CA). The free ends of the tubing were connected to 60 cm of PE-90. All three cannulas were filled with heparinized saline (100 USP units/ml heparin in 9.0 g/l NaCl) and connected to Gould P23 pressure transducers. A 35 Transonic flow probe (Transonic Systems, Ithaca, NY) was placed around the ventral aorta, distal to the cannula, and connected to a Transonic T206 flowmeter. The incision was closed with interrupted silk sutures and sealed with cyanoacrylate gel. Venous and ventral aortic cannulas and the flow probe lead were secured to the fish with silk sutures. The fish were revived and placed in black plastic tubs suspended in a 1,500-liter experimental aquarium with aerated, flowing well water at 15°C. Experiments were conducted 24–48 h after surgery.

Analog pressure signals were displayed with Hewlett-Packard 7853A patient monitors (Palo Alto, CA). Digitized signals of pressure and flow were collected at 0.1-s intervals, and 1-s averages were stored on computer. Resting pressure and cardiac output were visually monitored for 1–2 h before experimentation to ensure that they were stable. Cardiovascular variables were then continuously recorded for a 5-min control period, during 20 min of catecholamine infusion, and for an additional 15-min recovery period. Catecholamines were infused into the dorsal aortic cannula with a syringe pump in a 1,500-liter experimental aquarium with aerated, flowing well water at 15°C. Experiments were conducted 24–48 h after surgery.

Ventricular fibrillation. Trout were anesthetized in benzocaine, and the dorsal aorta and ductus Cuvier were cannulated as described above. Two coiled stainless steel wire stimulating electrodes (0.126 mm diameter) were placed in the pericardial cavity on either side of the ventricle and exteriorized and secured to the fish along with the venous cannula. The fish were revived and placed in black plastic tubes suspended in the experimental aquarium. Zero-flow conditions were produced by electrical fibrillation of the heart for 6–8 s with a 3.5- to 5.0-V, 40-ms-duration pulse administered at 50 Hz. This method was used for all Epi, low-dose NE (2.6 nmol·min⁻¹·kg body wt⁻¹), and autonomic blockade studies.

Ventricular aortic occlusion. A method to produce zero-flow conditions by occluding the ventral aorta was subsequently developed and employed in an additional group of experiments in which the effects of a high rate of NE infusion (10.4 nmol·min⁻¹·kg body wt⁻¹) were examined. A sleeve was constructed from a 7.5-mm-length of 6-gauge stainless steel tubing. A 2.5-mm-diameter hole was drilled through one wall in the middle of the sleeve, and a 1- to 2-mm-wide notch was cut down the length of the sleeve within 2–3 mm of the hole. A 90° bend was made 5 mm from the end of a 20-mm length of 20-gauge stainless steel tubing, and a piece of heat-flared polyethylene tubing (PE-90) was placed over the short end of the 20-gauge tubing. A piece of latex rubber was cut from a condom and secured over the flared end of the PE tubing with a silk ligature, and a 1-m length of PE-90 tubing was attached to the long end of the stainless steel tubing. The free end of the PE tubing was then inserted into the hole in the sleeve from the luminal side, and the tubing was pulled through the sleeve until the flared end was seated in the hole on the luminal side. A 1-ml syringe was attached to the other end of the PE-90 tubing, and the volume of air required to inflate the latex to the point where it occluded the sleeve was noted. The dorsal aorta and ductus Cuvier were cannulated as described above and the occluder sleeve was fitted over the anterior bulbus and ventral aorta by passing the vessel through the notch. Tubing from the occluder was exteriorized and secured to the fish along with the venous cannula. Inflation of the occluder produced changes in PDA and PVEN essentially identical to ventricular fibrillation without stimulation of nearby skeletal muscle.

PDA and PVEN were measured in unanesthetized fish before and within 5–7 s after initiation of zero-flow conditions. PVEN during zero-flow was assumed to be equal to MCFP. Blood pressures were restored within 2–3 s after cessation of ventricular fibrillation or ventral aortic occlusion. Vascular capacitance curves were obtained by measuring MCFP during normovolemia (30–35 ml/kg body wt; Ref. 15), and then again as blood volume was adjusted up or down in 10% increments between 120 and 80% of resting volume. Whole blood from a donor fish was used for volume expansion. Cardiac arrest or ventral aortic occlusion was initiated within 30 s after each volume manipulation, and blood volume was restored to 100% within 30 s after zero-flow pressure measurement. Zero-flow conditions did not exceed 15 s. The interval
The effects of volume expansion on PDA were more previously reported (7). Variable responses of individual fish, as has been this was not statistically significant because of the variations were qualitatively similar to those observed when C was uninterrupted, albeit at lower pressures. Epi infusion increased PVen at all blood volumes (Table 1), whereas NE infusion did not significantly affect PVen at any blood volume (not shown).

Chemicals

Composition of trout PBS in grams per liter was as follows: 7.37 NaCl, 0.31 KCl, 0.10 CaCl2, 0.14 MgSO4, 0.46 KH2PO4, 2.02 Na2HPO4, 0.9 glucose; pH 7.8. All chemicals were purchased from Sigma Chemical (St. Louis, MO).

Statistics

Comparisons of responses were made with appropriate paired or unpaired t-tests or repeated-measures analysis of variance. Significance was assumed at P ≤ 0.05. Values are expressed as means ± SE.

RESULTS

The effects of catecholamine infusion on cardiovascular parameters in unanesthetized trout are shown in Figs. 1 and 2. Epi infusion increased PVA, PDA, and PVen, whereas NE increased both arterial pressures but did not affect PVen. Epi also transiently decreased Rg and increased Rs, CO, HR, and SV appeared to change, but this was not statistically significant because of the variable responses of individual fish, as has been previously reported (7).

The effects of catecholamine infusion on PDA before and during zero-flow conditions and on vascular capacitance are shown in Fig. 3, and the effects on PVen during normal ventricular outflow are listed in Table 1. Blood volume expansion above 100% in saline-infused trout slightly increased PDA, whereas volume depletion did not appear to affect pressure (Fig. 3, A, C, and E). The effects of volume expansion on PDA were more pronounced and statistically significant during catecholamine infusion. Changes in PDA during zero-flow conditions were qualitatively similar to those observed when CO was uninterrupted, albeit at lower pressures. Epi infusion increased PVen at all blood volumes (Table 1), whereas NE infusion did not significantly affect PVen at any blood volume (not shown).

DISCUSSION

The results of the present study show that infusion of Epi into trout increases both arterial and central venous pressures, whereas NE infusion primarily affects arterial pressures. In a similar manner, Epi alone increases MCFP by increasing venous tone (which shifts blood from the unstressed into the stressed vascular compartments) and by decreasing C (which directly increases venous pressure at a constant SBV). The opposite effects, i.e., a decrease in venous tone and an increase in C, are produced by inhibition of α1-adrenoceptors with prazosin or by autonomic ganglionic blockade with hexamethonium. These studies indicate that the SNS is an important effector of venous function in trout and they provide the first evidence, in any fish, of tonic regulation of venous tone and compliance. Thus in fish, as in mammals, venous pressure appears to be an important, and regulated, determinant of cardiac filling and therefore CO.

The two primary determinants of CO in mammals are the heart and venous return. Although these two are intimately interrelated and their actions are difficult to separate, numerous studies (reviewed in Refs. 8, 20) support the hypothesis that the latter is perhaps more important in most situations, except for those that result from impaired cardiac function. In this context, the mammalian heart is considered to be more of a sump pump (i.e., filled through extracardiac factors) than a suction pump.

A number of arguments have been made for the opposite situation in fish, i.e., that the heart is the
primary determinant of CO (reviewed in Refs. 5, 24). These have been largely based on three observations. 1) Many fish have a rigid pericardium that could support a substantially negative pericardial pressure during ventricular contraction and thereby promote venous filling of the atrium. 2) Central venous pressure is near, or slightly below atmospheric, suggesting cardiac aspiration. 3) Fish in water are in an essentially gravity-free environment and therefore they will not experience orthostatic venous pooling. However, before the present study, active venous regulation in fish had not been directly examined.

The effects of Epi, prazosin, and hexamethonium on venous tone and compliance are consistent with the SNS acting as a tonically active anti-drop regulator of arterial blood pressure in trout. SNS activation can be significant under two circumstances (20): 1) if blood volume is reduced by hemorrhage or other factors, SNS activation will mobilize blood from the USBV to the SBV and thereby restore MCFP, venous return, and CO, and 2) if tissue metabolism is elevated in normovolemic fish, CO can be increased by SNS-stimulated elevation of MCFP and the resulting augmentation of venous return. SNS regulation of venous capacitance in...
concert with tonic SNS regulation of arteriolar resistance will enable the fish to adjust both systemic resistance and CO commensurate with the desire to maintain arterial blood pressure.

Infusion of Epi mobilizes ~2 ml/kg body wt of blood from the USBV in normovolemic fish (Table 2), which is nearly 7% of the total blood volume and, more importantly, ~15% of the hemodynamically active SBV. This increases central venous pressure in unfibrillated trout by 30–60% and accounts for nearly a 1-mmHg increase in pressure in normovolemic trout. A 1-mmHg increase in central venous pressure will increase CO by ~25% (6) in the isolated trout heart. In trout with an intact pericardium this increase in preload may have an even greater effect on CO (4).

It has been shown in the in situ-perfused trout heart model that central venous pressure becomes elevated when the pericardium is opened and that opening the pericardium lowers CO if preload is maintained constant. (4). However, resting CO (and arterial blood pressure) in trout with an intact pericardium (26) is essentially the same as that observed in trout with an open pericardium (present study). This suggests that CO (or more probably arterial pressure) is being maintained, and in order for this to be achieved central venous pressure may be adjusted upward to compen-

Table 1. Central venous pressure at 80–120% of resting blood volume before and after epinephrine infusion or injection of prazosin or hexamethonium

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<th>80%</th>
<th>90%</th>
<th>100%</th>
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<th>120%</th>
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<td>10</td>
<td>1.4 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>5.2 ± 0.3</td>
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<td>Epinephrine</td>
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<td>2.1 ± 0.3</td>
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<td>6.7 ± 0.6</td>
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<td>2.0 ± 0.2</td>
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<td>4.5 ± 0.4</td>
<td>5.9 ± 0.4</td>
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<td>Prazosin</td>
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<td>1.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>4.2 ± 0.4</td>
<td>5.4 ± 0.2</td>
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<tr>
<td>Control</td>
<td>8</td>
<td>1.5 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>7.0 ± 0.2</td>
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<tr>
<td>Hexamethonium</td>
<td>8</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>5.8 ± 0.2</td>
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Values are means ± SE in mmHg; n = number of trout. Epinephrine was infused at 1 nmol·min⁻¹·kg body wt⁻¹; prazosin (0.47 µmol/kg body wt) and hexamethonium (3.7 µmol/kg body wt) were given as single injections. All treatments are significantly different (P < 0.05) from respective control.
sate for reduced aspiration into the open pericardium. Thus the increase in venous pressure is more likely the direct result of a change in vascular capacitance, i.e., a peripheral response, and not due to blood passively damming up behind a mechanically inefficient heart. If the latter were the case, CO and arterial pressure would be expected to fall.

It is surprising that vascular capacitance and $P_{VEN}$ are refractory to NE even when NE is infused at 2.5–10 times the rate of Epi and, at the highest NE infusion rate, NE is a more potent arterial pressor (Fig. 3; Table 2). Differences in venous responsiveness are also evident when the two amines are infused at 3.3 nmol·min$^{-1}$·kg body wt$^{-1}$ (Figs. 1 and 2); i.e., although both have nearly the same effect on $P_{DA}$, only Epi appreciably affects $P_{VEN}$. These differences may be attributable to one or several factors, including different rates of catecholamine inactivation, different accessibility of the receptors to the two catecholamines, different receptors, or different receptor sensitivities. Radiolabeled NE is removed from the trout circulation somewhat faster than Epi (12) and if removal occurs in

<table>
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<th>90–110% Blood Volume</th>
<th>80–100% Blood Volume</th>
<th>100–120% Blood Volume</th>
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<td>USBV C</td>
<td>USBV C</td>
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<tr>
<td>Epinephrine</td>
<td>10 16.4 ± 0.5* 2.6 ± 0.2*</td>
<td>15.7 ± 0.5* 2.8 ± 0.1*</td>
<td>17.9 ± 1.0* 2.3 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>10 22.0 ± 0.5 2.0 ± 0.1</td>
<td>17.9 ± 1.2 3.2 ± 0.3</td>
<td>22.9 ± 0.6 1.8 ± 0.1</td>
</tr>
<tr>
<td>Norepinephrine 1</td>
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<td>19.3 ± 0.6 3.1 ± 0.2</td>
<td>23.1 ± 1.3 3.1 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>10 23.0 ± 0.9 2.2 ± 0.2</td>
<td>17.3 ± 1.5 4.4 ± 0.7</td>
<td>24.9 ± 1.2 1.9 ± 0.2</td>
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<td>Norepinephrine 2</td>
<td>10 23.3 ± 1.5 1.9 ± 0.3</td>
<td>18.1 ± 1.3 3.5 ± 0.4</td>
<td>23.8 ± 2.2 2.0 ± 0.4</td>
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Values are means ± SE; $n =$ number of trout. Unstressed blood volume (USBV) is in ml/kg body wt, vascular compliance (C) is in ml·mmHg$^{-1}$·kg body wt$^{-1}$. Epinephrine was infused at 1 nmol·min$^{-1}$·kg body wt$^{-1}$, and norepinephrine was infused at 2.6 (norepinephrine 1) and 10.4 (norepinephrine 2) nmol·min$^{-1}$·kg body wt$^{-1}$. Mean circulatory filling pressure (MCFP) was obtained at 80, 90, 100, 110, and 120% of estimated resting blood volume, and USBV and C were determined from 3 consecutive volumes. *Significant difference ($P < 0.05$) between control and respective catecholamine infusion.

Fig. 4. $P_{DA}$ (A, C, and E) and vascular capacitance curves (B, D, and F) before (Con; circles) and after (triangles) injection of 0.47 mol/kg body wt prazosin (Praz; A and B), 1 mol/kg body wt phentolamine (Phent; C and D), or 3.7 mol/kg body wt hexamethonium (Hexameth; E and F). Solid symbols in A, C, and D indicate pressure during cardiac fibrillation during control or experimental treatment; open symbols indicate pressure before fibrillation. Values are means ± SE; $n = 15, 12, and 8$ trout for Praz, Phent, and Hexameth, respectively.
prevenous systemic vessels this could account for some of the reduced NE effect, although it seems unlikely that differential inactivation kinetics could explain the total lack of NE effect on PVEN. Similarly, there does not appear to be a differential sensitivity of large vessels to catecholamines because Epi and NE are equipotent constrictors in propranolol-blocked large arteries and veins from trout (2). However, catecholaminergic control of venous capacitance may be a property of the venules, and their receptors have not been characterized. A significant venular contribution to venous capacitance has been proposed based on other studies in trout that have shown considerable differences between large vein responses, in vitro, and vascular capacitance in vivo to NPs and sodium nitroprusside (17). The fact that neither catecholamine affects rapid compliance of large systemic trout veins (2) provides additional support for a venular role in vivo.

It is also surprising that while both prazosin and phentolamine lowered PDA, only prazosin affected the capacitance curve and PVEN (Fig. 4, A-D; Table 1). It is tempting to speculate that in these experiments phentolamine’s actions are directed at NE effector sites, hence the consistent effects on PDA and lack of venous responsivity. Clearly, additional studies are needed to clarify the location of vascular receptors in these fish.

Perspectives

The effects of Epi and SNS blockade on MCFP, USBV, and C in trout are qualitatively the same as those observed in mammals (25). The ability of another teleost, the bluefish, Pomatomus saltatrix, to withstand head-up tilting out of water suggests that reflex regulation of venous capacitance is present in bony fish and it also suggests that this ability has been passed along
during the course of vertebrate evolution. It is not clear whether this regulatory capacity is universal among teleosts or subteleostean fish because capacitance curves have only been obtained in trout. The inability of elasmobranchs to tolerate head-up tilt (13) may indicate that reflex regulation of vascular capacitance is lacking in these fish, but it may also reflect the severity of the experimental procedure. Some degree of regulation of vascular capacitance would seem to be necessary for cardiovascular function in all soft-bodied animals, but this remains to be demonstrated.

Although SNS regulation of peripheral resistance and venous capacitance appears similar among trout and mammals, the sites and mechanisms of action of other classical cardiovascular regulatory systems are surprisingly different. For example, the renin-angiotensin system, pressor in both fish and mammals (15), has no effect on vascular capacitance in trout (27), whereas it is an important effector of capacitance in mammals (25). Conversely, arginine vasopressin does not affect capacitance in mammals (25), yet its evolutionary antecedent, arginine vasotocin, increases venous tone in the trout (1). Similarly, vasodilator NPs and the nitric oxide donor, sodium nitroprusside, have distinctly different effects in trout and mammals. In trout, NPs primarily affect venous compliance, and sodium nitroprusside decreases arteriolar resistance without altering the venous system (17), whereas in mammals, NPs reduce arteriolar resistance and NO donors primarily affect the venous system (1). Similarly, vasodilator NPs and the nitric oxide donor, sodium nitroprusside, have distinctly different effects in trout and mammals. In trout, NPs primarily affect venous compliance, and sodium nitroprusside decreases arteriolar resistance without altering the venous system (17), whereas in mammals, NPs reduce arteriolar resistance and NO donors primarily affect venous function (10). These differences point out the inherent dangers in generalization of cardiovascular control mechanisms across different vertebrate classes. However, they also show that there is a pervasive scheme for integrating cardiovascular function, albeit with different messengers, that is common among vertebrates.

This work was supported in part by National Science Foundation Grants IBN-9105247 and IBN-9723306.

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Received 10 September 1997; accepted in final form 14 January 1998.

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