Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Oncorhynchus mykiss*): role of neural and circulating catecholamines

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**Sandblom, Erik, and Michael Axelsson.** Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (*Oncorhynchus mykiss*): role of neural and circulating catecholamines. *Am J Physiol Regul Integr Comp Physiol* 291: R711–R718, 2006; doi:10.1152/ajpregu.00893.2005.—Central venous blood pressure (P<sub>ven</sub>) increases in response to hypoxia in rainbow trout (*Oncorhynchus mykiss*), but details on the control mechanisms of the venous vasculature during hypoxia have not been studied in fish. Basic cardiovascular variables including P<sub>ven</sub>, dorsal aortic blood pressure, cardiac output, and heart rate were monitored in vivo during normoxia and moderate hypoxia (P<sub>沃</sub>O<sub>2</sub> = ∼9 kPa), where P<sub>沃</sub>O<sub>2</sub> is water oxygen partial pressure. Venous capacitance curves for normoxia and hypoxia were constructed at 80–100, 90–110, and 100–120% of total blood volume by transiently (8 s) occluding the ventral aorta and measure P<sub>ven</sub> during circulatory arrest to estimate the mean circulatory filling pressure (MCFP). This allowed for estimates of hypoxia-induced changes in unstressed blood volume (USBV) and venous compliance. MCFP increased due to a decreased USBV at all blood volumes during hypoxia. These venous responses were blocked by α-adrenergic receptor blockade with prazosin (1 mg/kg body mass). MCFP still increased during hypoxia after pretreatment with the adrenergic nerve-blocking agent bretylium (10 mg/kg body mass), but the decrease in USBV only persisted at 80–100% blood volume, whereas vascular capacitance decreased significantly at 90–110% blood volume. In all treatments, hypoxia typically reduced heart rate while cardiac output was maintained through a compensatory increase in stroke volume. Despite the markedly reduced response in venous capacitance after adrenergic blockade, P<sub>ven</sub> always increased in response to hypoxia. This study reveals that venous capacitance in rainbow trout is actively modulated in response to hypoxia by an α-adrenergic mechanism with both humoral and neural components.

**IN CONTRAST TO MAMMALS, MANY fish regularly experience environmental hypoxia. Although large intraspecific variations exist, teleosts generally develop bradycardia during hypoxia, which is mediated by an increased cholinergic tone on the heart (47). An increased adrenergic tone with a resultant increase in systemic vascular resistance (R<sub>sys</sub>) is also common (17, 22, 29, 44), but this response is variable since local hypoxic vasodilation may, probably depending on the degree of hypoxia, offset the sympathetic response and leave overall R<sub>sys</sub> either unaltered, increased, or reduced (4, 10, 19, 29, 37, 41). Hypoxia generally results in an increased stroke volume (SV) that may increase cardiac output (Q) during mild hypoxia, or mitigate the effect of the bradycardia on Q during more severe hypoxia (10, 19, 30, 37, 45, 47). Given the very limited scope for decreasing end-systolic volume in rainbow trout (11, 15, 16), the increase in SV is likely to be mediated by a rise in cardiac preload and an increased end-diastolic volume, as supported by increasing central venous blood pressure (P<sub>ven</sub>) during hypoxia (29, 37). Even during relatively mild hypoxia, when no bradycardia and, therefore, no blood pooling occurs in the central veins, P<sub>ven</sub> can increase (37). This suggests that venospecific events are, at least in part, responsible for the changes in P<sub>ven</sub> during hypoxia in fish. In anaesthetized dogs, it has been demonstrated that hypoxic stimulation of chemoreceptors results in reflex-mediated increase in venous smooth muscle tone (34, 35). In fish, however, the neurohumoral control of the venous vasculature during hypoxia is not understood.

Vascular capacitance is dependent on vascular compliance (C) and tone and is proportional to the volume of blood contained at a given pressure in the circulation (26, 33). Because of the much higher compliance and volume of the venous compartment, total vascular capacitance essentially equals venous capacitance. A decrease in venous capacitance may either result in a net transfer of blood from the venules toward the central veins with a resultant increase in cardiac preload or, when Q is simultaneously increased, increase the upstream (venular) pressure that drives venous return to the heart (26, 33). The construction of vascular capacitance curves is probably the best tool available to monitor capacitance in vivo (25, 26). The method has previously been used in rainbow trout to investigate the venous effects for a range of vasoactive substances (6, 21, 24, 48, 49). Vascular capacitance curves are constructed by measuring the mean circulatory filling pressure (MCFP) at different blood volumes. MCFP is estimated as the plateau P<sub>ven</sub> during a short and transient circulatory stop (<10 s), assuming that transcapillary fluid shifts and small remaining pressure differences between arteries and veins are negligible (26, 33). The slope of the pressure-volume curve represents C, and the volume at the intercept on the volume axis is the unstressed blood volume (USBV). USBV represents the residual blood volume in the system that does not stretch the vasculature and actively contribute to the buildup of transmural pressure. The remainder of the blood volume, which stretches the vasculature and creates pressure, is called the stressed blood volume (26, 33). In fish, infusion of epinephrine, but not norepinephrine, decreases USBV and C (49).

Detailed investigations of the venous component in cardiovascular responses to naturally occurring stimuli, such as...

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exercise and hypoxia, are scarce for fish (38). In fact, there are no reports on measurements of venous capacitance changes in response to hypoxia in fish that we know of. The two main objectives of this study were therefore: 1) evaluate the general venous responses to hypoxia in rainbow trout by measuring MCFP, USBV, and C and link these changes to those of $P_{\text{ven}}$, SV, heart rate (fH), Q, and $R_{\text{sys}}$; 2) study the mechanisms of the hypoxia-induced changes. Given that adrenaline potently decreases venous capacitance in vivo in rainbow trout (49), experiments with adrenergic blockers were undertaken in two separate groups of fish. Pretreatment with the $\alpha$-adrenoceptor antagonist prazosin and the adrenergic nerve-blocking agent bretylium, allowed us to specifically pinpoint the role of neurally vs. humorally released catecholamines during hypoxia.

**MATERIAL AND METHODS**

*Animals.* Rainbow trout (*Oncorhynchus mykiss*) between 280 – 690 g were obtained from a local hatchery (Antens Laxodling) and kept in 2,000-liter fiberglass tanks, supplied with recirculating water at 15°C. The fish were subjected to a natural photoperiod and fed a maintenance diet of commercial trout pellets. Swedish laws on animal care and ethical permits on physiological experimentation on fish covered all procedures reported in this study (96/2001).

*Surgical procedures.* Before surgery, fish were netted from the holding tanks, anaesthetized in water containing NaHCO$_3$-buffered saline (0.9%) and moved to a surgery table covered with wet foam rubber. The gills were continuously irrigated, via the mouth, with aerated water (10°C) containing NaHCO$_3$-buffered MS-222 at a lower dose (150 and 75 mg/l respectively). The dorsal aorta was cannulated via the roof of the buccal cavity with a slightly tapered polyethylene (PE)-50 catheter using a guidewire (42). The catheter was subsequently filled with heparinized (~100 IU/ml) saline (0.9%). With the fish put on its left side, the operculum and the four gill arches were gently retracted and held in position using a crescent-shaped piece of plastic, and a silk suture that was pinched to the underlying foam rubber. The ventral aorta was exposed, and a 4-0 silk suture was positioned under the vessel, allowing it to be lifted for placement of a combined Doppler-flow/occlusion probe. This probe has been described previously (36). Subsequently, the fish was placed on its right side, and the operculum and gill arches were retracted as described above. The sinus venosus/ductus of Cuvier was nonocclusively cannulated with a polyurethane catheter (ID, ~1 mm; OD, ~1.1 mm) filled with heparinized (~100 IU/ml) saline (0.9%). To optimize patency and to facilitate blood withdrawal, 2–3 side holes were cut in the first 5–10 mm of the catheter with the use of a razor. An ~5- to 10-mm incision was made in the isthmus starting on top of the cleithrum and ending close to the angle of the fifth branchial arch. The lateral part of the vessel wall of the ductus of Cuvier was exposed, lifted with forceps, and secured with 4-0 silk. A small cut was made, and the catheter was inserted ~10 mm toward the sinus venosus (1). Following surgery, the fish were revived in fresh water, transported to opaque experimental chambers, and left to recover for 24–36 h before experimentation. Additionally, some fish were instrumented only with a venous catheter. These fish served as blood donors in the experiments outlined below.

*Data acquisition.* Blood pressure was recorded using pressure transducers (model DPT-6100; Medizintechnik, Kirchseeon, Germany) calibrated against static water columns with the water surface serving as zero pressure reference. The signals from the pressure transducers were amplified with a 4ChAmp amplifier (Somedic, Hörby, Sweden). Q was recorded with a directional-pulsed Doppler flow meter (model 545C-4; University of Iowa, Iowa City, IA), and water oxygen partial pressure ($P_{w}\text{O}_2$) was recorded with an oxygen meter (Ox1340i/SET; WTW, Weilheim, Germany) located at the inlet to the experimental chamber. The oxygen meter was calibrated to the ambient barometric pressure on a daily basis. Hypoxic water was prepared in a separate barrel (~50 liters) bubbled by a gas mixture of 75% N$_2$ and 25% air supplied by a gas mixing flow meter (model GF-3MP; Cameron Instruments, Port Aransas, TX). When water inflow to the chamber was changed to hypoxic water, $P_{w}\text{O}_2$ dropped rapidly and stabilized at the desired oxygen level ($P_{w}\text{O}_2 = \sim 9$ kPa; i.e., 8.4–10.7 kPa) within 1–2 min. The flow-velocity of normoxic water from the recirculating departmental water system was adjusted to equal the velocity of hypoxic water coming from the barrel to minimize disturbances due to changes in water flow in the experimental chamber. A Power Lab system (ADInstruments, Castle Hill, Australia) connected to a personal computer was used for digital acquisition and subsequent analysis of cardiovascular data.

*Experimental protocol.* MCFP was measured as $P_{\text{ven}}$ at 5–7 s after circulatory arrest by mechanical occlusion of the ventral aorta (36). A randomized protocol was used where the fish was exposed to either normoxia or a 3-min pulse of moderate hypoxia (~3 kPa) before blood volume was altered in 10% intervals between 80–120% of normal blood volume. A blood volume of 30 ml/kg body mass ($M_b$) for *O. mykiss* was assumed in these experiments (23). Manipulations were done by withdrawal or injection of blood via the venous catheter. Blood volume manipulations were separated by at least 15 min when fish were normoxic and normovolemic. The protocol was randomized with regard to the volume manipulations, but a hypoxic measurement was always followed by a normoxic measurement. Hence, the hypoxic exposures were separated by no less than 35–40 min.

The resultant data points for MCFP, between 80–120% of normal blood volume, were used to construct vascular capacitance curves to obtain USBV and C for individual fish during normoxia and hypoxia. The protocol described above is similar to previous protocols used to estimate vascular capacitance in trout (6, 21, 24, 49).

In two additional series of experiments, identical protocols were used in fish pretreated with either the $\alpha$-adrenoceptor antagonist prazosin (1 mg/kg $M_b$; Pfizer, Sandwich, UK) or the adrenergic nerve-blocking agent bretylium tosylate (10 mg/kg $M_b$; Sigma-Aldrich, St. Louis, MO). Bretylium blocks the release of catecholamines from nerve endings and thus selectively blocks the nervous component of an adrenergic response (39). Prazosin is an $\alpha$-adrenoceptor antagonist that blocks the effect of both circulating as well as neurally released catecholamines. In initial experiments, bretylium caused mortality during the injection in several fish. Therefore, fish were first instrumented with dorsal aortic catheters, and bretylium was injected slowly (during ~1–3 h). Only fish that did not exhibit apparent distress after the injection were reanesthetized and fully instrumented for subsequent experiments. The blood volume manipulation protocol was initiated roughly 24 h after bretylium injection, as this time span is sufficient for nerve blockade and surgical recovery (4, 5, 18, 39, 40). In the prazosin experiments, the drug was injected 2–4 h before experimentation. After drug injection, the catheter was flushed with heparinized saline.

*Calculation of cardiovascular variables and statistical analysis.* In mammals and fish, the vascular capacitance curve is not linear (33, 49). Capacitance curves for individual fish were therefore calculated by linear regression analysis at three points: 80–100, 90–110, and 100–120% of the total blood volume (49). In the resultant curves, USBV equals the blood volume at a MCFP of zero (the intercept of the y-axis) and C is the slope of the relationship between blood volume and MCFP. Individual USBV and C values, and not mean MCFP values, were used to calculate mean USBV and C. This approach enabled us to make statistical comparisons between normoxia and hypoxia for these variables. By assuming a blood volume of 30 ml/kg $M_b$ for *O. mykiss* (23), USBV and C were converted to real values (i.e., ml/kg $M_b$ and ml kPa/kg $M_b$, respectively). Mean USBV and C values were also used to construct capacitance curves to graphically illustrate the effects of hypoxia on venous capacitance.
VENOUS CAPACITANCE IN HYPOXIC TROUT

RESULTS

Cardiovascular responses to hypoxia in untreated fish. MCFP increased significantly at all blood volumes during hypoxia (Fig. 1A). The increase in MCFP was mediated by a shift of blood into the stressed vascular compartment because USBV decreased, whereas hypoxia did not change C at any blood volume interval (Table 1). This was also clearly visible on the vascular capacitance curves where hypoxia resulted in a rightward shift of the curve with apparently no change of the slope (Fig. 2A).

The cardiovascular responses to hypoxia in normovolemic fish are presented in Fig. 3. Hypoxia resulted in a bradycardia (resting \(f_H\) decreased from 66 ± 11 to 52 ± 11 beats/min) that was accompanied by an increase in \(P_{ven}\) (from 0.06 ± 0.07 to 0.16 ± 0.08 kPa) and a 22% increase in SV, Q, \(R_{sys}\), and \(P_{da}\) did not change.

Effect of adrenergic receptor blockade on the hypoxic response. Prazosin treatment abolished the changes in venous capacitance during hypoxia. Neither USBV nor C changed at any blood volume (Table 1), and MCFP increased only at 80% blood volume (Fig. 1B). After prazosin resting MCFP in normoxia, however, was significantly increased at 80, 90, and 110% blood volume, and USBV was significantly lower at the 80–100% blood volume interval (Fig. 1B and Table 1, respectively).

\(R_{sys}\) decreased by 30% during hypoxia after prazosin and, as a result, \(P_{da}\) decreased from a resting 2.9 ± 0.8 to 1.8 ± 0.4 kPa. The bradycardic response to hypoxia was qualitatively identical to untreated fish as \(f_H\) dropped significantly from a resting 64 ± 17 to 49 ± 13 beats/min. Routine \(P_{ven}\) was significantly higher after prazosin compared with the untreated value. The hypoxic response, however, mimicked untreated fish, and \(P_{ven}\) increased from 0.17 ± 0.11 to 0.26 ± 0.09 kPa. Again, this was associated with an increased SV by 20% and no significant change in Q during hypoxia (Fig. 3).

Effects of adrenergic nerve blockade on the hypoxic response. Blockade of adrenergic nerves with bretylium had no significant effect on MCFP, USBV, and C compared with normoxic control fish (Fig. 1 and Table 1). However, the venous capacitance responses to hypoxia were not as clear-cut after adrenergic nerve blockade (Table 1). Although MCFP increased significantly, and, with a similar magnitude during hypoxia (Fig. 1C), USBV only increased significantly at 80–100% blood volume, whereas C decreased significantly at 90–110% blood volume, a response markedly different from untreated fish (Table 1). This was manifested as a flatter appearance of the vascular capacitance curve during hypoxia, whereas the y-axis intercept was unchanged (Fig. 2C).

Resting cardiovascular variables were not significantly different after adrenergic nerve blockade compared with untreated fish (Figs. 1 and 3). Similar to prazosin, however, the arterial
response to hypoxia was different after bretylium (Fig. 3). Hypoxia resulted in an 18% reduction in Rsys, which reduced Pda from 3.2 ± 0.3 to 2.7 ± 0.5 kPa. Similar to untreated fish, hypoxia triggered a bradycardia from 70 ± 9 to 59 ± 9 beats/min. Again, Q remained unchanged because of a 22% increase in SV that was accompanied by an increased Pven from 0.09 ± 0.06 to 0.15 ± 0.07 kPa (Fig. 3).

**DISCUSSION**

*Venous capacitance changes during hypoxia.* The increase in Pven during hypoxia found in the present study (Fig. 3) has been demonstrated previously in rainbow trout (29, 37). Since factors, such as reduced peripheral resistance and bradycardia, could account for this response (1, 37), it is not possible to directly interpret an increased Pven as an increase in venomotor tone. The present study, however, reveals that some of the venous blood volume, which is hemodynamically inactive during normoxia, is actively shifted into the stressed vascular compartment by an increased venous smooth muscle activity during hypoxia. This increases MCFP at all blood volumes and decreases USBV with a resultant rightward displacement of the vascular capacitance curve (Table 1 and Figs. 1A, 1B, and 2A). The rise in Pven during hypoxia (29, 37) could therefore, at least in part, be mediated by venospecific events (i.e., venoconstriction) and not only as a result of passive venous blood pooling during the hypoxic bradycardia. Interestingly, hypoxia only decreased venous capacitance by reducing USBV, whereas C did not change. Zhang et al. (49) reported that both USBV and C decreased in response to adrenaline infusion in rainbow trout. Although beyond the scope of the present investigation, it may be speculated that a more severe hypoxia would have resulted in reduced C as well. Furthermore, the vascular resistance response to hypoxia appears to be quite variable in fish (4, 10, 19, 29, 37, 41), and in the present study, there was no significant change in untreated fish during hypoxia (Fig. 3). The reason for this variation between studies is unknown, but it may be attributed to differences in the experimental protocols.

In mammals, vascular capacitance is primarily a function of the smooth muscle activity of venules and small veins (20, 26, 33). A decreased venous capacitance therefore results in a net transfer of blood from the periphery toward the central venous compartment and increases cardiac filling pressure if \( f_H \) is maintained. The relative contribution from different vascular beds to venous blood volume mobilization in fish is still unknown. During hypoxia, for example, splanchic blood volume and flow would be expected to decrease to favor perfusion of more vital vascular beds, such as those of respiratory, cardiac, and locomotory muscles, as well as central nervous tissues. Blood flow to the gastrointestinal tract does indeed decrease during hypoxia and exercise in fish (3, 4, 14, 46). According to Poiseuille’s law, decreased blood flow through a vascular bed would, per se, result in a decreased

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**Table 1. Effects of hypoxia on USBV and C in rainbow trout (Oncorhynchus mykiss) in vivo**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>USBV, ml/kg Mb</th>
<th>C, ml/kg Pa/kg Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80–100%</td>
<td>90–110%</td>
</tr>
<tr>
<td>Normoxia</td>
<td>11</td>
<td>25.0±2.4</td>
<td>25.2±2.8</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>11</td>
<td>20.6±5.9*</td>
<td>22.5±2.8*</td>
</tr>
<tr>
<td>Normoxia + prazosin</td>
<td>9</td>
<td>21.0±3.8†</td>
<td>21.3±5.4</td>
</tr>
<tr>
<td>Hypoxia + prazosin</td>
<td>9</td>
<td>21.9±1.6</td>
<td>22.8±3.6</td>
</tr>
<tr>
<td>Normoxia + bretylium</td>
<td>9</td>
<td>24.4±1.3*</td>
<td>24.1±3.4</td>
</tr>
<tr>
<td>Hypoxia + bretylium</td>
<td>9</td>
<td>22.2±4.1*</td>
<td>24.3±2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0±11.3</td>
<td>21.2±4.4</td>
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<td>20.7±6.7</td>
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<td>24.9±17.6</td>
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<td>19.5±7.6</td>
<td>20.3±11.9</td>
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<td>24.3±7.9</td>
<td>24.4±6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.4±10.0</td>
<td>17.6±3.3*</td>
</tr>
</tbody>
</table>

Mean values (±SD) of unstressed blood volume (USBV) and total vascular compliance (C) during normoxia and hypoxia (≥9 kPa) in untreated, prazosin-treated [1 mg/kg body mass (Mb)] and bretylium-treated (10 mg/kg Mb) rainbow trout (Oncorhynchus mykiss) *Statistical difference between normoxia and hypoxia (≤0.05)."
distending pressure and passive recoil of the vasculature with a resultant reduction in contained blood volume of that tissue (20, 26, 32). This effect is known as the De Jager-Krogh phenomenon (32). In fish, it remains to be investigated whether, or to what extent, also active changes in smooth muscle activity of the splanchnic capacitance vessels contribute to blood mobilization during exercise and hypoxia. In our opinion, it appears technically very difficult to address this question in vivo. Determinations of blood volume changes in vitro/in situ perfused organ preparations may generate some interesting information to this topic.

**Circulating and neural catecholamines during hypoxia.** The other focus of this study was to investigate the adrenergic control of the venous vasculature during hypoxia. Since prazosin treatment almost completely abolished all changes in MCFP and USBV, it is clear that the acute venous response to hypoxia is primarily mediated via α-adrenergic stimulation. Given the short duration of the hypoxic exposure in the present study (3 min), any venous response to hypoxia most likely originated from nervous or humoral mechanisms, because potential blood volume changes would be too slow to develop. Adrenergic nerve blockade with bretylium did not fully abolish the venous responses to hypoxia; as MCFP still decreased at all blood volumes, C decreased at 90–110% blood volume, and there was only a partial blockade of the hypoxia-induced decrease in USBV. This leads us to conclude that both circulating, as well as neural catecholamines, contribute to the venous responses to hypoxia. Furthermore, the degree of hypoxia used in the present study (PWO₂ = ~9 kPa) is within the range where humoral catecholamines are released in rainbow trout at similar temperatures (27, 28, 31). Prazosin was clearly not able to block the hypoxic increase in Pven. As discussed above, this highlights the fact that regulation of central venous pressure is very labile and is affected not only by venous events but by cardiac and arterial peripheral changes as well. The persistent increase in Pven after prazosin and bretylium is therefore best explained by venous blood pooling due to the bradycardia and possibly also by the reduction in Rsys. Similarly, the increased SV and the unchanged Q during hypoxia after adrenergic blockade was probably because of the inverse relationship between fH and SV, which operates by a mechanical autoregulatory mechanism in rainbow trout (1). It is possible that a more severe hypoxia after prazosin would have decreased Q as a result of an inability to increase cardiac preload and SV sufficiently after α-adrenoceptor blockade. In fact, in a previous study where we exposed rainbow trout to a slightly more severe hypoxia (PWO₂ = ~7.3 kPa for 8 min), untreated fish maintained Q during hypoxia, whereas there was a marked reduction in Q after prazosin, despite the fact that Pven increased in both treatments (37). The arterial hypotensive
response that was unmasked during hypoxia after adrenergic nerve or $\alpha$-adrenoceptor blockade has been reported previously and is presumably mediated by an unmasking of a $\beta$-dilatory response and/or the direct effect of low blood oxygen levels on the resistance vasculature (4, 18, 37, 43, 44, 47).

Circulating and neural catecholamines during normoxia.

With the exception of the increased MCFP and $P_{ven}$, and the reduced USBV at 80–100% blood volume, resting cardiovascular variables after prazosin did not differ from untreated fish (see Figs. 1 and 3 and Table 1). The somewhat unexpected increases in $P_{ven}$ and MCFP after $\alpha$-adrenoceptor blockade, however, have been reported previously (36–38). With only speculating on the mechanism(s), an increased blood volume due to a shift in the Starling equilibrium, altered branchial water uptake and/or reduced systemic resistance, may contribute to these responses. Zhang et al. (49) reported a slightly increased USBV after prazosin in trout. The different response in USBV to prazosin in the present study may be explained by the comparatively long time period of 2–4 h that was allowed after prazosin injection in our study compared with 20–40 min in the study by Zhang et al. (49) before the capacitance curves were constructed. This could indicate that it takes up to several hours before a new blood pressure/volume equilibrium is established after $\alpha$-adrenoceptor blockade.

The ability of bretylium to block transmitter release from adrenergic presynaptic nerve endings has been validated previously in fish (2, 4, 5, 18, 39, 40). Resting $P_{da}$, $P_{ven}$, and $f_H$ after bretylium was not different from the untreated group (Fig. 3). This may suggest a low routine adrenergic nervous tone on the vasculature and heart in rainbow trout and is in agreement with a previous study on cod (18) but different from other studies on cod and rainbow trout where bretylium treatment resulted in mild hypotension (5, 39, 40). It should be emphasized, however, that since only relative blood flow values are presented, it cannot be excluded that a compensatory increase in $Q$ after adrenergic blockade served to offset any potential decrease in $P_{sys}$, leaving $P_{da}$ unchanged. Given the very variable nature of the resting venous responses to adrenergic blockade in vivo, further experiments are required to resolve the apparently complex relationship between altered vascular tone and potential changes in fluid volume, vascular resistance, and/or activity of compensatory neurohumoral control systems.

Methodological considerations. The construction of vascular capacitance curves represents the best method available to estimate USBV and $C$ in vivo (25, 26). This method requires that total blood volume is manipulated and that the circulation is transiently stopped. In the present study, a comparatively large-bore venous catheter (ID, 1 mm) was used for blood volume manipulation, the advantage being that both injection and withdrawal of blood becomes fast (ideally completed within 3–15 s). Hence, fast alterations in blood volume serve to minimize the confounding effects on venous capacitance from barostatic reflexes (36). When central venous blood volume is rapidly increased, however, there may be a possibility that stretch-induced release of vasodilating atrial natriuretic peptides occurs (7, 8, 13, 24). On the other hand, if the narrower (PE-50) dorsal aortic catheter is used, the increase in cardiac preload will be less, but particularly, blood withdrawal becomes slow (~15–30 s in preliminary experiments) and barostatic reflexes will inevitably increase venous tone (36).

For the 90–110% blood volume interval in untreated fish, USBV was 25.23 (+2.83) ml/kg $M_b$ and $C$ was 21.22 (+4.42) ml kg/kg $M_b$ (Tables 1 and 2). Using similar techniques, previous investigations of USBV and $C$ in rainbow trout have, with a few exceptions, produced slightly lower values of USBV and $C$ (Table 2). These differences could result from differences between various strains of $O$. mykiss but may also reflect differences between surgical methods and experimental protocols. When the pericardium is surgically opened, there is a resultant increase in $P_{ven}$ (for discussions, see Refs. 12, 37, and 49). With the observation that resting values of $Q$ and $P_{da}$ are maintained within physiological limits in trout with surgically opened pericardia, Zhang et al. (49) hypothesized that this increase in $P_{ven}$ is the result of an active venous compensation (i.e., an increased venous smooth muscle activity) to increase cardiac preload and not due to central venous blood pooling as cardiac aspiration is impaired. This seems likely considering that the vascular limb of the baroreflex controls venous capacitance and a decreased arterial/branchial blood pressure will result in a reflex-mediated decrease in venous capacitance in trout (36). In contrast to previous studies, the surgical methods used in the present study left the pericardium intact and $P_{ven}$ in resting fish was therefore very low, in fact slightly subambient in some fish (Fig. 3, Table 2). In light of the present study, the

Table 2. Summary of previous studies on vascular capacitance in rainbow trout (Oncorhynchus mykiss)

<table>
<thead>
<tr>
<th>Reference</th>
<th>USBV, ml/kg $M_b$</th>
<th>$C$, ml kPa/kg $M_b$</th>
<th>$P_{ven}$, kPa</th>
<th>Temp, °C</th>
<th>Blood Volume Manipulated by</th>
<th>Pericardium Opened</th>
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<td>Zhang et al. (48)</td>
<td>13.3</td>
<td>25.5</td>
<td>~0.40</td>
<td>12</td>
<td>dorsal aortic catheter</td>
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<td>Conklin et al. (6)</td>
<td>20.7±0.6</td>
<td>12.8±1.5</td>
<td>0.27±0.08</td>
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<td>18.8±0.5</td>
<td>17.2±0.7</td>
<td>0.47±0.03</td>
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<td>Zhang et al. (49)</td>
<td>19.6±0.8</td>
<td>18.0±3.7</td>
<td>0.40±0.03</td>
<td>15</td>
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<tr>
<td>Hoagland et al. (21)</td>
<td>20.6±0.6</td>
<td>16.5±1.5</td>
<td>0.47±0.03</td>
<td>12</td>
<td>yes</td>
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</tr>
<tr>
<td>Present study</td>
<td>25.2±2.8</td>
<td>21.2±4.4</td>
<td>0.06±0.07</td>
<td>15</td>
<td>venous catheter</td>
<td>no</td>
</tr>
</tbody>
</table>

Comparison of previous studies with the present study regarding methodology and cardiovascular data for USBV, $C$, and venous pressure ($P_{ven}$) in untreated resting $O$. mykiss. Values for USBV, $C$, and $P_{ven}$ are mean values (+SE or +SD in the present study). In cases where no absolute values were given, $P_{ven}$ was estimated from mean pressure traces.
conclusion by Zhang et al. (49) thus appears to be supported, as also routine USBV and C in our study were in the high range (Table 2), indicating a low level of venous compensation.

In conclusion, the present study demonstrates for the first time that venous capacitance in fish is actively modulated in response to hypoxia. The decreased capacitance is mediated by α-adrenergic control systems, including both neural and humoral components. During exercise, a decreased venous capacitance (manifested as an increased MCFP) has been interpreted as a mechanism to prevent passive venular blood pooling when Q is increased but also to increase the peripheral venous driving pressure to match venous return to the increased Q (37a, 38). During hypoxia, however, Q remains unaltered because SV increases and offsets the bradycardia. Therefore, we suggest that the two major physiological functions of the reduction in venous capacitance during hypoxia are to shift blood into the central venous compartment to increase cardiac preload and SV to facilitate the maintenance of Q when f\textsubscript{1H} drops and to quickly redistribute blood volume to the most vital organ systems. Future experiments will have to explore from what region(s) in the venous circulation this blood is being mobilized.

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