

CARDIOVASCULAR EFFECTS OF ARGININE VASOTOCIN IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

The physiological functions of the neurohypophyseal hormone arginine vasotocin (AVT) in teleosts are not clear. In the present studies, the sites and mechanisms of action of AVT on the rainbow trout *Oncorhynchus mykiss* cardiovascular system were examined in unanesthetized instrumented fish, perfused organs and isolated vessels. Injection of AVT (1, 10 or 100 pmol kg⁻¹ body mass) into trout with dorsal aortic cannulas produced a modest, but dose-dependent, increase in dorsal aortic pressure (P_{DA}). Bolus injection of AVT (100 pmol kg⁻¹ body mass), or continuous infusion (6.7 pmol kg⁻¹ min⁻¹), into trout instrumented with dorsal aortic, ventral aortic and central venous cannulas and a ventral aortic flow probe significantly increased P_{DA} as well as ventral aortic (P_{VA}) and central venous (P_{VEN}) blood pressure. Bradycardia accompanied the rapid rise in P_{VA} while gill resistance (R_G) increased. Maximum response to the AVT bolus was reached within 13–21 min and the response decayed slowly over the ensuing 90 min. AVT infusion (6.7 pmol kg⁻¹ min⁻¹) significantly increased P_{VEN} and mean circulatory filling pressure and decreased unstressed blood volume, whereas venous compliance was unaffected. These *in vivo* studies indicate that AVT increases venous tone, thereby mobilizing blood from the unstressed compartment into the stressed compartment. This increases P_{VEN} , which increases venous return and helps maintain, or slightly elevate, cardiac output. This,

combined with an elevated R_G and slightly elevated systemic resistance (R_S), increases both P_{VA} and P_{DA} ; however, the rise in P_{DA} is mitigated by a disproportionate increase in R_G relative to R_S . *In vitro*, the effects of AVT are consistent with *in vivo* responses. AVT increased vascular resistance in the perfused gill and perfused trunk and contracted isolated vascular rings from both rainbow and steelhead trout. The general order of sensitivity of isolated vessels to AVT was (in decreasing order): anterior cardinal vein, celiacomesenteric artery, ductus Cuvier, efferent branchial artery, ventral aorta and coronary artery. Extracellular Ca²⁺ accounted for over 70 % of the tension in the AVT-contracted efferent branchial artery, but only 57 % of the tension in the anterior cardinal vein. Vascular AVT receptor sensitivity (EC_{50}) *in vitro* ranged from 0.3 to 6 nmol l⁻¹ and was similar to the estimated ED_{50} for the dose-dependent increase in P_{DA} *in vivo* (approximately 1 nmol l⁻¹). AVT was not inotropic in paced ventricular rings nor did it exhibit vasorelaxant activity in perfused organs or vascular rings. These results show that AVT is a potent vasoconstrictor in trout and that its two primary cardiovascular targets are the systemic veins and the branchial vasculature.

Key words: blood pressure, cardiac output, vascular capacitance, smooth muscle, venous function, arginine vasotocin, rainbow trout, steelhead trout, *Oncorhynchus mykiss*.

Introduction

Arginine vasotocin (AVT) is the ancestral peptide from which all other neurohypophyseal hormones arose (for reviews, see Sawyer, 1977; Acher, 1996). AVT is a pressor in both teleosts and sub-teleostean fish, and this has led to the hypothesis that one of the earliest sites of AVT activity was the cardiovascular system. This tenet is supported by the

following observations: (1) AVT is the only neurohypophyseal peptide found in agnathans (Acher, 1996), (2) it has a long-lasting pressor effect in primitive fish, including lamprey and lungfish (Sawyer *et al.* 1976; Chan, 1977; Pang *et al.* 1983; Uchiyama and Murikama, 1994), and (3) it contracts isolated ventral and dorsal aortic strips from the hagfish and lamprey

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(Somlyo and Somlyo, 1968; Uchiyama and Murakami, 1994). However, while AVT contracts isolated arteries from the holostean gar, it also relaxes gar veins (Conklin *et al.* 1996). Thus, even in sub-teleostean species, the actions of AVT may be quite diverse.

Pressor effects of AVT have been observed in higher teleosts, and especially detailed studies have been performed on eels. An AVT bolus increases ventral and dorsal aortic pressure (P_{VA} and P_{DA} , respectively) in both *Anguilla japonica* and *A. rostrata* (Chan, 1977; Oudit and Butler, 1995), increases cardiac output (\dot{Q}) in *A. rostrata* (Oudit and Butler, 1995) but has no effect on sinus venosus blood pressure (P_{VEN}) in *A. japonica* (Chan, 1977). AVT produces branchial shunting *in vivo* in *A. rostrata* (Oudit and Butler, 1995) and constricts the isolated perfused gill of the European eel *A. anguilla* (Rankin and Maetz, 1971; Bennett and Rankin, 1986). It also has positive chronotropic and inotropic effects in isolated atria of *A. japonica* (Chiu and Lee, 1990). Thus, AVT appears to exert multiple effects on the eel heart and arterial circulation.

Less is known regarding the cardiovascular actions of AVT in trout. Intra-arterial (dorsal aortic) and intracerebroventricular injection of AVT produce dose-dependent increases in P_{DA} in both anesthetized and conscious rainbow trout *Oncorhynchus mykiss* (LeMevel *et al.* 1991, 1993), and intra-arterial AVT injection is associated with an apparent reflex bradycardia in anesthetized trout (LeMevel *et al.* 1991). *In vitro*, AVT produces a concentration-dependent increase in vascular resistance in the constant-flow perfused rainbow trout trunk preparation and reduces urine flow in the constant-pressure perfused preparation (Pang *et al.* 1983; Pang and Furspan, 1984). Isolated trout veins are strongly contracted by AVT (Conklin and Olson, 1994a), which implies a venous as well as an arterial site of AVT action.

The current study was undertaken to localize cardiovascular effectors of AVT actions in trout *in vivo* and to characterize further the actions of AVT on select tissues *in vitro*. In the first of two *in vivo* studies, five cardiovascular parameters (\dot{Q} , P_{VA} , P_{DA} , P_{VEN} and heart rate, f_H) were simultaneously measured during AVT injection into unanesthetized trout. Gill and systemic vascular resistances (R_G and R_S , respectively) and stroke volume (V_S) were derived from these five parameters. In the second set of *in vivo* studies, mean circulatory filling pressure (P_{MCF}), stressed (V_{SB}) and unstressed (V_{USB}) blood volume, and vascular compliance (C) were examined during transient cardiac fibrillation (zero-flow conditions) to identify the specific actions of AVT on the venous system. Perfused organs and isolated arteries, veins and cardiac tissue were also examined to localize the actions of AVT further and to quantify the sensitivity of vascular responses.

Materials and methods

Animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (Kamloops strain, 0.3–1.0 kg) of both sexes were used in all but a few *in vitro* vessel studies. Trout were purchased from a

local hatchery (Robinson Trout Farm, Grand Haven, MI) and housed at the University of Notre Dame in 20001 circular aquaria in aerated, through-flowing well water (12 °C). They were fed a maintenance diet of commercial trout pellets (Ralston-Purina, St Louis, MO) and exposed to a 12h:12h or 16h:8h light:dark photoperiod appropriate for the season.

Steelhead trout (*Oncorhynchus mykiss*, Skamania strain, 3–7 kg) of both sexes were used for *in vitro* vessel studies because of their larger vessels. They were captured locally by the Indiana Department of Natural Resources (DNR) and were housed at the Richard Clay Bodine State Fish Hatchery. The steelhead were anesthetized in tricaine (ethyl-*m*-aminobenzoic acid; MS-222; 1:5000, w:v) by the DNR prior to removal of the vessels.

In vivo experiments

Pressure and flow

The methods for cannulation of the dorsal and ventral aortas and ductus Cuvier and for the placement of flow probe have been described previously (Olson *et al.* 1997c). Trout were anesthetized in benzocaine (ethyl-*p*-aminobenzoic acid, 1:6000, w:v), and the dorsal aorta was cannulated with heat-tapered polyethylene tubing (PE 60). This procedure took less than 1 min, and the gills were not irrigated. During subsequent instrumentation, the gills were continuously irrigated with aerated water at 10 °C containing 1:24 000 (w:v) benzocaine.

The pericardial cavity was exposed with a mid-line ventral incision, and both the right horn of the ductus Cuvier and the bulbus arteriosus were cannulated with 5 cm long, 0.51 mm i.d. silicone tubing (Dow Corning veterinary grade, Konigsberg Instruments, Inc., Pasadena, CA). The free ends of the tubing were connected to 60 cm of PE 90 tubing. All three cannulas were filled with heparinized saline (100 USP units ml⁻¹ heparin in 9.0 g l⁻¹ NaCl) and connected to Gould P23 pressure transducers. A 3S Transonic flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the ventral aorta, distal to the cannula, and connected to a Transonic T101 or T206 flow meter. The incision was closed using interrupted silk sutures and sealed with cyanoacrylate gel. Venous and ventral aortic cannulas and the flow probe lead were secured to the fish using silk sutures. The fish were revived, placed in black plastic tubes and immersed in a 15001 experimental aquarium containing aerated, through-flowing well water. Experiments were conducted 48 h after surgery.

Analog pressure signals were displayed with Hewlett Packard 7853A patient monitors and recorded continuously with a Grass 7 or 79P polygraph. Digitized signals of pressure and flow were collected at 0.1 s intervals, and 1 s averages were stored in an 486 IBM-compatible computer. Pressure transducers were calibrated using a water manometer, and the flow probe was calibrated *in situ* by saline perfusion at the end of the experiment.

Drugs were administered *via* the dorsal aortic cannula either by bolus injection or by infusion. Each injection was followed by 0.25 ml of saline to flush the cannula, and subsequent injections were separated by 30–90 min to permit recovery of

measured variables. In the infusion studies, the dead space of the cannula (approximately 0.2 ml) was flushed by a 1 min priming infusion at 0.3 ml min^{-1} AVT. Thereafter, saline or AVT was infused at $1 \text{ ml kg}^{-1} \text{ h}^{-1}$. The concentration of peptide infused was adjusted for body mass to give an infusion rate of $6.7 \text{ pmol kg}^{-1} \text{ min}^{-1}$.

Mean P_{DA} , P_{VA} and P_{VEN} were calculated as the arithmetic averages of the respective systolic and diastolic pressures (Olson *et al.* 1991). $f\text{H}$ was calculated from the pulse interval of either dorsal or ventral aortic pressure. \dot{Q} was normalized to body mass (kg), and V_{S} was calculated using the formula: $V_{\text{S}} = \dot{Q} / f\text{H}$. Resistances, R_{G} and R_{S} , were calculated from the pressure drop across the respective vasculature relative to \dot{Q} , i.e. $R_{\text{G}} = (P_{\text{VA}} - P_{\text{DA}}) / \dot{Q}$ and $R_{\text{S}} = (P_{\text{DA}} - P_{\text{VEN}}) / \dot{Q}$. In initial experiments designed to determine the effective dose for an AVT bolus, only dorsal aortic pressure was measured.

Vascular capacitance

Venous function, *in vivo*, was determined from vascular capacitance curves obtained in a second series of experiments. Details of this method have been reported previously (Zhang *et al.* 1995). 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 in diameter) were placed in the pericardial cavity on either side of the ventricle. The fish were revived and placed in black plastic tubes suspended in the experimental aquarium. Experiments were conducted 48 h after surgery.

P_{DA} and P_{VEN} were measured in unanesthetized fish before and during cardiac arrest, the latter produced by electrical fibrillation of the heart for 6–8 s with a 3.5–5.0 V voltage pulse, 40 ms duration, administered at 50 Hz. During fibrillation, there was a rapid loss of arterial pulse as P_{DA} fell while P_{VEN} increased. P_{DA} and P_{VEN} became stable (zero-flow pressure) within 5 s of fibrillation; at this time, P_{VEN} was assumed to be equal to P_{MCF} . Ventricular fibrillation was then stopped, the heart resumed beating immediately and blood pressures were restored within 2–3 beats.

Vascular capacitance curves were obtained by measuring P_{MCF} during normovolemia (100 % blood volume) and then again as blood volume was adjusted up or down in 10 % increments between 120 and 80 % of resting volume. A resting blood volume of 30 ml kg^{-1} body mass was assumed (Olson, 1992), and whole blood from a donor fish was used for volume expansion. Cardiac arrest was initiated within 30 s after each volume manipulation, and blood volume was restored to 100 % within 30 s after zero-flow pressure measurement. The interval between each volume perturbation, during which the fish were normovolemic, was 15 min. Control capacitance curves were obtained from fish infused with saline at 0.25 ml h^{-1} . The fish were then infused, at the same flow rate, with $6.7 \text{ pmol kg}^{-1} \text{ min}^{-1}$ AVT, and the entire pressure–volume protocol was repeated 30 min after the onset of AVT infusion. The cannula was not flushed with a priming dose in these experiments.

Because the capacitance curve is not linear, vascular

compliance and unstressed volume were determined at three blood volume intervals, 80–90–100 %, 90–100–110 % and 100–110–120 % by regression analysis of the three consecutive pressure–volume data points within each interval. By convention, P_{MCF} is treated as the independent variable; therefore, the slope of the resultant volume–pressure line ($\Delta V / \Delta P$) is equal to vascular compliance, and the intercept of this line with the blood volume axis at $P_{\text{MCF}} = 0$ is the percentage of the total blood volume in the unstressed compartment (Rothe, 1993). The product of per cent blood volume and estimated actual blood volume (30 ml kg^{-1} body mass) permits the conversion of compliance and unstressed volume into actual volumes, i.e. $\text{ml mmHg}^{-1} \text{ kg}^{-1}$ and ml kg^{-1} , respectively.

In vitro experiments

Trout were anesthetized as described above or killed by a blow to the head. Heparin (1000 USP units in 1 ml of 0.9 % NaCl) was injected into the bulbus arteriosus *via* a mid-ventral incision and allowed to circulate for 2–3 min before the organs were cannulated.

Isolated gill perfusion

The second and third pair of gill arches were isolated, and the afferent branchial artery (ABA) and efferent branchial artery (EBA) were cannulated with bevelled PE 60 and PE 50 tubing, respectively, as described by Olson (1984). Gill venous effluent was collected from a latex collar glued around the posteriodorsal end of the arch using cyanoacrylate tissue cement. The arch was suspended in a 125 ml tapwater bath and perfused using a peristaltic pump. A T-junction was inserted in the ABA cannula near the arch and connected to a pressure transducer. The outflow end of the EBA cannula was elevated by 20 cm to produce approximately 15 mmHg back pressure, and venous outflow pressure was assumed to be atmospheric. Arches were perfused with phosphate-buffered saline (PBS; see below) at a flow sufficient to produce an input pressure of 25–40 mmHg. Effluent from the EBA cannula (arterioarterial, A–A pathway) and the latex collar (arteriovenous, A–V pathway) was collected in tared vials at 1, 2 or 5 min intervals. After a 30 min control perfusion period, AVT was added to the perfusate in tenfold increasing increments from 10^{-12} to $10^{-8} \text{ mol l}^{-1}$. Each dose was infused for 15 min.

Perfused trunk

The dorsal aorta (DA) and celiacomesenteric (CMA) artery were cannulated with PE 190 and PE 50 tubing and independently secured by 0 and 3/0 gauge silk ligatures, respectively (Olson and Villa, 1991). The sinus venosus was removed to allow unimpeded venous drainage. Using this method, the cannulated DA perfuses skeletal muscle, the kidney and a small portion of the distal intestine, and the CMA perfuses the splanchnic circulation, excluding the distal intestine (Olson and Villa, 1991). The cannulas were connected to individual peristaltic pumps, and a T-junction in the cannula near the site of cannulation was connected to a pressure

transducer. Pump flow rate was adjusted to produce perfusion pressures of 15–25 mmHg. Both circuits were perfused for 20–30 min with PBS prior to experimentation.

Cumulative dose–response properties of the CMA and DA were obtained by perfusion with AVT in tenfold incremental doses (10^{-12} to 10^{-7} mol l⁻¹). To determine whether AVT produced relaxations in trout trunk vasculature, the CMA and DA circuits were perfused with epinephrine (EPI; 10^{-7} or 10^{-6} mol l⁻¹) for 10–15 min, to precontract the vasculature (Olson *et al.* 1994), and AVT was infused in tenfold cumulative dose increments (10^{-15} to 10^{-7} mol l⁻¹) against the EPI background.

Isolated ventricular cardiac muscle

The effects of AVT on ventricular rings were examined using the methods described by Olson *et al.* (1994). Ventricular rings (approximately 2 mm wide) were cut from the middle of the ventricle perpendicular to the base–apex axis (Olson *et al.* 1994). Rings were connected to a Grass FT03C force-displacement transducer and suspended in an aerated, 20 ml water-jacketed (12 °C) smooth muscle chamber containing PBS. Rings were equilibrated to 500–1000 mg of diastolic tension. Two coiled platinum electrodes were placed on either side of the rings, and the muscle was paced with 5 V voltage pulses of 40 ms duration at 0.33 Hz. Ventricular rings were exposed to a single concentration of AVT (10^{-7} mol l⁻¹), washed three times with PBS and stimulated with a single concentration of EPI (10^{-5} mol l⁻¹).

Isolated vascular smooth muscle

Rainbow trout vessels were examined between March and December, steelhead vessels were examined from January to March.

Rings from CMA, coronary artery (COR; from steelhead only), EBA, ventral aorta (VA), anterior cardinal vein (ACV) and ductus Cuvier (DOC; from steelhead only) were cut transaxially into 2–3 mm rings or, in the case of DOC into 2 mm × 4 mm strips. Vessel rings and strips were mounted in the same chambers as cardiac muscle, but they were not electrically paced. Resting tensions of 150–200 mg (DOC), 200–300 mg (ACV, COR), 500 mg (CMA and EBA) or 750 mg (VA) were applied to the vessel for at least 1 h prior to experimentation. During this time, vessels were precontracted with 10^{-5} mol l⁻¹ acetylcholine (ACh) or EPI, washed three times, and baseline tension was re-established for at least 30 min before further experimentation (Olson and Meisheri, 1989).

Cumulative dose–response curves were obtained from isolated vessels over the AVT concentration range 10^{-13} to 10^{-7} mol l⁻¹. Tension developed during contraction was normalized for vessel wet mass and expressed as mg tension mg⁻¹ wet mass. The effective AVT concentration required to produce a half-maximal response (EC₅₀) was obtained from dose–response curves of individual vessels using an automated curve-fitting computer program (TableCurve 2D, Jandel Scientific, San Rafael, CA). Data

obtained from rainbow and steelhead trout rings were not combined because, in a few instances, the order of AVT potency was slightly different.

To determine whether AVT could also produce vasodilation, EBA, CMA and ACV were maximally precontracted with the Na⁺/K⁺-ATPase inhibitor sodium orthovanadate, EPI or ACh (all at 10^{-5} mol l⁻¹) and, after stabilization of the precontraction (10–15 min), AVT was added in tenfold incremental doses from 10^{-15} to 10^{-9} mol l⁻¹. To characterize the AVT contraction further, the contribution of extracellular Ca²⁺ was determined by incubating vessels for 30 min in a Ca²⁺-free PBS in the presence of the Ca²⁺ chelator EGTA (see below). The vessels were then contracted with 10^{-9} mol l⁻¹ AVT and, when tension had stabilized, 0.10 g l⁻¹ CaCl₂ was added to the PBS. The contribution of extracellular Ca²⁺ was determined as the percentage tension developed after addition of Ca²⁺, relative to the total tension developed. The role of extracellular Ca²⁺ in the maintenance phase of an AVT contraction was examined by addition of the L-type Ca²⁺ channel blocker verapamil (10^{-5} mol l⁻¹) during a sustained contraction produced by 10^{-9} mol l⁻¹ AVT.

Chemicals

The composition of trout PBS (in g l⁻¹) 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. Ca²⁺-free PBS in EGTA was prepared by substituting 0.1 g l⁻¹ EGTA for CaCl₂ (Olson and Meisheri, 1989). Other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

Statistics

Comparisons of responses were made using appropriate paired or unpaired *t*-tests (two-tailed). Multiple comparisons between vessels were made using one-way analysis of variance (ANOVA) with Bonferroni adjustment. Significance was assumed at $P \leq 0.05$. Values are expressed as means ± standard error (S.E.M.).

Results

In vivo experiments

Bolus injection of 1 pmol kg⁻¹ body mass AVT into trout with dorsal aortic cannulas did not significantly affect P_{DA} , whereas 10 and 100 pmol kg⁻¹ body mass AVT increased P_{DA} in an apparently dose-dependent manner (data not shown). Higher AVT doses appeared to irritate the fish and were not examined further. The estimated ED₅₀ for the P_{DA} response, assuming that maximum, non-irritant effects were reached at 100 pmol kg⁻¹ body mass, was 8.3 pmol kg⁻¹ body mass ($N=7$). If rapidly and thoroughly mixed in blood, this would produce a plasma concentration of approximately 4.7×10^{-10} mol l⁻¹ (based on 30 ml kg⁻¹ blood volume and 33 % hematocrit).

Injection of 100 pmol kg⁻¹ body mass AVT into fully instrumented fish (Fig. 1; Table 1) increased P_{DA} by 20 %, P_{VA} by 41 %, P_{VEN} by 61 % and R_G by 130 %, while f_H

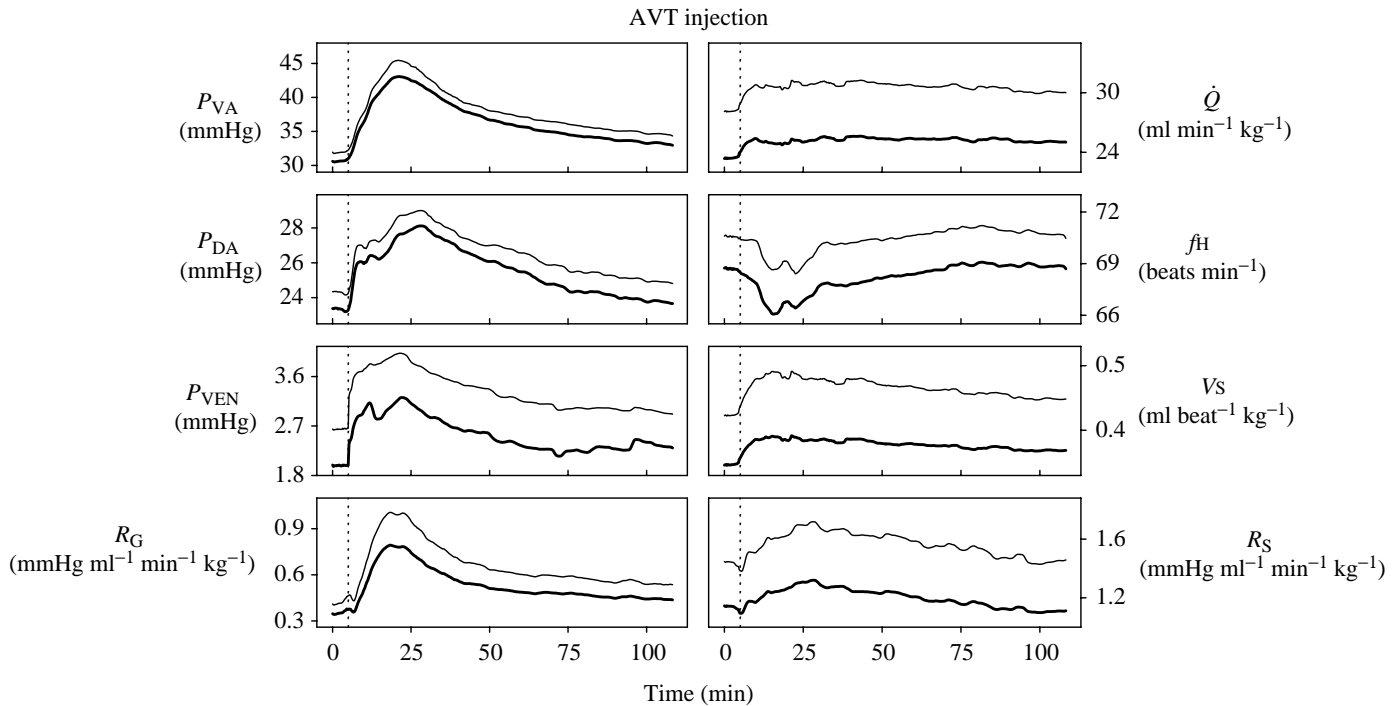


Fig. 1. Effects of bolus arginine vasotocin (AVT; 100 pmol kg^{-1} body mass) injection on cardiovascular variables in unanesthetized trout. Thick lines represent mean values and thin lines indicate 95% confidence limits for $N=5$ trout; AVT was injected at the vertical dotted line. P_{VA} , ventral aortic pressure; P_{DA} , dorsal aortic pressure; P_{VEN} , central venous pressure (all mmHg); \dot{Q} , cardiac output ($\text{ml min}^{-1} \text{ kg}^{-1}$); f_H , heart rate (beats min^{-1}); V_s , stroke volume ($\text{ml beat}^{-1} \text{ kg}^{-1}$); R_G , branchial resistance; R_S , systemic resistance (both $\text{mmHg ml}^{-1} \text{ min}^{-1} \text{ kg}^{-1}$). $1 \text{ mmHg}=0.133 \text{ kPa}$.

Table 1. Cardiovascular variables before and after bolus injection of 100 pmol kg^{-1} body mass AVT into unanesthetized rainbow trout

	Control	AVT	Response time (min)	Recovery time (min)
P_{DA} (mmHg)	23.4 ± 1.0	$28.1 \pm 0.9^*$	21.4 ± 3.0	64 ± 16
P_{VA} (mmHg)	30.5 ± 1.3	$43.1 \pm 1.3^*$	16.8 ± 1.2	71 ± 12
P_{VEN} (mmHg)	2.0 ± 0.6	$3.2 \pm 0.6^*$	$13.5 \pm 5.2^\dagger$	84 ± 15
\dot{Q} ($\text{ml min}^{-1} \text{ kg}^{-1}$)	23.4 ± 4.8	25.6 ± 5.1		
f_H (beats min^{-1})	68.7 ± 1.8	$66.0 \pm 1.5^*$	$13.4 \pm 4.0^\dagger$	55 ± 24
V_s (ml kg^{-1})	0.34 ± 0.01	0.39 ± 0.10		
R_G ($\text{mmHg ml}^{-1} \text{ min}^{-1} \text{ kg}^{-1}$)	0.34 ± 0.06	$0.79 \pm 0.21^*$	15.4 ± 2.0	66 ± 15
R_S ($\text{mmHg ml}^{-1} \text{ min}^{-1} \text{ kg}^{-1}$)	1.14 ± 0.1	1.32 ± 0.40		

AVT values reflect maximum response; Response time, time until maximum response; Recovery time, time from response to restoration of parameter to within 90% of control value.

AVT, arginine vasotocin; P_{DA} , dorsal aortic pressure; P_{VA} , ventral aortic pressure; P_{VEN} , sinus venosus pressure; \dot{Q} , cardiac output; f_H , heart rate; V_s , stroke volume; R_G , R_S , gill and systemic resistance, respectively.

*Significant difference from control value ($P \leq 0.05$); † significantly different from value for P_{DA} ($P \leq 0.05$).

Values are means \pm S.E.M.; $N=5$ trout.

decreased by 4%. \dot{Q} initially began to increase; however, this increase was not significant as the increase in stroke volume was offset by an ensuing bradycardia. R_S also appeared to increase; however, this increase was not statistically significant. Maximum responses were recorded between 13 and 21 min after injection, and recovery took another 55–85 min. The time to reach maximum P_{DA} appeared to be

delayed and was significantly longer than the time for maximum P_{VEN} and minimum f_H to be achieved. There were no significant differences in the time for recovery of the cardiovascular variables. Continuous AVT infusion for 2 h (Fig. 2) produced responses similar to the AVT bolus, although P_{VEN} and R_G remained significantly elevated for the entire 2 h infusion period.

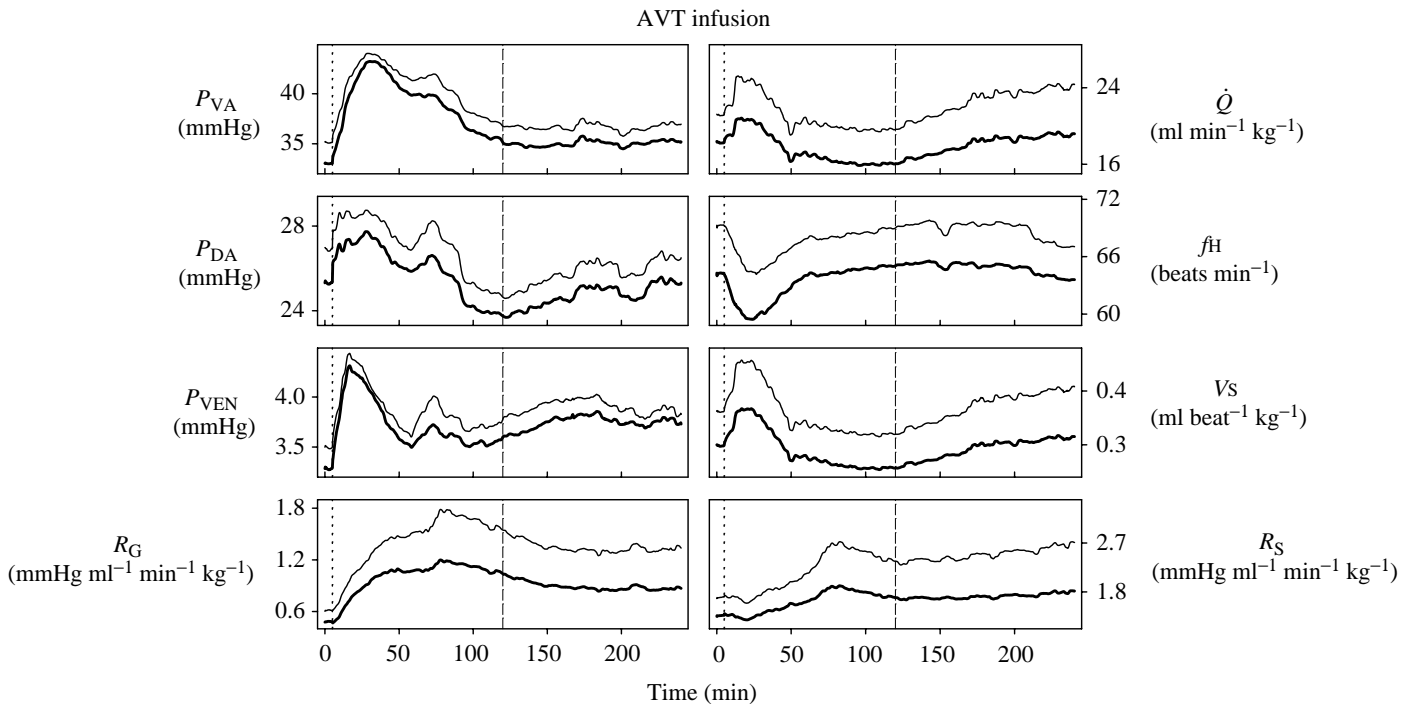


Fig. 2. Effects of arginine vasotocin (AVT) infusion ($6.7 \text{ pmol kg}^{-1} \text{ min}^{-1}$) on cardiovascular variables in unanesthetized trout. Infusion started at the vertical dotted line and stopped at the vertical dashed line. Abbreviations as in Fig. 1; $N=6$ trout. $1 \text{ mmHg}=0.133 \text{ kPa}$.

Table 2. Effects of $6.7 \text{ pmol kg}^{-1} \text{ body mass min}^{-1}$ arginine vasotocin infusion on unstressed blood volume and vascular compliance in rainbow trout in vivo

	80–100%		90–110%		100–120%	
	V_{USB} (ml kg^{-1})	C ($\text{ml mmHg}^{-1} \text{ kg}^{-1}$)	V_{USB} (ml kg^{-1})	C ($\text{ml mmHg}^{-1} \text{ kg}^{-1}$)	V_{USB} (ml kg^{-1})	C ($\text{ml mmHg}^{-1} \text{ kg}^{-1}$)
Control	16.2 ± 1.0	2.9 ± 0.3	20.7 ± 0.6	1.7 ± 0.2	21.5 ± 0.6	1.7 ± 0.2
AVT	$14.6 \pm 0.7^*$	2.5 ± 0.3	$18.6 \pm 0.5^*$	1.8 ± 0.1	20.0 ± 0.7	1.7 ± 0.2

V_{USB} , unstressed blood volume; C , compliance; AVT, arginine vasotocin.

Values were determined from three 10% increments in blood volume, where 100% blood volume equals 30 ml kg^{-1} body mass. V_{USB} is measured in ml kg^{-1} body mass, C in $\text{ml mmHg}^{-1} \text{ kg}^{-1}$ body mass.

Means \pm S.E.M., $N=7$ trout.

*Significantly different ($P \leq 0.05$) from the control value.

The effects of AVT infusion on P_{MCF} , C and V_{SB} are presented in Fig. 3 and Table 2. AVT infusion significantly ($P \leq 0.05$) increased P_{VEN} from 4.0 ± 0.2 to $5.0 \pm 0.5 \text{ mmHg}$ (not shown) and mean circulatory filling pressure from 5.0 ± 0.2 to $6.1 \pm 0.5 \text{ mmHg}$ (Fig. 3) in normovolemic trout. AVT infusion also significantly decreased the unstressed blood volume over the ranges 80–100% and 90–110% of control blood volume, but did not affect unstressed blood volume when total blood volume was adjusted above the control value (100–120%; Table 2). AVT infusion did not affect vascular compliance at any blood volume (Table 2). P_{DA} , zero-flow P_{DA} and f_{H} remained at, or near, control levels during the infusion period, in spite of the sustained effect of AVT infusion on P_{VEN} . Arterial pulse pressure, however, was significantly increased

from 4.8 ± 0.4 to $6.7 \pm 0.9 \text{ mmHg}$ throughout the AVT infusion period.

In vitro experiments

Two distinct AVT effects were observed in the isolated constant-flow perfused gill (Fig. 4). Perfusion with AVT at $10^{-12} \text{ mol l}^{-1}$ significantly increased flow through the A–A pathway and decreased flow through the A–V pathway, while total flow and input pressure (not shown) were unaffected. Total flow, the sum of effluent collected from the A–A and A–V pathways, was more than 80% of pump flow and remained constant throughout this period. At higher concentrations ($>10^{-9} \text{ mol l}^{-1}$), AVT significantly increased A–V flow, whereas both A–A flow and total flow fell.

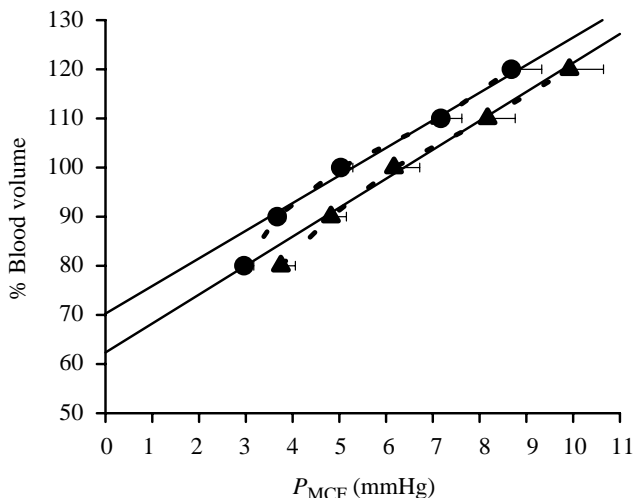


Fig. 3. Vascular capacitance curve showing the effects of saline (Control; ●) or arginine vasotocin (AVT; ▲) infusion ($6.7 \text{ pmol kg}^{-1} \text{ min}^{-1}$) on mean circulatory filling pressure (P_{MCF} ; = zero-flow venous pressure) at blood volumes ranging from 80 to 120% of control in the unanesthetized trout. Vascular compliance is the slope of the linear regression of P_{MCF} over a range of blood volumes; in this example, the solid lines were determined from 90 to 110% of the estimated blood volume. The unstressed volume is obtained from the intersection of the compliance line with the blood volume axis at $P_{MCF}=0 \text{ mmHg}$. This figure shows that AVT infusion increased P_{MCF} at all blood volumes and decreased unstressed blood volume but did not affect compliance over the volume range analyzed. Means \pm S.E.M.; $N=7$ trout. $1 \text{ mmHg}=0.133 \text{ kPa}$.

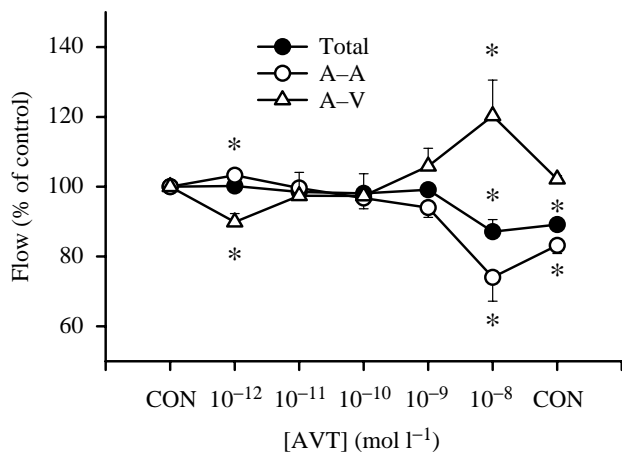


Fig. 4. Effects of arginine vasotocin (AVT) on flow recovery from the isolated constant-flow perfused gill. Values are expressed as a percentage of control flow of effluent collected from the arterioarterial (A-A; ○) and arteriovenous (A-V; △) pathways or total flow (sum of A-A and A-V; ●). Means \pm S.E.M.; *significantly different ($P \leq 0.05$) from the control value; $N=5$ trout. CON, perfusion with physiological saline without AVT.

Perfusion with AVT concentrations above $10^{-11} \text{ mol l}^{-1}$ produced a dose-dependent increase in input pressure. At the

Table 3. Sensitivity of constant-flow perfused gill arch, perfused celiacomesenteric artery and dorsal aorta to arginine vasotocin

	Variable (change)	EC ₅₀ (nmol l ⁻¹)
Whole gill	Input pressure (↑)	0.8 \pm 0.02
Gill arterioarterial	Outflow (↓)	1.4 \pm 0.5
Gill arteriovenous	Outflow (↑)	1.0 \pm 0.7
Uncontracted CMA	Input pressure (↑)	4.4 \pm 1.5
Contracted CMA	Input pressure (↑)	2.6 \pm 0.5
Uncontracted DA	Input pressure (↔)	—
Contracted DA	Input pressure (↑)	2.6 \pm 1.9

CMA, celiacomesenteric artery; DA, dorsal aorta. Mean \pm S.E.M. for $N=5-8$ trout.

Contracted denotes constant perfusion with 10^{-7} to $10^{-6} \text{ mol l}^{-1}$ epinephrine prior to and during arginine vasotocin exposure.

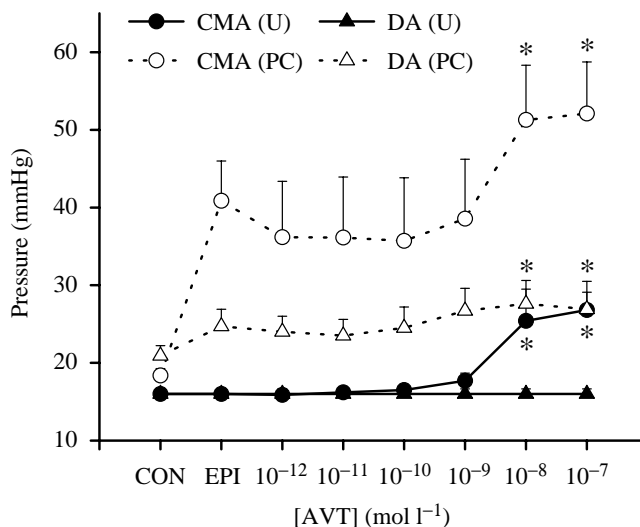


Fig. 5. Effects of arginine vasotocin (AVT) on input pressure in the constant-flow perfused trunk of trout. The perfused celiacomesenteric artery (CMA, circles) or dorsal aorta (DA; triangles) was either uncontracted (U; filled symbols) or precontracted (PC; open symbols) by continuous infusion of 10^{-7} to $10^{-6} \text{ mol l}^{-1}$ epinephrine (EPI) prior to AVT exposure. Means \pm S.E.M.; *significant effect of AVT ($P \leq 0.05$) on input pressure compared with control perfusion (CON) in uncontracted trout or compared with pressure during EPI exposure in precontracted trout. $N=5-7$ trout. $1 \text{ mmHg}=0.133 \text{ kPa}$.

higher AVT concentrations ($>10^{-11}$), the estimated sensitivities of the gill pressure and flow distribution responses were similar (Table 3). Perfusion with PBS at the end of the experiment partially reversed the effects of AVT on flow (Fig. 3) and pressure.

The effects of AVT in the constant-flow perfused trunk preparations are shown in Fig. 5, and data for the sensitivity of the preparation are provided in Table 3. AVT significantly increased input pressure when perfused into the CMA but did

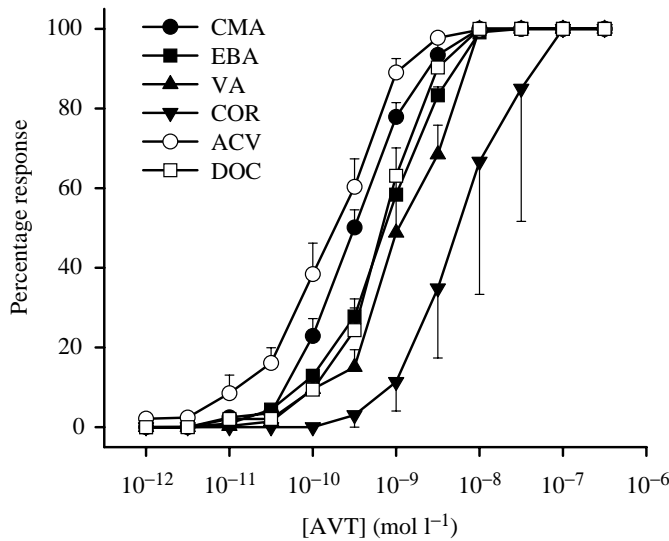


Fig. 6. Cumulative arginine vasotocin (AVT) concentration–response curves for isolated arteries (filled symbols) or veins (open symbols) from steelhead trout. CMA, celiacomesenteric artery (●); EBA, efferent branchial artery (■); VA, ventral aorta (▲); COR, coronary artery (▼); ACV, anterior cardinal vein (○); DOC, ductus Cuvier (□). Values are expressed as a percentage of maximal contraction; means \pm S.E.M., $N=4-10$ trout.

not affect pressure when infused into the DA. However, AVT augmented input pressure in both the CMA and the DA when they were precontracted with 10^{-7} to 10^{-6} mol l $^{-1}$ EPI. The sensitivity of CMA and DA to AVT was not affected by EPI precontraction.

The contractility of paced ventricular rings was unaffected by 10^{-7} mol l $^{-1}$ AVT, whereas 10^{-5} mol l $^{-1}$ EPI applied 10 min after AVT removal increased ventricular contraction by approximately 200% ($N=2$). The effects of AVT on cardiac tissue were not pursued further.

All vascular rings were contracted by AVT in a clear dose-dependent manner, although some differences in vessel sensitivity were observed (Fig. 6; Table 4). In general, the anterior cardinal veins had the highest sensitivity to AVT and the coronary arteries and ventral aortas the lowest. Steelhead anterior cardinal veins and celiacomesenteric arteries were more sensitive to AVT than the corresponding vessels from rainbow trout. AVT-induced contractions of isolated vessels were often slow in onset (5–30 min from exposure to plateau

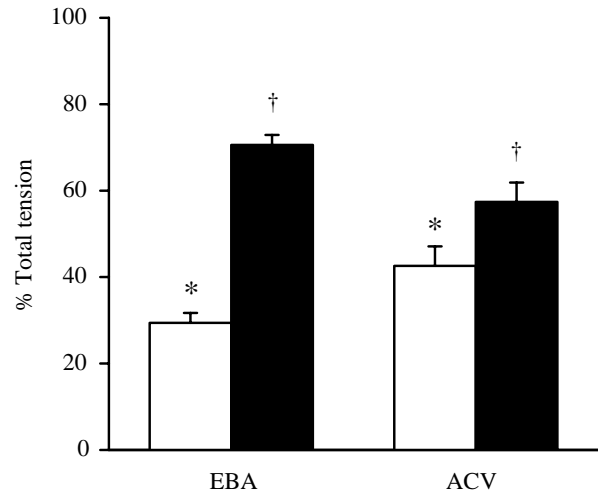


Fig. 7. Percentage contribution of intracellular (open bars) and extracellular (filled bars) Ca $^{2+}$ to arginine vasotocin (AVT; 10^{-9} mol l $^{-1}$) contraction of steelhead efferent branchial arteries (EBA; $N=5$ trout) and anterior cardinal veins (ACV; $N=4$ trout). Means \pm S.E.M.; * and † denote significant differences between EBA and ACV values ($P \leq 0.05$).

of tension), and tension could be sustained at nearly 90% of maximum for 60–120 min during continuous exposure to AVT at concentrations below 10^{-8} mol l $^{-1}$. AVT (10^{-15} to 10^{-9} mol l $^{-1}$) did not produce relaxation in ACV, CMA or EBA precontracted with ACh, EPI or orthovanadate, respectively.

Maximal contraction of both EBA and ACV required extracellular Ca $^{2+}$. However, extracellular Ca $^{2+}$ accounted for significantly more of the total tension developed by EBA in response to 10^{-9} mol l $^{-1}$ AVT than by ACV (Fig. 7). Verapamil (10^{-5} mol l $^{-1}$) added during a contraction in EBA produced by 10^{-9} mol l $^{-1}$ AVT reduced tension by over 90% ($N=5$).

Discussion

The results from the present experiments indicate that AVT is a pressor peptide in trout *in vivo*. AVT acts at a number of effector sites including systemic resistance vessels, systemic veins and gills, with effects on the latter two being most prominent. These *in vivo* responses are consistent with *in vitro* vasoconstriction of perfused systemic organs and gills and contraction of isolated vessels. There is no evidence to suggest that AVT has either vasodilatory activity in trout vessels or a

Table 4. Sensitivity (EC_{50}) of steelhead and rainbow trout arteries and veins to arginine vasotocin

	ACV	EBA	DOC	CMA	VA	COR
Steelhead trout	0.25 ± 0.08^a	0.63 ± 0.08^b	$0.71 \pm 0.15^{b,c}$	$0.79 \pm 0.34^{a,b}$	$1.6 \pm 0.4^{c,d}$	18.6 ± 15.7^d
Rainbow trout	$0.87 \pm 0.19^{e,\dagger}$	$1.8 \pm 0.9^{f,g}$	–	$1.3 \pm 0.2^{e,f,\dagger}$	6.1 ± 2.8^f	–

ACV, anterior cardinal vein; EBA, efferent branchial artery; DOC, ductus Cuvier; CMA celiacomesenteric artery; VA, ventral aorta; COR, coronary artery.

Values are given in nmol l $^{-1}$; mean \pm S.E.M., $N=4-10$ trout.

Values with same letter are not significantly different within strain; † significant difference ($P \leq 0.05$) from the value for steelhead trout.

direct inotropic effect on the ventricle. In addition to identifying the cardiovascular actions of AVT in trout, these experiments are the first, in any fish, to show that venous tone *in vivo* is actively regulated by vasoactive substances.

AVT effects on venous function

It is apparent from the *in vivo* studies on fully instrumented trout that AVT increases central venous pressure (Figs 1, 2), but it is not evident how this is achieved. The venous contribution to cardiovascular function in fish has been questioned on the grounds that fish veins are poorly endowed with smooth muscle and that, because the aqueous medium provides an essentially gravity-free environment, orthostatic venous pooling is never encountered (Satchell, 1991, 1992). However, a number of studies have shown that isolated trout veins actively respond to a variety of vasoactive stimuli (Olson and Villa, 1991; Olson *et al.* 1991; Conklin and Olson, 1994a,b). More recently, the generation of vascular capacitance curves for intact, unanesthetized trout has provided evidence that the venous system may be an important effector of cardiovascular function in fish (Zhang *et al.* 1995; Olson *et al.* 1997b) as well as in mammals (Hainsworth, 1986; Rothe, 1986, 1993).

The trout vascular capacitance curve (Fig. 3) illustrates the relationship between blood volume and P_{MCF} , and it is an indicator of the venous contribution to venous return and cardiac output. The total vascular compliance *in vivo* is the slope of the regression line through several (usually three) consecutive blood volume– P_{MCF} determinations that constitute the capacitance curve. Since venous compliance is over twenty times greater than arterial compliance (Conklin and Olson, 1994a), it can be assumed that total vascular compliance is approximately equal to venous compliance. Thus, as shown in Table 2, venous compliance at normovolemia is $1.7 \text{ ml mmHg}^{-1} \text{ kg}^{-1}$ body mass. The intersection of the compliance line with the blood volume axis (when P_{MCF} is zero) indicates the fraction of total blood volume in the unstressed compartment. The unstressed volume is the volume of blood required to fill the dead space of the vasculature and, because this volume does not create a positive pressure in the vessels, it is hemodynamically inert. In other words, only approximately 30% of the blood volume is in the stressed compartment, where it distends the vessels and creates a favorable pressure gradient for venous return to the heart.

Blood can be shifted from the unstressed compartment to the stressed compartment by an increase in venous tone, i.e. displacement of the compliance line down and to the right, or by a decrease in venous compliance, i.e. clockwise rotation of the compliance line at a constant unstressed volume (Hainsworth, 1986; Rothe, 1993). Because infusion of AVT into trout causes a parallel displacement of the compliance line without changing its slope (Fig. 3; Table 2), it can be concluded that AVT mobilizes blood from the unstressed compartment into the stressed compartment without affecting vascular compliance. This promotes venous return. It has been predicted that a change in venous tone will be most significant

in a hypovolemic state, whereas a change in venous compliance will have the greatest impact when total blood volume is greater than normal (Conklin and Olson, 1994a). The general pressor effects of AVT indicate that this peptide is predominantly an anti-drop effector of cardiovascular homeostasis in fish, as it is in mammals. It is not unexpected then that, if AVT secretion is responsive to hypovolemia or hypotension, the greatest benefit will be incurred through an increase in tone, rather than compliance. Indeed, this appears to be the case in trout. Perhaps not coincidental in this regard is the observation that natriuretic peptides, which may be more relevant in hypervolemic states, exert considerable effects on the trout venous system by increasing venous compliance (Olson *et al.* 1997b).

The ability of AVT to increase P_{MCF} through an increase in venous tone, as inferred from the venous capacitance curves, is substantiated by the direct constrictory effect of AVT on isolated cardinal veins and strips from the ductus Cuvier (Fig. 6). In fact, cardinal veins are more sensitive to AVT than any other isolated vessel (Table 4), and they are more sensitive than the resistance vessels of perfused organs (cf. Tables 3, 4).

The present studies show that the response of large veins *in vitro* is consistent with increased venous tone *in vivo*. The contribution of small veins and venules to the *in vivo* response is not known. Small veins and venules, however, are major determinants of venous compliance in trout (Olson *et al.* 1997b). It will be interesting to determine whether compliance and tone are distinct properties of venules and veins, respectively.

The effects of AVT infusion on central venous pressure in trout (Fig. 2) and the stimulatory effects of AVT on venous tone and P_{MCF} (Fig. 3) should increase venous return and, thereby, increase cardiac output. However, cardiac output appears to be only modestly affected by AVT infusion. This apparent dichotomy may be caused by two interactive factors. First, in addition to its venous action, AVT is also a potent systemic arterial and gill vasoconstrictor. Second, AVT was administered to normotensive fish, not to fish in a hypotensive state where AVT would be expected to be released endogenously. Together, these factors resulted in considerable prebranchial and systemic hypertension. This appeared to elicit other reflexes, such as bradycardia, in an apparent attempt to avert the hypertension. This, and perhaps other reflexes as well, may have mitigated the effects on cardiac output that would otherwise have occurred in response to a hypotensive situation.

Effects of AVT on vascular resistance

Bolus injection (Fig. 1) or infusion (Fig. 2) of AVT into the dorsal aorta increased gill resistance twofold, while systemic resistance increased by less than 50%. Thus, even though AVT was administered into the systemic arterial circuit, it had a greater impact on branchial resistance than it did on systemic resistance.

Both the A–A and A–V pathways of the isolated perfused gill were affected by AVT. Low AVT concentrations ($10^{-12} \text{ mol l}^{-1}$) increased flow through the A–A pathway,

while decreasing A–V flow; higher AVT levels ($>10^{-9}$ mol l⁻¹) increased A–V flow but decreased A–A flow (Fig. 4). In both instances, the increase in flow probably resulted from constriction of the alternative pathway, rather than a direct AVT-mediated dilation, because AVT never decreased perfusion pressure in this constant-flow preparation. The comparatively high sensitivity of the A–V pathway is well within reported plasma AVT levels *in vivo* (10^{-11} to 2×10^{-10} mol l⁻¹; Perrott *et al.* 1991; Warne and Balment, 1997) suggesting that AVT may tonically increase resistance in this pathway *in vivo*. Elevated perfusion pressures produced by vasoconstriction at high AVT concentrations, coupled with the constant-flow perfusion method, probably created some vascular instability, resulting in leakage of perfusate from preparation. Subsequent perfusion with PBS partially restored pressure and diminished the leak (Fig. 4).

Numerous studies have shown that AVT increases vascular resistance in the teleost branchial vasculature (Chan and Chester Jones, 1969; Rankin and Maetz, 1971; Maetz and Lahlou, 1974; Chan, 1977; Bennett and Rankin, 1986; Oudit and Butler, 1995), although the effects on intrafilamental perfusion distribution and its physiological implication are unclear. Chan and Chester Jones (1969) observed that AVT increased A–V blood flow in eel gills *in vivo*, whereas Bennett and Rankin (1986) found that AVT decreased A–A flow without affecting A–V flow in constant-pressure perfused eel gills (Bennett and Rankin, 1986). Our present experiments with the trout gill, where all A–V flow has a post-lamellar origin, clearly indicated that AVT-stimulated vasoactivity occurs in post-lamellar vessels because there were reciprocal changes in outflow from the two pathways. If vasoactivity were at a prelamellar site, flow from both pathways would be similarly affected, i.e. they would either remain constant or both would decrease if constriction elevated pressure enough to affect perfusate extravasation. In the trout gill, vasoconstriction probably occurs at the origin of the A–V anastomoses at low AVT concentrations and in the distal efferent filamental artery sphincter during exposure to elevated AVT levels (Olson, 1991). AVT-induced vasoconstriction of the A–A pathway is probably also responsible for increased branchial shunting in eel *in vivo* (Chan and Chester Jones, 1969; Oudit and Butler, 1995).

The physiological effects of AVT on the gill may be both vascular and extravascular, e.g. osmoregulatory. It has been suggested that increasing A–V flow delivers more AVT to the ionocytes and possibly influences ion regulation (Maetz and Lahlou, 1974). However, it remains to be shown how (or if) alterations in A–V perfusion affect osmoregulation (Olson, 1991). Perfusion redistribution could also affect AVT delivery to other effector cells. Isolated eel gill cells have high-affinity AVT binding sites and, when stimulated by AVT, decrease basal and glucagon-stimulated cellular cyclic AMP levels (Guibbolini and Lahlou, 1987, 1990; Guibbolini *et al.* 1988). Moreover, rapid transfer of euryhaline teleosts between freshwater and saltwater environments is associated with

elevated plasma AVT concentrations (Perrott *et al.* 1991; Balment *et al.* 1993).

The action of AVT on systemic resistance vessels appears to be less significant than its actions on either venous capacitance or branchial resistance vessels. The effect of AVT on trout P_{DA} is, at best, modest. AVT increased P_{DA} in trout by only 5–10 mmHg (Figs 1, 2, this study; LeMevel *et al.* 1991, 1993), which is a considerably smaller effect than that produced by either catecholamines (approximately 30–40 mmHg; Xu and Olson, 1992) or angiotensin II (30–40 mmHg; Olson *et al.* 1994) in the same species. Similar (<10 mmHg) pressor responses have been observed in eels (Chan, 1977; Oudit and Butler, 1995). The relative ineffectiveness of AVT on P_{DA} may be due both to the inability of AVT to constrict the skeletal muscle–renal vasculature (dorsal aorta-perfused trunk; Fig. 5) and to its potent constrictory effect on the gills. Because the fish gill circulation is in-series with the systemic circulation, branchial vasoconstriction will actually reduce systemic arterial pressure. However, systemic hypotension is prevented by the combined increase in both cardiac output and systemic vascular resistance, the latter presumably due primarily to splanchnic vasoconstriction (Fig. 5).

The present studies with isolated vascular rings indicate that AVT directly stimulates blood vessels. Although isolated foci of AVT-vasodilator activity cannot be ruled out, the predominant response to AVT is vasoconstriction. Vasoconstriction is also a characteristic response of perfused organs, and thus AVT has similar effects on both resistance and conductance vessels. This single effect of AVT contrasts with the paradoxical angiotensin-II-induced large-vessel relaxation and arteriolar contraction found in rainbow trout (Conklin and Olson, 1994b).

AVT contractions were dependent on extracellular Ca²⁺, a common feature shared by a number of agonists including catecholamines (Olson and Meisheri, 1989) and endothelin (Olson *et al.* 1991). The ability of verapamil to almost completely relax AVT-contracted EBA confirms our previous observation that most, if not all, maintenance tension in trout arteries is dependent on extracellular Ca²⁺ (Olson and Meisheri, 1989).

AVT-induced contractions of isolated vessels and perfused organs were unaffected by the degree of pre-existing tonus produced by stimulatory doses of other vasoconstrictors. Thus, the effects of AVT and other vascular smooth muscle agonists (including endothelin; Smith and K. R. Olson, unpublished observation) on contraction are additive. This suggests that the AVT excitation–contraction pathways, although similar, are not shared with other agonists. The time course for AVT contraction is generally longer than that observed for other agonists except endothelin (Olson *et al.* 1997a), which provides further evidence for the independence of AVT effects.

A primary role for AVT in arterial blood pressure regulation has not been substantiated experimentally; however, it has been proposed that AVT has a secondary ‘back-up’ role if other, more active, systems are incapacitated (Pang *et al.* 1983). For

example, arterial blood pressure can be reduced in the African lungfish *Protopterus africanus* by treatment with the angiotensin converting enzyme inhibitor captopril, and it can then be further reduced by KB-IV-24, an AVT analog that inhibits AVT effects (Pang *et al.* 1983), whereas initial treatment with KB-IV-24 is ineffective (Pang *et al.* 1983). The weak pressor activity of AVT in trout supports this contention. However, the effects of AVT may be substantial during hypotensive/hypovolemic crises, or AVT may serve other important roles in flow distribution or the regulation of venous return. The latter two are not as obvious when the efficacy of a cardiovascular regulator is assessed by its impact on arterial pressure.

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