The Journal of Experimental Biology 202, 55–66 (1999)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1731

CARDIOVASCULAR EFFECTS OF ANGIOTENSIN-II-MEDIATED ADRENALINE RELEASE IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

NICHOLAS J. BERNIER AND STEVE F. PERRY*

Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5

*Author for correspondence (e-mail: sfperry@science.uottawa.ca)

Accepted 14 October; published on WWW 7 December 1998

Summary

To determine the contribution of plasma catecholamines to the cardiovascular effects of elevated levels of angiotensin II (Ang II) in trout, this study investigated (1) the stimulatory effects of [Asn^1-Val^5]-Ang II on plasma catecholamine levels, (2) the cardiovascular effects of Ang II with and without α-adrenoceptor blockade and (3) the relationship between plasma adrenaline concentrations and their cardiovascular effects. Bolus intravascular injections of Ang II (25–1200 pmol kg^{-1}) elicited dose-dependent (between 75 and 1200 pmol kg^{-1}) increases in plasma adrenaline levels; mean plasma noradrenaline levels only increased in response to a dose of 1200 pmol kg^{-1}. Ang-II-elicited increases in plasma adrenaline levels ranged from 3.3±0.3 nmol l^{-1} for 75 pmol kg^{-1} Ang II to 125.1±40.0 nmol l^{-1} for 1200 pmol kg^{-1} Ang II. Injections of Ang II (25–1200 pmol kg^{-1}) also elicited dose-dependent increases in dorsal aortic pressure (P_{DA}), systemic resistance (R_S), cardiac output ($\dot{Q}$) and stroke volume (V_S). In fish first treated with the α-adrenoceptor blocker phenoxybenzamine, Ang II injections elicited a decrease in $\dot{Q}$ and V_S, and the increases in P_{DA} and R_S following administration of the 600 and 1200 pmol kg^{-1} Ang II doses were significantly reduced. Bolus injections of adrenaline (1.8×10^{-10} to 1.4×10^{-8} mol kg^{-1}) elicited dose-dependent increases in P_{DA} at a plasma adrenaline concentration of 16.5 nmol l^{-1} and in R_S at a plasma adrenaline concentration of 50.5 nmol l^{-1}. Adrenaline injections also elicited increases in $\dot{Q}$ and V_S at plasma adrenaline concentrations of 50.5 nmol l^{-1}; however, higher plasma adrenaline concentrations were not associated with further increases in either $\dot{Q}$ or V_S. These results demonstrate that, *in vivo*, Ang II can act as a potent non-cholinergic secretagogue of humoral adrenaline in trout and that some of the cardiovascular effects of exogenous Ang II can be attributed to increased levels of plasma adrenaline. Our data also indicate that the cardiovascular effects of Ang-II-mediated humoral catecholamines are recruited in a dose-dependent manner and, as such, may require an acute stimulation of the renin–angiotensin system to contribute significantly to the pressor activity of endogenous angiotensins.

Key words: fish, catecholamine, angiotensin II, blood pressure regulation, cardiovascular control, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Regulation of the cardiovasculature by the sympathetic nervous system in mammals is modulated at various levels by the renin–angiotensin system (for reviews, see Peach, 1977; Saxena, 1992; Reid, 1992; Head, 1996). Angiotensins, the active products of the renin–angiotensin system, are involved centrally in the autonomic control of the cardiovasculature and peripherally in enhancing sympathetic neurotransmission and eliciting secretion from the adrenal medulla. Interactions between the renin–angiotensin system and the sympathetic nervous system have also been reported in all non-mammalian vertebrate classes (Carroll and Opdyke, 1982; Wilson, 1984; Nishimura, 1985), suggesting that significant relationships may exist between these two mediators of cardiovascular homeostasis throughout vertebrates.

In teleosts, peripheral injection of angiotensin II (Ang II) elicits a pressor response that is partially inhibited by α-adrenoceptor blockade (*Amia calva*, Butler et al., 1995; *Anguilla rostrata*, Nishimura et al., 1978; Nishimura, 1985; Oudit and Butler, 1995; *Cyclopterus lumpus*, Carroll and Opdyke, 1982; *Gadus morhua*, Platzack et al., 1993; *Oncorhynchus mykiss*, Lipke et al., 1990; Olson et al., 1994). While these studies implicated the sympathetic nervous system in the cardiovascular effects of Ang II in fish, they could not reveal whether the interaction between the renin–angiotensin system and the sympathetic nervous system occurs at the level of the adrenergic nerve terminals, the chromaffin tissue or both (Nishimura et al., 1978).

In previous experiments, we have provided immunohistochemical evidence for the presence of Ang II binding sites on the catecholamine-containing chromaffin cells

of *Oncorhynchus mykiss* (Bernier and Perry, 1997). Furthermore, bolus injections of Ang II in an *in situ* perfused posterior cardinal vein preparation of *O. mykiss* elicit a dose-dependent release of catecholamines (Bernier and Perry, 1997). The only *in vivo* evidence that Ang II can evoke catecholamine secretion in fish stems from a single study in which intravascular injections of high doses of heterologous Ang II (approximately 1940 pmol kg^{-1}) increased plasma catecholamine levels in *Cyclopterus lumpus* (Carroll and Opdyke, 1982). However, the maximum plasma catecholamine concentrations recorded in *C. lumpus* following Ang II injection (adrenaline approximately 3.2 nmol l^{-1}; noradrenaline approximately 0.7 nmol l^{-1}; Carroll and Opdyke, 1982) are similar to the basal resting catecholamine levels reported in most teleost species (Randall and Perry, 1992). Therefore, while it is generally accepted that some of the cardiovascular effect of Ang II in teleosts is mediated through an interaction with the sympathetic nervous system, it remains to be determined whether humoral catecholamines play a significant role in this interaction.

Although it is known that angiotensins can elicit catecholamine release from the chromaffin tissue of teleosts, it is unclear whether catecholamines of humoral origin can contribute to cardiovascular regulation. There is convincing evidence that in resting *O. mykiss* the adrenergic control of vascular resistance has a neuronal origin (Wood and Shelton, 1975; Smith, 1978; Xu and Olson, 1993a). However, there is considerable debate as to whether physiological concentrations of circulating catecholamines can influence systemic resistance (R_S). Although the results obtained from perfused tissues and isolated vessels (Wood and Shelton, 1975; Xu and Olson, 1993a) indicate that plasma catecholamines are of minor importance in the regulation of R_S, intravascular injections of adrenaline doses ($\leqslant$3 nmol kg^{-1}) that presumably yield physiological plasma concentrations have resulted in significant increases in R_S (Wood and Shelton, 1980; Gamperl et al., 1994a,b). Previous studies have identified the problems associated with estimating plasma catecholamine levels after such injected doses (Gamperl et al., 1994c). Thus, in studies employing catecholamine injections, there is a clear need to correlate the realized (i.e. measured) plasma concentrations with the resultant physiological effects to determine the true contribution of humoral catecholamines. This approach, however, has not been used in any previous investigation.

Therefore, while there is some evidence to suggest that a portion of the cardiovascular effects of Ang II in teleosts may be mediated through plasma catecholamines, this hypothesis has yet to be tested rigorously. Towards this goal, the present study (1) assessed whether homologous rainbow trout angiotensin II can elicit a dose-dependent increase in plasma catecholamine levels, (2) determined the cardiovascular effects of Ang II with and without α-adrenoceptor blockade and (3) established relationships between intravascular doses of adrenaline, realised plasma levels and cardiovascular responses. These experiments were performed to assess whether Ang-II-elicited increases in humoral catecholamine levels can contribute to cardiovascular control.

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex were obtained from Linwood Acres Trout Farms (Campbellcroft, Ontario, Canada) and transported to the fish-rearing facilities of the University of Ottawa. The fish were held indoors in large fibreglass tanks supplied with flowing, aerated and dechlorinated city of Ottawa tap water (pH 6.8; $[Ca^{2+}]$, 0.433 mmol l^{-1}; $[Na^{+}]$, 0.135 mmol l^{-1}; $[K^{+}]$, 0.023 mmol l^{-1}; $[Cl^{-}]$, 0.140 mmol l^{-1}; water CO_2 content, 0.44 mmol l^{-1}). The fish were maintained at a temperature of 14 °C, on a 12 h:12 h light:dark photoperiod, and fed daily with a commercial salmonid diet. The trout of series 1 (see below) had a mean mass of 325.4±9.6 g (experimental N=56), and the trout of series 2 and 3 together had a mean mass of 743.0±39.7 g (experimental N=26) (means ± S.E.M.).

Surgical procedures

Series 1: the effects of Ang II on plasma catecholamine levels

Rainbow trout were anaesthetised in an oxygenated and buffered ($NaHCO_3$; 0.16 g l^{-1}) solution of ethyl-*m*-aminobenzoate (0.08 g l^{-1}; MS-222; Syndel, Vancouver, British Columbia, Canada) until cessation of breathing movements. The fish were then transferred to an operating table where the gills were force-ventilated with the same anaesthetic solution. To permit injections and repeated blood sampling, the dorsal aorta was cannulated non-occlusively with polyethylene tubing (PE 50; Clay Adams) using the technique of Soivio et al. (1975). After surgery, fish were placed into individual flow-through opaque Perspex boxes and left to recover for 48 h before experimentation.

Series 2 and 3: cardiovascular responses to Ang II and to adrenaline

Rainbow trout were anaesthetized as described above before surgery. To measure dorsal aortic blood pressure (P_{DA}) or to carry out repeated blood sampling, fish were equipped with a dorsal aortic cannula (PE 50; Sovio et al., 1975). To permit drug injections, a lateral incision was made in the caudal peduncle to expose the caudal vein, to dissect it free from overlying tissue and to cannulate (PE 50) it in the direction towards the heart. In addition, the pericardial cavity was exposed with a midline ventral incision, and the pericardium was dissected to expose the bulbus arteriosus. To allow measurement of cardiac output ($\dot{Q}$), a 3S or 4S ultrasonic flow probe (Transonic Systems Inc., Ithaca, NY, USA) was placed non-occlusively around the bulbus arteriosus. Lubricating jelly was used with the perivascular flow probe as an acoustic coupling agent. Silk sutures were used to close the ventral and caudal peduncle incisions and to anchor the cardiac output probe lead and the caudal vein cannula to the skin. After surgery, fish were placed into individual flow-through Perspex

boxes and left to recover for 24 h before experimentation. All cannulae were filled and flushed with heparinized (50 i.u. ml^{-1} sodium heparin; Sigma, St Louis, MI, USA) teleost Cortland saline (Wolf, 1963).

Experimental protocol

Series 1: the effects of Ang II on plasma catecholamine levels

Seven different experimental groups of eight fish each (N=56) were used to investigate the effects of homologous [Asn^1-Val^5]-Ang II (Conlon et al., 1996) (0, 25, 75, 150, 300, 600 and 1200 pmol kg^{-1}; Sigma) on the circulating plasma catecholamine concentrations of rainbow trout. Within a given trial, after removal of a blood sample (0.3 ml) to assess basal plasma catecholamines, fish were given a bolus injection (0.3 ml) of [Asn^1-Val^5]-Ang II over a period of 30 s, and the injection was followed by 0.2 ml of saline to clear the cannula. Five more blood samples (0.3 ml) were then taken 1, 2, 3, 5 and 10 min after the beginning of the injection. Each blood sample was replaced by an equivalent volume of saline, collected in a 1.5 ml microcentrifuge tube, immediately centrifuged at 10 000 ***g*** for 15 s, and the plasma was frozen in liquid nitrogen and stored at −80 °C for later analysis of catecholamines.

Series 2: the cardiovascular effects of Ang II with and without α-adrenoceptor blockade

After monitoring stable P_{DA} and $\dot{Q}$ traces for 1 h, control levels of variables were recorded for 5 min, and the fish (N=10) were given a bolus injection (0.3 ml) of [Asn^1-Val^5]-Ang II through the caudal vein cannula over a period of 30 s. Cardiovascular responses to the injection were monitored continuously and, following recovery of cardiovascular variables to control levels for a 1 h period, a second dose of [Asn^1-Val^5]-Ang II was then injected. Repeating this protocol, seven doses of [Asn^1-Val^5]-Ang II (0, 25, 75, 150, 300, 600 and 1200 pmol kg^{-1}) were randomly and sequentially tested on each fish. Each injection was followed by 0.2 ml of saline to clear the cannula.

In a second group (N=8) of fish, the same experimental protocol and doses of [Asn^1-Val^5]-Ang II as above were tested on fish first treated with the α-adrenoceptor blocker phenoxybenzamine hydrochloride (RBI, Natick, MA, USA). α-Adrenergic blockade was achieved by slowly (over a 15 min period) giving two doses of 3 mg kg^{-1} phenoxybenzamine 12 and 6 h before experimentation (Xu and Olson, 1993b). Phenoxybenzamine was dissolved in 100 μl of ethanol and diluted in saline before injection (3 mg ml^{-1}). Before and after the seven Ang II injections, the effectiveness of the α-adrenergic blockade was tested by injection of a catecholamine cocktail (0.375 ml kg^{-1}) prepared in a 0.9 % NaCl solution and consisting of 3.5×10^{-6} mol l^{-1} noradrenaline bitartrate (Arterenol, Sigma) and 1.1×10^{-5} mol l^{-1} adrenaline bitartrate (Sigma).

Series 3: cardiovascular responses to adrenaline

Once stable baseline levels of cardiovascular variables had been established, fish were given a bolus injection (0.3 ml) of either saline or adrenaline bitartrate *via* the caudal vein cannula, and the responses were monitored. The doses of adrenaline ranged from 1.8×10^{-10} to 1.4×10^{-8} mol kg^{-1}. Spaced by inter-injection periods that allowed cardiovascular variables to recover to control levels for 60 min, four injections of adrenaline were tested on each fish (N=8). The range of adrenaline doses tested in this series was selected to achieve a continuum of arterial plasma adrenaline concentrations between 10 and 1000 nmol l^{-1}. This was achieved by varying the lowest dose tested in each fish (1.8×10^{-10} to 1.1×10^{-9} mol kg^{-1}) and multiplying this dose by 2, 5 and 12.5 to prepare the three other doses. All injections were randomly tested, given through the caudal vein cannula over a period of 30 s and followed by 0.2 ml of saline to clear the cannula.

To determine the maximum arterial plasma adrenaline concentration achieved with each injection, each fish was allowed to recover for 24 h and then once again received an injection of saline and the same four doses of adrenaline, in the same sequence and with the same time interval between each injection. Within a given trial, a control blood sample (0.3 ml) was taken to assess basal plasma catecholamine levels, the fish were then given a bolus injection of adrenaline or saline over a period of 30 s, and four more blood samples (0.3 ml) were taken 1, 2, 3 and 4 min after the beginning of the injection. While the adrenaline injections were made *via* the caudal vein cannula, all the blood samples were collected *via* the dorsal aortic cannula. Each blood sample, which was replaced by an equivalent volume of saline, was collected in a 1.5 ml microcentrifuge tube, immediately centrifuged at 10 000 ***g*** for 15 s, and the plasma was then frozen in liquid nitrogen and stored at −80 °C for later analysis of catecholamines.

Analytical procedures

Plasma catecholamine levels (adrenaline and noradrenaline) were determined on alumina-extracted plasma samples (0.2 ml) using high-pressure liquid chromatography (HPLC) with electrochemical detection (Bernier and Perry, 1997). P_{DA} was measured with a pressure transducer (UFI model 1050BP; UFI, Morro Bay, CA, USA) which was calibrated against a static water column. Mean blood pressure was calculated as (systolic pressure + diastolic pressure)/2. The perivascular flow probes used to measure $\dot{Q}$ were connected to a Transonic T106 small-animal blood flow meter. These probes were precalibrated in the factory and verified in the laboratory by pump perfusion of the heart with saline at known flow rates in an immersed killed fish. Both P_{DA} and $\dot{Q}$ signals were recorded with a data acquisition system (Biopac System Inc., Goleta, CA, USA) and collected at intervals of 0.04 s using Acknowledge III (Biopac System Inc.) data acquisition software. Systemic resistance (R_S) was calculated as mean P_{DA} divided by $\dot{Q}$ (i.e. $R_S=P_{DA}/\dot{Q}$), heart rate (f_H) was derived from the dorsal aortic pressure pulse trace, and stroke volume (V_S) was calculated as $\dot{Q}$ divided by f_H (i.e. $V_S=\dot{Q}/f_H$).

Statistical analyses

Data are presented as mean values ± one standard error of

the mean (S.E.M.). The statistical significance of the observed effects of a given Ang II injection within a group were tested using one-way repeated-measures analysis of variance (ANOVA). Dunnett's *post-hoc* multiple-comparison test was used to compare the pre-injection control data point with values at subsequent and previous times. Control and maximum plasma catecholamine levels following a given Ang II injection were analysed by paired *t*-test. Differences between the changes in a cardiovascular variable after the different Ang II injections within a given treatment were determined using one-way repeated-measures ANOVA followed by the Student–Newman–Keuls test for multiple comparisons. The statistical significance of observed differences between the means of two treatments was determined by *t*-test. To assess the effects of the adrenaline injections on the cardiovasculature, post-injection increases for a given cardiovascular variable were separated into four groups on the basis of the maximum arterial plasma adrenaline concentration achieved by each adrenaline injection (0–30 nmol l^{-1}, 31–100 nmol l^{-1}, 101–250 nmol l^{-1} and 251–1000 nmol l^{-1} adrenaline). Differences between the mean increases in a cardiovascular variable after the adrenaline injections were determined using one-way ANOVA followed by the Student–Newman–Keuls test for multiple-comparison. The significance level for all statistical tests was $P<0.05$.

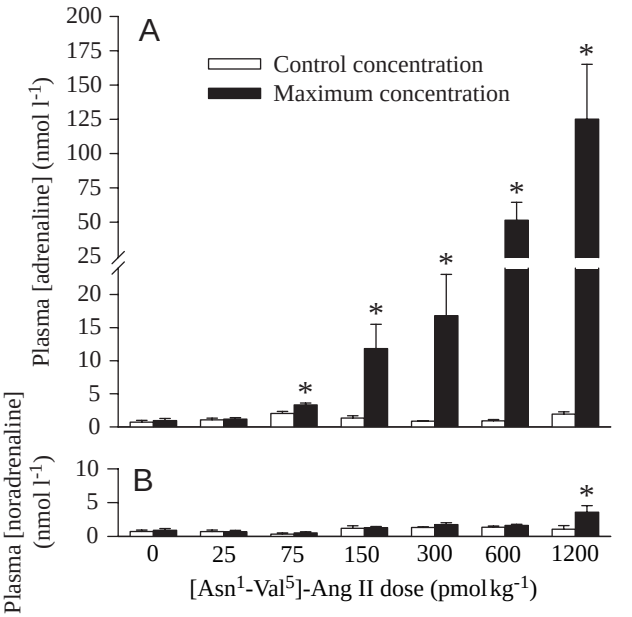

Fig. 1. Effects of bolus injections of homologous [Asn^1-Val^5]-Ang II on (A) plasma adrenaline and (B) plasma noradrenaline concentrations in rainbow trout (N=8 for each Ang II dose). The open columns indicate the catecholamine concentrations before the Ang II injection, and the filled columns indicate the maximum catecholamine concentrations in response to the injection. An asterisk denotes a significant ($P<0.05$) difference between the maximum and the control concentration for a given dose of Ang II. Values are means + 1 S.E.M.

Results

Series 1: the effects of Ang II on plasma catecholamine levels

Bolus injections of Ang II between 75 and 1200 pmol kg^{-1} elicited a dose-dependent increase in plasma adrenaline level (Fig. 1A). The times taken to attain the peak plasma adrenaline concentration were similar for all Ang II doses, with peak values occurring 2 min post-injection (data not shown). In contrast, only the 1200 pmol kg^{-1} Ang II dose elicited a small, but significant, increase in the mean maximum plasma noradrenaline concentration (Fig. 1B). Bolus injection of saline had no effect on basal plasma adrenaline and noradrenaline levels (Fig. 1A,B).

Series 2: the cardiovascular effects of Ang II with and without α-adrenoceptor blockade

In comparison with the resting cardiovascular variables of the control group, fish treated with the α-adrenergic antagonist phenoxybenzamine had significantly lower resting mean P_{DA} and R_S, higher resting mean $\dot{Q}$ (Table 1) and V_S, and similar mean f_H (Table 2). Relative to the control group, phenoxybenzamine treatment reduced the pressure response to a bolus injection of catecholamines by 67 %, abolished the increase in R_S and reduced the increase in $\dot{Q}$ by 42 % (Table 1).

Bolus injections of homologous rainbow trout Ang II resulted in a dose-dependent increase in P_{DA} (Fig. 2A) and R_S (Fig. 2B) in both the control and phenoxybenzamine-treated fish. A significant increase in P_{DA} was elicited by 25–1200 pmol kg^{-1} Ang II and a significant increase in R_S was elicited by 75–1200 pmol kg^{-1} Ang II. The absolute changes in P_{DA} and R_S elicited by the injection of 600 pmol kg^{-1} Ang II (representative dose) are shown in Fig. 3. For any given Ang II dose, the duration of the pressor response in the control treatment was longer than the duration of the increase in R_S (Fig. 3). In contrast, the duration of the Ang-II-mediated pressor response in the phenoxybenzamine-treated fish was of equal duration to the increase in R_S (Fig. 3). In comparison with the control group, the increases in mean P_{DA} and R_S were significantly reduced by 33 % and 43 %, respectively, following the 600 pmol kg^{-1} dose of Ang II, and by 33 % and 53 %, respectively, following the 1200 pmol kg^{-1} dose of Ang II in the phenoxybenzamine-treated fish (Fig. 2A,B). Saline injection alone had no significant effect on mean P_{DA} and R_S in the control and phenoxybenzamine-treated fish.

The administration of graded Ang II doses elicited an increase in $\dot{Q}$ in the control fish at all doses (25–1200 pmol kg^{-1}; Figs 3C, 4A), and the maximum increase in $\dot{Q}$ was attained with the 600 pmol kg^{-1} dose (Figs 3C, 4A). In the phenoxybenzamine-treated fish, Ang II injections elicited a significant decrease in $\dot{Q}$ at the two highest doses (600 and 1200 pmol kg^{-1}; Figs 3C, 4A), and the reduction in $\dot{Q}$ was dose-dependent between 25 and 1200 pmol kg^{-1} Ang II (Fig. 4A). While the Ang II injections had no significant effect on the f_H of the control and phenoxybenzamine-treated fish, they elicited an increase in V_S in the control fish and a decrease

Table 1. *The effects of a bolus injection of catecholamines on the cardiovascular variables of intact control and phenoxybenzamine-treated rainbow trout*

Treatment	*N*	P_{DA} (cmH_2O)	R_S ($cmH_2O\ ml^{-1}\ min^{-1}\ kg^{-1}$)	$\dot{Q}$ ($ml\ min^{-1}\ kg^{-1}$)
Control	10			
Resting values		30.4±2.0	1.3±0.2	25.3±2.5
Post-injection		41.1±4.4*	1.7±0.3*	29.5±2.1*
Phenoxybenzamine	8			
Resting values		22.5±2.6‡	0.6±0.1‡	44.6±3.8‡
Post-injection		26.0±3.3*	0.6±0.1	47.1±3.8*

The catecholamine injection consisted of a $0.375\ ml\ kg^{-1}$ dose of $3.5\times10^{-6}\ mol\ l^{-1}$ noradrenaline bitartrate and $1.1\times10^{-5}\ mol\ l^{-1}$ adrenaline bitartrate.

Values are mean ± 1 S.E.M.

*Significantly different from the resting value for a given treatment, ‡Significantly different from the resting value of the control treatment ($P<0.05$).

P_{DA}, mean dorsal aortic pressure; R_S, systemic resistance; $\dot{Q}$ cardiac output.

$1\ cmH_2O=98.1\ Pa$.

in V_S in the phenoxybenzamine-treated fish (Table 2; Fig. 4B). Injection of saline alone elicited a significant increase in $\dot{Q}$ of 1.00 and $1.14\ ml\ min^{-1}\ kg^{-1}$ in the control and phenoxybenzamine-treated fish, respectively, and had no statistical effect on f_H or V_S in either group (Table 2).

The temporal features of the Ang-II-elicited changes in P_{DA}, R_S and $\dot{Q}$ are shown in Fig. 5. In the control treatment, the times taken to achieve peak pressor response (2.5–3.0 min) and peak R_S (1.9–2.4 min) remained constant for the different Ang II doses, and the time taken to achieve peak $\dot{Q}$ (3.4–7.1 min) increased in a dose-dependent manner (Fig. 5A). In the phenoxybenzamine-treated fish, the times taken to achieve peak pressor response (3.0–3.8 min), peak R_S (2.4–4.2 min) and peak $\dot{Q}$ (2.9–5.4 min) remained constant for the different Ang II doses (Fig. 5B). While the times taken to achieve peak P_{DA} and peak R_S were shorter in the control group than in the phenoxybenzamine-treated group (75–1200 $pmol\ kg^{-1}$), the time taken to achieve peak $\dot{Q}$ at the three highest Ang II doses (300–1200 $pmol\ kg^{-1}$) was longer in the control group than in the phenoxybenzamine-treated group (Fig. 5). Although the increase in $\dot{Q}$ in the control group lagged significantly behind the increase in P_{DA} and R_S at all doses, and in general peak R_S occurred before peak P_{DA}, in the phenoxybenzamine treatment, all three cardiovascular variables peaked at similar times (Fig. 5).

Series 3: cardiovascular responses to adrenaline

Caudal vein injection of adrenaline doses ranging between 1.8×10^{-10} and $1.4\times10^{-8}\ mol\ kg^{-1}$ resulted in arterial plasma adrenaline concentrations between $9.2\ nmol\ l^{-1}$ and $785.6\ nmol\ l^{-1}$ ($r^2=0.941$, Fig. 6). The dose–response relationship between injected adrenaline dose (y; in $nmol\ kg^{-1}$) and realised plasma concentration (x; in $nmol\ l^{-1}$) is described by the following linear equation: $y=56.62x-4.27$. The adrenaline injections had no effect on basal plasma noradrenaline concentrations ($r^2=0.028$; Fig. 6). Adrenaline injections elicited significant and dose-dependent increases in mean P_{DA} between the plasma adrenaline concentrations

Table 2. *The effects of a bolus injection of homologous [Asn^1-Val^5]-Ang II on the heart rate and stroke volume of intact control and phenoxybenzamine-treated rainbow trout*

		Heart rate ($beats\ min^{-1}$)				Stroke volume ($ml\ kg^{-1}\ beat^{-1}$)			
			Post-injection				Post-injection		
Ang II dose ($pmol\ kg^{-1}$)	Treatment	Pre-injection 0 min	2.5 min	5 min	10 min	Pre-injection 0 min	2.5 min	5 min	10 min
0	Control	72.0±2.2	73.4±2.4	71.4±2.5	73.7±2.6	0.36±0.05	0.37±0.04	0.37±0.04	0.36±0.04
	Phen.	72.2±3.9	74.1±3.1	72.5±3.7	73.4±3.9	0.60±0.06‡	0.59±0.06‡	0.61±0.07‡	0.61±0.07‡
25	Control	73.1±3.0	72.3±2.6	72.6±2.6	71.8±2.4	0.39±0.04	0.42±0.05*	0.41±0.05*	0.40±0.04
	Phen.	77.9±2.9	76.2±3.3	74.2±2.6	77.7±3.8	0.53±0.04‡	0.56±0.04‡	0.56±0.04‡	0.54±0.04‡
75	Control	74.5±2.7	72.8±2.5	73.9±2.5	74.6±3.0	0.37±0.04	0.42±0.05*	0.42±0.05*	0.39±0.05
	Phen.	75.3±2.3	77.6±3.3	76.0±3.2	75.0±2.6	0.54±0.05‡	0.51±0.05	0.53±0.05	0.54±0.05‡
150	Control	73.7±2.8	71.6±2.5	71.0±2.1	71.9±2.2	0.38±0.05	0.43±0.06*	0.45±0.06*	0.41±0.05*
	Phen.	76.1±3.4	75.9±3.9	74.9±2.8	76.3±2.7	0.56±0.05‡	0.52±0.06	0.54±0.05	0.55±0.05
300	Control	75.1±3.1	77.6±1.8	74.0±2.9	75.8±4.3	0.35±0.04	0.37±0.04	0.43±0.05*	0.39±0.05*
	Phen.	71.2±2.3	73.8±3.2	74.9±2.4	72.3±3.0	0.60±0.06‡	0.54±0.07‡	0.54±0.06	0.59±0.06‡
600	Control	72.3±2.3	72.8±1.8	72.3±2.0	72.6±2.9	0.37±0.05	0.38±0.04	0.43±0.05*	0.41±0.05*
	Phen.	73.9±3.0	74.2±3.1	74.3±3.4	76.1±3.1	0.59±0.05‡	0.53±0.06	0.54±0.06	0.56±0.06
1200	Control	73.7±2.7	72.7±3.2	71.6±3.4	72.7±3.8	0.37±0.05	0.36±0.05	0.42±0.05*	0.42±0.05*
	Phen.	73.8±2.8	74.5±3.7	74.6±1.7	76.2±2.9	0.55±0.05‡	0.49±0.06	0.48±0.05	0.52±0.05

Values are mean ± 1 S.E.M. Sample size for control and phenoxybenzamine (Phen.) treatments are 10 and 8, respectively.

‡Significantly different from the control treatment at given time; *significantly different from the pre-injection value for a given treatment ($P<0.05$).

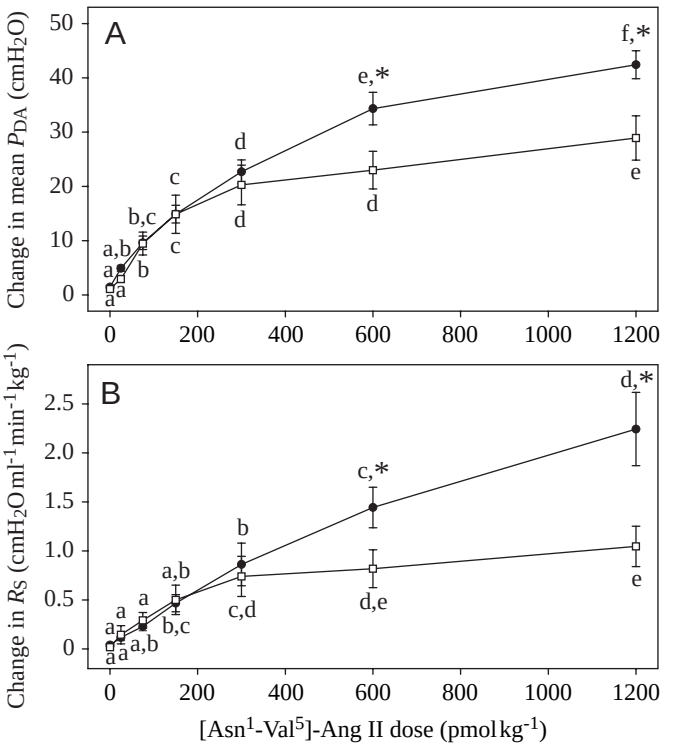

Fig. 2. Changes in mean dorsal aortic pressure (P_{DA}; A) and systemic resistance (R_S; B) in intact control (N=10; filled circles) and phenoxybenzamine-treated (N=8; open squares) rainbow trout after intravenous injections of graded homologous [Asn^1-Val^5]-Ang II doses. An asterisk denotes a significant (P<0.05) difference between the control and phenoxybenzamine-treated fish at a given dose of Ang II. Dissimilar letters indicate significant (P<0.05) differences between doses within a group. Values are means ±1 S.E.M. 1 cmH$_2$O=98.1 Pa.

16.5±1.8 and 487.6±98.2 nmol l^{-1} (Fig. 7A). The increases in R_S following administration of adrenaline were significant and dose-dependent between the plasma adrenaline concentrations 50.5±5.2 and 487.6±98.2 nmol l^{-1} (Fig. 7B). Significant increases in $\dot{Q}$ and V_S were achieved with adrenaline injections which yielded plasma adrenaline concentrations of 50.5±5.2 nmol l^{-1} (Fig. 7C,D). Higher plasma adrenaline concentrations did not result in further increases in either $\dot{Q}$ or V_S (Fig. 7C,D). Although, in some fish, adrenaline injections were followed by a drop in f_H, the changes in f_H were not significant overall. While injections of saline alone had no significant effect on mean P_{DA}, R_S, f_H or V_S, they elicited a small increase in $\dot{Q}$ (Fig. 7A–D).

Discussion

The effects of Ang II on plasma catecholamine levels

The stimulatory effects of Ang II on plasma adrenaline levels reported in the present study support the original observation of Carroll and Opdyke (1982) that Ang II can elicit the release of catecholamines in teleost fish *in vivo* and establish for the first time the dose-dependent nature of the

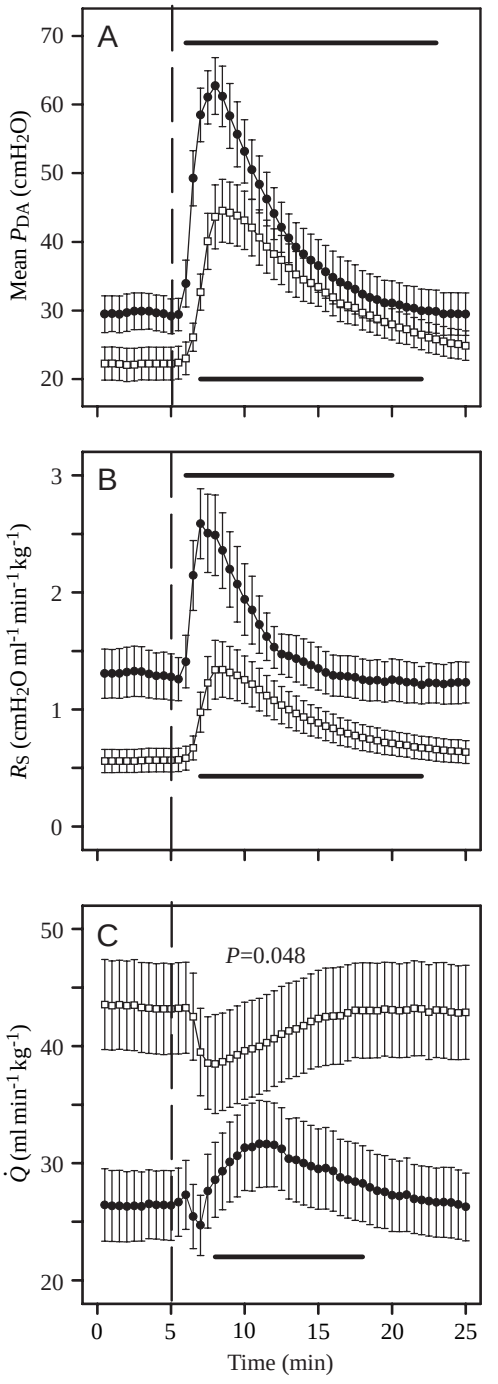

Fig. 3. Mean dorsal aortic pressure (P_{DA}; A), systemic resistance (R_S; B) and cardiac output ($\dot{Q}$; C) in intact control (N=10; filled circles) and phenoxybenzamine-treated (N=8; open squares) rainbow trout given a 600 pmol kg^{-1} bolus injection of homologous [Asn^1-Val^5]-Ang II. The broken vertical line in each graph indicates the time of Ang II injection. The solid lines above and below the control and phenoxybenzamine-treated groups, respectively, indicate the time period during which the post-injection values were significantly different from the resting value immediately preceding the broken line (P<0.05). The P value resulting from the one-way repeated-measures ANOVA analysis of the $\dot{Q}$ data from the phenoxybenzamine-treated fish (C) is shown because results from the multiple-comparison test failed to determine the area of significance. Values are means ±1 S.E.M. 1 cmH$_2$O=98.1 Pa.

relationship. The preferential increase in plasma adrenaline level over noradrenaline level in response to Ang II injections also supports the findings of Carroll and Opdyke (1982) in the lumpfish (*C. lumpus*) and our previous observations in *in situ* preparations of rainbow trout (Bernier and Perry, 1997). Since a 1200 pmol kg^{-1} dose of Ang II elicited an increase in plasma noradrenaline level that was only marginally greater than basal resting levels, our results suggests that, under physiological conditions, the cardiovascular effects of Ang-II-mediated catecholamine release can be attributed primarily to plasma adrenaline in rainbow trout. While an Ang II dose of 1940 pmol kg^{-1} was required to elicit a plasma adrenaline concentration of 3.3 nmol l^{-1} in *C. lumpus*, the same adrenaline concentration was achieved with an Ang II dose of 75 pmol kg^{-1} in the trout. Hence, although results are available from only two species, they do suggest that there may be considerable variability in the responsiveness of the chromaffin tissue to Ang II among teleosts. Relative to other secretagogues of the chromaffin tissue (Fritsche et al., 1993; Reid and Perry, 1994; Reid et al., 1996, 1998), the maximum adrenaline concentration achieved with Ang II in the present study, 125 nmol l^{-1}, suggests that Ang II can be a potent secretagogue of humoral adrenaline in rainbow trout.

Although few studies have measured circulating levels of Ang II in teleosts, the available data suggest that an Ang-II-elicited increase in plasma adrenaline level can be achieved with physiological concentrations of Ang II. While basal plasma Ang II concentrations vary from approximately 10 to 220 pmol l^{-1} in eels (Henderson et al.,1985; Kobayashi et al., 1980; Okawara et al., 1987; Takei et al., 1988; Tierney et al., 1995a,b), Lipke et al. (1990) reported a mean resting value of 824 pmol l^{-1} in rainbow trout. From these resting values, experiments carried out with eels have shown that plasma Ang II concentrations can increase more than 15-fold (from approximately 380 to 3490 pmol l^{-1}) following various acute hypotensive stresses (Henderson et al., 1985; Kobayashi et al., 1980; Tierney et al., 1995a,b). In the present study, Ang II doses ranging from 75 to 1200 pmol kg^{-1} elicited a significant increase in plasma adrenaline level. Given that the half-life of Ang II in trout is 3–7 min (Olson, 1992) and assuming that Ang II, after injection, was distributed rapidly throughout the

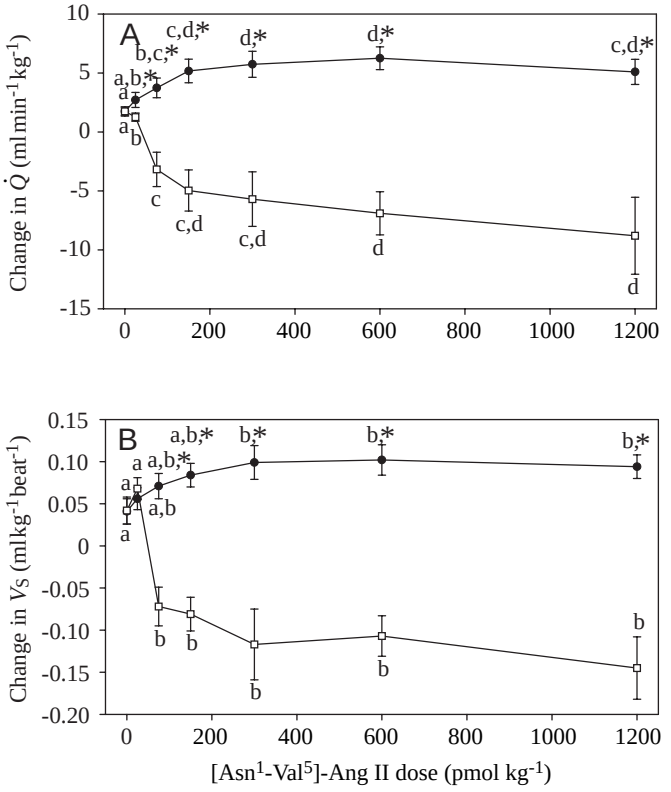

Fig. 4. Changes in cardiac output ($\dot{Q}$; A) and stroke volume (V_S; B) in intact control (N=10; filled circles) and phenoxybenzamine-treated (N=8; open squares) rainbow trout after intravenous injections of graded homologous [Asn^1-Val^5]-Ang II doses. An asterisk denotes a significant (P<0.05) difference between the control and phenoxybenzamine-treated fish at a given dose. Dissimilar letters indicate significant (P<0.05) differences between doses within a group. Values are means ±1 S.E.M.

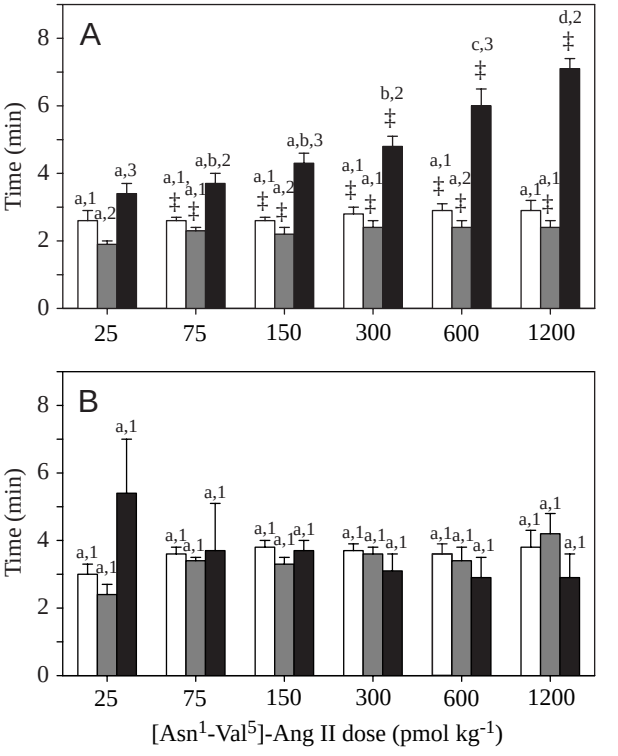

Fig. 5. Effects of intravenous injections of graded homologous [Asn^1-Val^5]-Ang II doses on the time taken to reach peak pressure (open columns), peak systemic resistance (grey columns) and peak cardiac output (filled columns) in intact control (A; N=10) and phenoxybenzamine-treated (B; N=8) rainbow trout. Dissimilar letters indicate significant (P<0.05) differences between doses for a given cardiovascular variable and treatment. Dissimilar numbers indicate significant (P<0.05) differences between cardiovascular variables for a given dose and treatment. ‡ denotes a significant (P<0.05) difference between the control and phenoxybenzamine treatments at a given dose. Values are means +1 S.E.M.

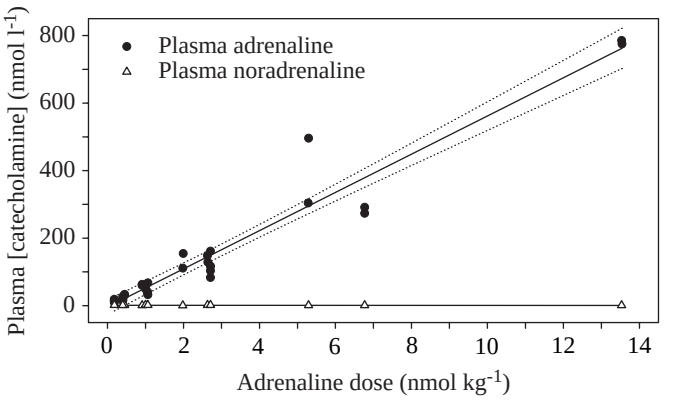

Fig. 6. Effects of intravenous injections of adrenaline on maximal arterial plasma adrenaline (filled circles) and noradrenaline (open triangles) concentrations in resting rainbow trout (N=8). The linear regression for plasma adrenaline is y=56.62x–4.27 (r^2=0.941); for plasma noradrenaline, the linear regression is y=–0.03x+0.10 (r^2=0.028). The 95 % confidence interval for adrenaline is indicated by the dotted line.

extracellular fluid volume (300 ml kg^{-1}) before reaching the chromaffin tissue, the 75–1200 pmol kg^{-1} Ang II doses probably elicited physiological levels of Ang II in the plasma. Whether activation of the proposed intrarenal renin–angiotensin system of rainbow trout (Brown et al., 1995; Bernier and Perry, 1997) results in higher concentrations of Ang II in the vicinity of the chromaffin tissue has yet to be investigated.

The cardiovascular effects of Ang II with and without α-adrenoceptor blockade

Once Q_{10} effects are taken into consideration, the resting cardiovascular variables (mean values of P_{DA}, R_S, $\dot{Q}$, f_H and V_S) of the cannulated and instrumented fish in the control group are comparable with those reported previously for rainbow trout (Kiceniuk and Jones, 1977; Wood and Shelton, 1980; Gamperl et al., 1994a,b). Phenoxybenzamine treatment, in addition to blocking humoral α-receptor-mediated increases in R_S and P_{DA} (Wood, 1976; Randall and Stevens, 1967; Wood and Shelton, 1980; Xu and Olson, 1993b), caused a reduction in resting mean P_{DA} and R_S and an increase in resting $\dot{Q}$ and V_S. While phenoxybenzamine-associated vasodepression has been observed by some authors (Wood and Shelton, 1980), others have concluded that phenoxybenzamine has no significant effect on blood pressure (Randall and Stevens, 1967; Olson and Duff, 1992; Xu and Olson, 1993b). On the basis of the latter observations, Xu and Olson (1993b) suggested that the α-antagonistic effects of phenoxybenzamine are limited to the luminal receptors of the vasculature that are exposed only to humoral catecholamines. In contrast, our results suggest that phenoxybenzamine treatment, like treatment with other α-adrenergic antagonists (Wood and Shelton, 1980; Xu and Olson, 1993b), blocks both neuronal and humoral α-adrenoceptors.

The pressor effect of intravascular Ang II injections has been documented in a variety of teleost species (for reviews, see Nishimura, 1985; Olson, 1992) including rainbow trout (Le Mevel et al., 1993, 1994; Olson et al., 1994; Fuentes and Eddy, 1998). A significant component of this Ang-II-elicited pressor response is mediated indirectly through an activation of the sympathetic nervous system (Nishimura et al., 1978; Carroll and Opdyke, 1982; Nishimura, 1985; Lipke et al., 1990; Platzack et al., 1993; Olson et al., 1994; Butler et al., 1995; Oudit and Butler, 1995). In the dorsal aorta of perfused trunk preparations of rainbow trout, approximately 40 % of the overall vasoconstrictory effect of a pharmacological dose of Ang II (10^{-7} mol l^{-1}) is abolished by either the α-adrenoceptor antagonist phentolamine or the adrenergic nerve toxin bretylium (Olson et al., 1994). Since catecholamines from the chromaffin tissue do not circulate in the isolated trunk preparation, and bretylium only blocks the release of catecholamines from adrenergic neurones, these results suggest that a significant portion of the Ang II pressor response can be mediated *via* sympathetic nerves. However, since the Ang II pressor response in the coeliacomesenteric artery of the same preparation was unaffected by pharmacological blockade, the interaction between Ang II and sympathetic nerves may be vessel-specific (Olson et al., 1994). *In vivo*, α-adrenoceptor blockade with phenoxybenzamine also inhibits a significant portion (33 %, this study; 60 %, Lipke et al., 1990) of the Ang-II-mediated pressor response in rainbow trout. While this may result in part from the blockade of a possible interaction between Ang II and the sympathetic nerves, our results suggest that the reduced Ang-II-elicited pressor response with α-adrenoceptor blockade can also be ascribed to an inhibition of the Ang-II-mediated increase in humoral catecholamine levels. Indeed, the maximum plasma adrenaline concentrations recorded following the 600 and 1200 pmol kg^{-1} doses of Ang II are concentrations that can elicit significant increases in P_{DA}, R_S and $\dot{Q}$.

In the trout, increases in both R_S and $\dot{Q}$ contribute to the Ang-II-elicited pressure response. However, since the increase in R_S slightly precedes peak P_{DA} and the increase in $\dot{Q}$ lags behind peak P_{DA}, R_S is the principal cause of the pressor response in trout. Comparisons of Ang II pressor responses in perfused tissues and large isolated vessels of rainbow trout have shown that the direct vasoconstrictory response to Ang II occurs in the systemic microcirculation (Conklin and Olson, 1994a; Olson et al., 1994). In contrast, experiments using ventricular rings *in vitro* and *in situ* perfused heart preparations of rainbow trout have concluded that Ang II does not directly affect cardiac performance in this species (Olson et al., 1994). In the American eel (*Anguilla rostrata*), increases in R_S and $\dot{Q}$ also contribute to the Ang-II-elicited pressure response, and the temporal changes in R_S, P_{DA} and $\dot{Q}$ are similar to those in the trout (Oudit and Bulter, 1995). However, in the eel, Ang II has both direct and indirect stimulatory effects on the heart (Oudit and Butler, 1995). Ang II injections in the Antarctic fish *Pagothenia borchgrevinki* also induced increases in R_S and P_{DA}, but unlike either the eel or the trout, they elicited no

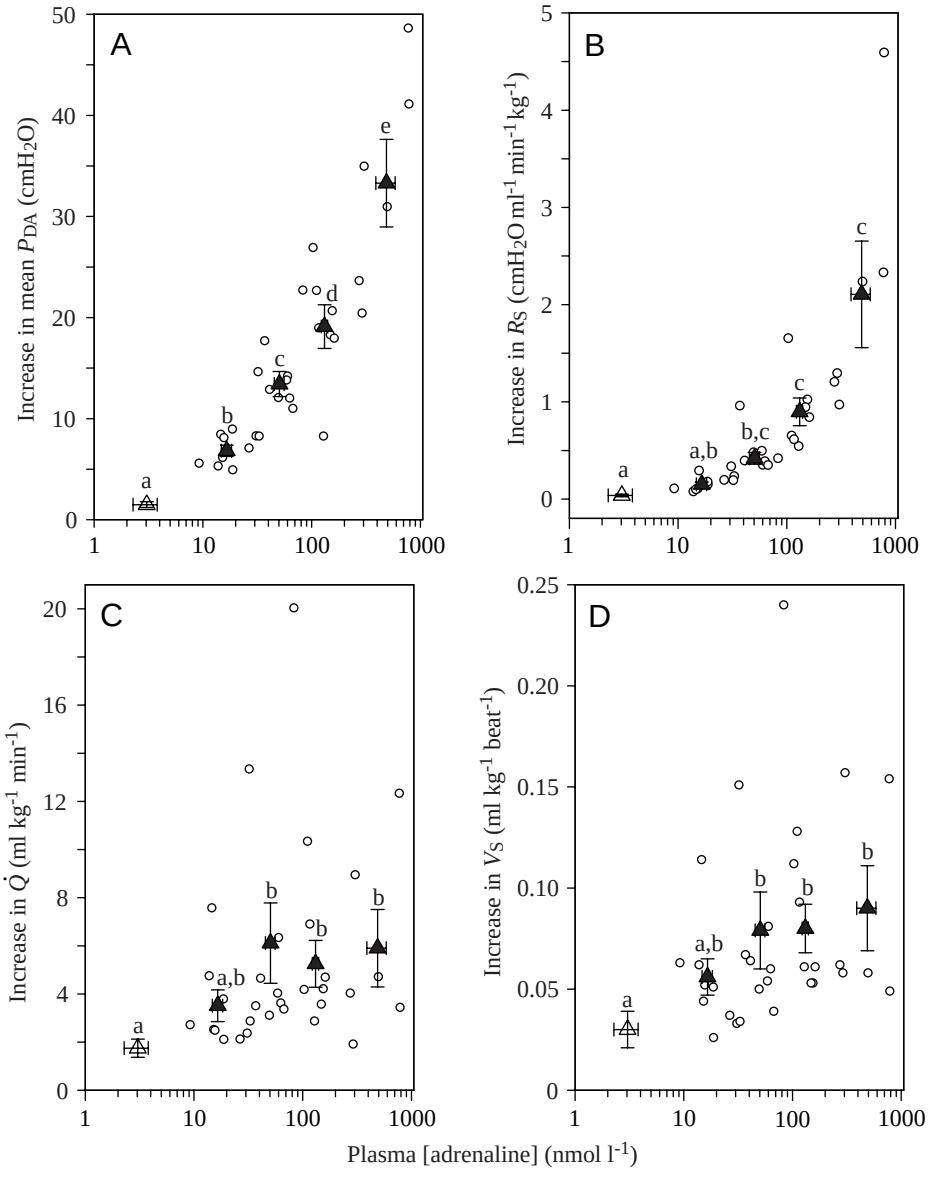

Fig. 7. Increases in mean dorsal aortic pressure (P_{DA}; A), systemic resistance (R_S; B), cardiac output ($\dot{Q}$; C) and stroke volume (V_S; D) as a function of arterial plasma adrenaline concentration in rainbow trout (N=8). Open circles represent individual adrenaline injections at doses ranging between 1.8×10^{-10} and 1.4×10^{-8} mol kg^{-1}. Open triangles represent mean values (±1 S.E.M.) for saline injections. Filled triangles represent mean values (±1 S.E.M.) for adrenaline injections. Dissimilar letters indicate significant (P<0.05) differences between means for a given cardiovascular variable. 1 cmH$_2$O=98.1 Pa.

change in $\dot{Q}$ because bradycardia cancelled out a rise in V_S (Axelsson et al., 1994).

Although there is no evidence for a direct effect of Ang II on the cardiac performance of the trout (Olson et al., 1994), several indirect mechanisms may contribute to the stimulatory effects of Ang II on $\dot{Q}$. For example, Ang-II-elicited catecholamine levels may have chronotropic and inotropic effects (Farrell and Jones, 1992; Gamperl et al., 1994a). Ang II can also act centrally to suppress vagal tone (Le Mevel et al., 1994) or to facilitate sympathetic neurotransmission (Reid, 1992). In addition, Ang II may increase $\dot{Q}$ by affecting venous return (Oudit and Butler, 1995). However, while an Ang II dose of 25 pmol kg^{-1} elicited a significant increase in $\dot{Q}$, it had no effect on plasma adrenaline levels. Also, although near-maximal stimulation of $\dot{Q}$ was attained with a 300 pmol kg^{-1} dose of Ang II, this dose elicited a plasma adrenaline concentration of only 16.8 nmol l^{-1}. So, while humoral catecholamines may contribute to the stimulatory effects of Ang II on $\dot{Q}$, the importance of circulating catecholamines may be relatively minor and secondary to other mechanisms. Even though in trout (Le Mevel et al., 1994) and mammals (Reid, 1992) Ang II can act centrally to suppress baroreceptor-mediated reflexive bradycardia, it is unlikely that peripheral injections of Ang II inhibited vagal tone or reduced the baroreceptor reflex. The Ang-II-elicited increase in $\dot{Q}$ was mediated entirely by an increase in V_S in the present study, and peripheral injections of Ang II in trout have also been associated with moderate bradycardia (Le Mevel et al., 1993). Although inotropic and chronotropic effects have been attributed to interactions between Ang II and sympathetic ganglia in mammals (Saxena, 1992), overall the role of adrenergic nerves in teleost cardiac control is not clear (Nilsson, 1994). Even though interactions between Ang II and sympathetic nerves have been observed in parts of the vasculature of rainbow trout *in situ* (Olson et al., 1994), the specific effects of Ang II on the adrenergic nervous control of

the heart remain to be investigated. In *A. rostrata*, Ang II may also increase $\dot{Q}$ by affecting venous return (Oudit and Butler, 1995). In rainbow trout, this is supported by the importance of cardiac filling pressures in determining V_S (Graham and Farrell, 1989). However, the venous system of trout appears to be refractory to Ang II *in vitro* (Conklin and Olson, 1994a,b), and inhibition of angiotensin-converting enzyme does not affect mean circulatory filling pressure *in vivo* (Zhang et al., 1995). Nevertheless, the stimulatory effects of Ang II on sympathetic nerves and humoral catecholamine levels may increase the sensitivity of the rainbow trout heart to filling pressure (Graham and Farrell, 1989) and increase venous return through a mobilization of blood from the unstressed volume (Conklin and Olson, 1994b; Zhang et al., 1998).

The dose-dependent delay in the peak $\dot{Q}$ response to Ang II, which lags behind peak P_{DA}, probably results from a passive inhibition of systolic emptying caused by the elevated outflow pressures (Wood and Shelton, 1980), especially at higher Ang II concentrations (600 and 1200 pmol kg^{-1}, see Fig. 5). Although the end-systolic volume of the ventricle is usually independent of mean aortic output pressure (homeometric regulation), at very high pressures the intrinsic ability of the heart to maintain V_S breaks down and end-systolic volume increases (Farrell and Jones, 1992). The breakdown of homeometric regulation and the resulting decrease in V_S are especially noticeable in α-adrenoceptor-blocked fish. Without the potential stimulation of $\dot{Q}$ resulting from the interaction between Ang II and the sympathetic nervous system, $\dot{Q}$ and V_S of phenoxybenzamine-treated fish decreased in proportion to and simultaneously with the increases in P_{DA} and R_S. The response is also probably exacerbated by the higher resting V_S that α-adrenoceptor-blocked fish must maintain to compensate for the loss of neuronal tone.

Cardiovascular responses to adrenaline

In support of other *in vivo* studies that have investigated the cardiovascular responses to physiological doses of adrenaline (Wood and Shelton, 1980; Gamperl et al., 1994a,b), our data provide direct evidence that physiological levels of plasma adrenaline can contribute to cardiovascular control in rainbow trout. The discrepancy between these *in vivo* results and the observation that physiological concentrations of catecholamine do not contribute to systemic vascular resistance *in vitro* (Wood and Shelton, 1975) may be resolved by the observation that neuronal sympathetic tone is enhanced by circulating catecholamines (Xu and Olson, 1993a). As previously observed (Randall and Stevens, 1967; Wood and Shelton, 1980; Gamperl et al., 1994a), the effects of adrenaline on P_{DA} are dose-dependent within and beyond the physiological range of plasma adrenaline levels. Our results also confirm the previously described variable nature of the response of $\dot{Q}$ to adrenaline in trout (Wood and Shelton, 1980; Gamperl et al., 1994a) and support the observations that near-maximal increases in $\dot{Q}$ and V_S are achieved at relatively low physiological concentrations of adrenaline (Farrell et al., 1986; Gamperl et al., 1994a).

The plasma adrenaline levels that elicit significant increases in P_{DA}, R_S and $\dot{Q}$ are characteristic of the circulating catecholamine levels observed following a variety of disturbances (for reviews, see Randall and Perry, 1992; Gamperl et al., 1994c). The common link between these physical and environmental disturbances is their severity. As observed by Randall and Perry (1992), plasma catecholamine levels do not rise substantially unless the degree of stress is severe. In support of this, we observed that only Ang II doses of 600 pmol kg^{-1} or above elicit plasma adrenaline levels that contribute to the cardiovascular effects of Ang II. On the basis of experiments carried out on eels (Henderson et al., 1985; Kobayashi et al., 1980; Tierney et al., 1995a,b), the plasma Ang II concentrations that correspond to these Ang II doses may only be approached following acute haemorrhage, hypotension or transfer to sea water. Hence, solely on the basis of circulating Ang II levels, our results suggest that the cardiovascular effects of Ang II will only be supplemented by humoral catecholamines under conditions that acutely stimulate the renin–angiotensin system. Whether circulating catecholamine levels are elevated during such disturbances remains to be investigated.

Finally, our dose–response relationship between caudal-vein-injected adrenaline dose and realised arterial plasma concentration can only be used for predictive purposes if the same drug injection and blood sampling sites are used. In comparison with the study of Gamperl et al. (1994c), in which both adrenaline injection and blood sampling were performed *via* a dorsal aortic cannula, the realised plasma concentration for a given adrenaline dose was approximately 10 times lower than in the current study. Also, in support of other studies in the trout (Perry and Vermette, 1987; Gamperl et al., 1994c), but in contrast to the observation of Epple and Nibbio (1985) in the American eel (*Anguilla rostrata*), adrenaline injections had no catecholaminotropic effect on plasma noradrenaline level.

In summary, this study demonstrates that Ang II can be a potent non-cholinergic secretagogue of adrenaline secretion from the chromaffin tissue of the trout *in vivo*. However, given the exogenous Ang II doses required to elicit plasma adrenaline levels that contribute to cardiovascular regulation, the interaction between Ang II and adrenaline secretion may only have physiological significance following stressful disturbances that acutely stimulate the renin–angiotensin system. Since mammalian studies suggest that the interactions between Ang II and the sympathetic nervous system that result from exogenous Ang II are of little significance in comparison with those from endogenous Ang II (Reid, 1992), it is possible that the present results underestimate the impact of Ang-II-elicited catecholamine release on fish cardiovascular control. In addition to the role of endogenous plasma Ang II, future experiments investigating Ang-II-elicited catecholamine release should take into account the role of the intrarenal renin–angiotensin system and the potential neuromodulatory actions of centrally generated Ang II in the neural regulation of catecholamine release.

This study was supported by NSERC of Canada operating and equipment grants to S.F.P. N.J.B. was the recipient of an NSERC Postgraduate Scholarship. The animals were cared for in accordance with the principles of the Canadian Council for Animal Care *Guide to the Care and Use of Experimental Animals*, vol. 1 (1980) and vol. 2 (1984).

References

Axelsson, M., Davidson, B., Forster, M. and Nilsson, S. (1994). Blood pressure control in the antartic fish *Pagothenia borchgrevinki*. *J. Exp. Biol*. **190**, 265–279.

Bernier, N. J. and Perry, S. F. (1997). Angiotensins stimulate catecholamine release from the chromaffin tissue of the rainbow trout. *Am. J. Physiol.* **273**, R49–R57.

Brown, J. A., Paley, R. K., Amer, S. and Aves, S. J. (1995). Evidence for an intrarenal renin–angiotensin system in the rainbow trout. *J. Endocrinol*. **147**, P79.

Butler, D. G., Oudit, G. Y. and Cadinouche, M. Z. A. (1995). Angiotensin I- and II- and norepinephrine-mediated pressor responses in an ancient holostean fish, the bowfin (*Amia calva*). *Gen. Comp. Endocrinol*. **98**, 289–302.

Carroll, R. G. and Opdyke, D. F. (1982). Evolution of angiotensin II-induced catecholamine release. *Am. J. Physiol.* **243**, R54–R69.

Conklin, D. J. and Olson, K. R. (1994a). Angiotensin II relaxation of rainbow trout vessels *in vitro*. *Am. J. Physiol.* **266**, R1856–R1860.

Conklin, D. J. and Olson, K. R. (1994b). Compliance and smooth muscle reactivity of rainbow trout (*Oncorhynchus mykiss*) vessels *in vitro*. *J. Comp. Physiol.* B **163**, 657–663.

Conlon, J. M., Yano, K. and Olson, K. R. (1996). Production of [Asn^1,Val^5]angiotensin II and [Asp^1,Val^5]angiotensin II in kallikrein-treated trout plasma (T60K). *Peptides* **17**, 527–530.

Epple, A. and Nibbio, B. (1985). Catecholaminotropic effects of catecholamines in a teleost fish, *Anguilla rostrata*. *J. Comp. Physiol*. B **155**, 285–290.

Farrell, A. P. and Jones, D. R. (1992). The heart. In *Fish Physiology*, vol. XIIA, *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1–88. New York: Academic Press.

Farrell, A. P., MacLeod, K. and Chancey, B. (1986). Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. *J. Exp. Biol.* **125**, 319–345.

Fritsche, R., Reid, S. G., Thomas, S. and Perry, S. F. (1993). Serotonin-mediated release of catecholamines in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **178**, 191–204.

Fuentes, J. and Eddy, F. B. (1998). Cardiovascular responses *in vivo* to angiotensin II and the peptide antagonist saralasin in rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol*. **201**, 267–272.

Gamperl, A. K., Pinder, A. W. and Boutilier, R. G. (1994a). Effect of coronary ablation and adrenergic stimulation on *in vivo* cardiac performance in trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **186**, 127–143.

Gamperl, A. K., Pinder, A. W., Grant, R. R. and Boutilier, R. G. (1994b). Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **193**, 209–232.

Gamperl, A. K., Vijayan, M. M. and Boutilier, R. G. (1994c). Experimental control of stress hormones levels in fishes: techniques and applications. *Rev. Fish Biol. Fisheries* **4**, 215–255.

Graham, M. S. and Farrell, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38–61.

Head, G. A. (1996). Role of AT_1 receptors in the central control of sympathetic vasomotor function. *Clin. Exp. Pharmacol. Physiol.* **Suppl. 3**, S93–S98.

Henderson, I. W., Hazon, N. and Hughes, K. (1985). Hormones, ionic regulation and kidney function in fishes. In *Physiological Adaptations of Marine Animals* (ed. M. S. Laverack), pp. 245–265. Cambridge: The Company of Biologists Ltd.

Kiceniuk, J. W. and Jones, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. Exp. Biol.* **69**, 247–260.

Kobayashi, H., Uemura, H. and Takei, Y. (1980). Physiological role of the renin–angiotensin system during dehydration. In *Avian Endocrinology* (ed. A. Epple and M. H. Stetson), pp. 319–330. New York: Academic Press.

Le Mevel, J.-C., Pamantung, T.-F., Mabin, D. and Vaudry, H. (1993). Effects of central and peripheral administration of arginine vasotocin and related neuropeptides on blood pressure and heart rate in the conscious trout. *Brain Res.* **610**, 82–89.

Le Mevel, J.-C., Pamantung, T.-F., Mabin, D. and Vaudry, H. (1994). Intracerebroventricular administration of angiotensin II increases heart rate in the conscious trout. *Brain Res.* **654**, 216–222.

Lipke, D. W., Meisheri, K. D. and Olson, K. R. (1990). Generation of vasoactive substances in trout and rat plasma by trypsin and kallikrein. *Am. J. Physiol*. **258**, R507–R514.

Nilsson, S. (1994). Evidence for adrenergic nervous control of blood pressure in teleost fish. *Physiol. Zool.* **67**, 1347–1359.

Nishimura, H. (1985). Evolution of the renin–angiotensin system and its role in control of cardiovascular functions in fishes. In *Evolutionary Biology of Primitive Fishes* (ed. R. E. Foreman, A. Gorbman, J. M. Dodd and R. Olsson), pp. 275–293. New York: Plenum Press.

Nishimura, H., Norton, V. M. and Bumpus, F. M. (1978). Lack of specific inhibition of angiotensin II in eels by angiotensin antagonists. *Am. J. Physiol.* **235**, H95–H103.

Okawara, Y., Karakida, T., Aihara, M., Yamaguchi, K. and Kobayashi, H. (1987). Involvement of angiotensin II in water intake in the Japanese eel, *Anguilla japonica*. *Zool. Sci*. **4**, 451–454.

Olson, K. R. (1992). Blood and extracellular fluid volume regulation: role of the renin–angiotensin, kallikrein–kinin system and atrial natriuretic peptides. In *Fish Physiology*, vol. XIIB, *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 136–254. New York: Academic Press.

Olson, K. R., Chavez, A., Conklin, D. J., Cousins, K. L., Farrell, A. P., Ferlic, R., Keen, J. E., Kne, T., Kowalski, K. A. and Veldman, T. (1994). Localization of angiotensin II responses in the trout cardiovascular system. *J. Exp. Biol.* **194**, 117–138.

Olson, K. R. and Duff, D. W. (1992). Cardiovascular and renal effects of eel and rat atrial natriuretic peptide in rainbow trout, *Salmo gairdneri*. *J. Comp. Physiol.* B **162**, 408–415.

Oudit, G. Y. and Butler, D. G. (1995). Angiotensin II and cardiovascular regulation in a freshwater teleost, *Anguilla rostrata* LeSueur. *Am. J. Physiol.* **269**, R726–R735.

Peach, M. J. (1977). Renin–angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.* **57**, 313–370.

Perry, S. F. and Vermette, M. G. (1987). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout *Salmo gairdneri*. I. Blood respiratory, acid–base and ionic states. *J. Exp. Biol*. **128**, 235–253.

Platzack, B., Axelsson, M. and Nilsson, S. (1993). The renin–angiotensin system in blood pressure control during exercise in the cod *Gadus morhua*. *J. Exp. Biol*. **180**, 253–262.

Randall, D. J. and Perry, S. F. (1992). Catecholamines. In *Fish Physiology*, vol. XIIB, *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 255–300. New York: Academic Press.

Randall, D. J. and Stevens, E. D. (1967). The role of adrenergic receptors in cardiovascular changes associated with exercise in salmon. *Comp. Biochem. Physiol.* **21**, 415–424.

Reid, I. A. (1992). Interactions between ANG II, sympathetic nervous system and baroreceptor reflexes in regulation of blood pressure. *Am. J. Physiol.* **262**, E763–E778.

Reid, S. G., Bernier, N. J. and Perry, S. F. (1998). The adrenergic stress response in fish: control of catecholamine storage and release. *Comp. Biochem. Physiol*. (in press).

Reid, S. G. and Perry, S. F. (1994). Storage and differential release of catecholamines in rainbow trout (*Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*). *Physiol. Zool.* **67**, 216–237.

Reid, S. G., Vijayan, M. M. and Perry, S. F. (1996). Modulation of catecholamine storage and release by the pituitary–interrenal axis in the rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol.* B **165**, 665–676.

Saxena, P. R. (1992). Interaction between the renin–angiotensin–aldosterone and sympathetic nervous systems. *J. Cardiovasc. Pharmacol.* **19**, S80–S88.

Smith, D. G. (1978). Neural regulation of blood pressure in rainbow trout (*Salmo gairdneri*). *Can. J. Zool*. **56**, 1678–1683.

Soivio, A., Nynolm, K. and Westman, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. Exp. Biol.* **62**, 207–217.

Takei, Y., Okubo, J. and Yamaguchi, K. (1988). Effects of cellular dehydration on drinking and plasma angiotensin II level in eel, *Anguilla japonica. Zool. Sci*. **5**, 43–51.

Tierney, M. L., Cramb, G. and Hazon, N. (1995a). Stimulation of the renin–angiotensin system and drinking by papaverine in the seawater eel, *Anguilla anguilla*. *J. Fish Biol*. **46**, 721–724.

Tierney, M. L., Luke, G., Cramb, G. and Hazon, N. (1995b). The role of the renin–angiotensin system in the control of blood pressure and drinking in the European eel, *Anguilla anguilla*. *Gen. Comp. Endocrinol*. **100**, 39–48.

Wilson, J. X. (1984). Coevolution of the renin–angiotensin system and the nervous control of blood circulation. *Can. J. Zool*. **62**, 137–147.

Wolf, K. (1963). Physiological salines for fresh-water teleosts. *Progve Fish Cult.* **25**, 135–140.

Wood, C. M. (1976). Pharmacological properties of the adrenergic receptors regulating systemic vascular resistance in the rainbow trout. *J. Comp. Physiol.* B **107**, 211–228.

Wood, C. M. and Shelton, G. (1975). Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. Exp. Biol.* **63**, 505–523.

Wood, C. M. and Shelton, G. (1980). Cardiovascular dynamics and adrenergic responses of the rainbow trout *in vivo*. *J. Exp. Biol*. **87**, 247–270.

Xu, H. Y. and Olson, K. R. (1993a). Significance of circulating catecholamines in regulation of trout splanchnic vascular resistance. *J. Exp. Zool*. **267**, 92–96.

Xu, H. Y. and Olson, K. R. (1993b). Evidence for multiple adrenoreceptor sites in rainbow trout vasculature. *J. Comp. Physiol.* B **163**, 470–476.

Zhang, Y., Jenkinson, E. and Olson, K. R. (1995). Vascular compliance and mean circulatory filling pressure in trout: effects of ACE inhibition. *Am. J. Physiol*. **268**, H1814–H1820.

Zhang, Y., Weaver, L., Ibeawuchi, A. and Olson, K. R. (1998). Catecholaminergic regulation of venous function in the rainbow trout. *Am. J. Physiol.* **274**, R1195–R1202.