The Journal of Experimental Biology 202, 1677–1690 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB1973

BLOOD PRESSURE REGULATION DURING HYPOTENSION IN TWO TELEOST SPECIES: DIFFERENTIAL INVOLVEMENT OF THE RENIN–ANGIOTENSIN AND ADRENERGIC SYSTEMS

NICHOLAS J. BERNIER, JOHN E. McKENDRY 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 17 March; published on WWW 20 May 1999

Summary

The stimulatory effects of angiotensin II (Ang II) on catecholamine release and the contributions of the renin–angiotensin system, humoral catecholamines and adrenergic nerves to blood pressure regulation were investigated in rainbow trout (*Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*). In trout, bolus injections of homologous [Asn^1,Val^5]-Ang II (100 or 500 pmol kg^{-1}) increased catecholamine secretion rates and plasma catecholamine concentrations from *in situ* posterior cardinal vein preparations and chronically cannulated fish, respectively. In contrast, *in situ* or *in vivo* injections of similar doses of Ang II in eel did not affect catecholamine release. α-Adrenoceptor blockade (prazosin; 1 mg kg^{-1}) reduced the pressor effect of exogenous Ang II (500 pmol kg^{-1}) in both species. In eel, intravenous injection of the smooth muscle relaxant papaverine (10 mg kg^{-1}) elicited a rapid decrease in dorsal aortic pressure (P_{DA}; 58 %) followed by a gradual recovery back to the baseline value 85 min after the treatment. In trout, papaverine elicited a similar decrease in blood pressure (62 %); however, P_{DA} recovered fully 20 min after treatment. Blockade of either α-adrenoceptors with prazosin or adrenergic nerves with bretylium (10 mg kg^{-1}) prior to papaverine treatment did not alter P_{DA} recovery in eel. In trout, α-adrenoceptor and adrenergic nerve blockade prior to the papaverine treatment prevented and attenuated P_{DA} recovery, respectively. In both species, papaverine treatment elicited significant increases in plasma catecholamine and Ang II concentrations. However, the increases in plasma catecholamine concentrations were markedly greater in trout than in eel. Similarly, the papaverine-elicited increase in plasma Ang II levels occurred earlier and was greater in trout than in eel. Thus, while Ang II stimulates humoral catecholamine release in trout, there is no evidence for a similar interaction in eel. Moreover, during hypotensive stress, although the renin–angiotensin system is recruited in both species, an essential involvement of adrenergic nerves and humoral catecholamines in the restoration of blood pressure is only apparent in trout.

Key words: fish, teleost, angiotensin II, catecholamine, adrenergic nerve, American eel, *Anguilla rostrata*, rainbow trout, *Oncorhynchus mykiss*, cardiovascular control.

Introduction

The control of catecholamine release from the chromaffin tissue of fish, as in mammals (Livett and Marley, 1993), may be achieved through a variety of cholinergic and non-cholinergic pathways (for a review, see Reid et al., 1998). In addition to the cholinergic control provided by the preganglionic fibres that innervate the chromaffin tissue in the head kidney (Nilsson, 1976; Nilsson et al., 1976), various hormones are known to stimulate catecholamine release in fish (Reid et al., 1998). One such humoral factor that has been implicated in the control of catecholamine release in various species is the biologically active product of the renin–angiotensin system, angiotensin II (Ang II; Opdyke et al., 1981; Carroll and Opdyke, 1982; Bernier and Perry, 1997; Bernier et al., 1999a).

In rainbow trout (*Oncorhynchus mykiss*), Ang II directly elicits catecholamine release from the chromaffin tissue *via* specific Ang II binding sites (Bernier and Perry, 1997). In other fish species, however, although Ang II may also stimulate catecholamine release, the evidence is indirect. While exogenous Ang II injections increase plasma catecholamine concentrations in some fish (Opdyke et al., 1981; Carroll and Opdyke, 1982; Bernier et al., 1999a), in others the implication that Ang II is involved in the control of catecholamine release is based on the observation that Ang-II-elicited pressor responses are partially inhibited by α-adrenoceptor blockade (Nishimura et al., 1978; Nishimura, 1985; Platzack et al., 1993; Butler et al., 1995; Oudit and Butler, 1995b). In the American eel (*Anguilla rostrata*), for example, although a portion of the cardiovascular effect of exogenous Ang II is indirectly mediated by catecholamines (Nishimura et al., 1978; Nishimura, 1985; Oudit and Butler, 1995b), it is not known whether the adrenergic effects are caused by humoral

catecholamines arising from chromaffin tissue, adrenergic nerves or both. Furthermore, marked differences in the cholinergic control of humoral catecholamine release between *O. mykiss* and *A. rostrata* (Reid and Perry, 1994, 1995; Al-Kharrat et al., 1997; Abele et al., 1998; Julio et al., 1998) suggest that there may also be similar inter-species differences in the Ang-II-mediated control of catecholamine release from the chromaffin tissue.

The physiological significance of an interaction between Ang II and catecholamine release stems from the importance of the renin–angiotensin system and the adrenergic system in the homeostatic regulation of blood pressure in teleosts (Olson, 1992; Nilsson, 1994; Bernier and Perry, 1999). In *O. mykiss* and *A. rostrata* both Ang II and catecholamines are potent vasopressors (Nishimura et al., 1978; Gamperl et al., 1994; Oudit and Butler, 1995a,b; Fuentes and Eddy, 1998; Bernier and Perry, 1999) and thereby key effectors of cardiovascular control. During hypotensive stress in trout, the renin–angiotensin system and humoral catecholamines are both recruited and play significant roles in the compensatory response to hypotension (Bernier et al., 1999b). However, the contribution of the renin–angiotensin system to blood pressure recovery in trout is largely indirect and relies on an Ang-II-mediated secretion of catecholamines (Bernier et al., 1999b). In the European eel (*Anguilla anguilla*), the renin–angiotensin system plays a significant role in the compensatory response to hypotension (Tierney et al., 1995). However, the contribution of the adrenergic system to blood pressure regulation during hypotension or the significance of a potential interaction between the renin–angiotensin system and the adrenergic system during such conditions have not been investigated in the eel. Lastly, the relative importance of neurally derived catecholamines to blood pressure regulation during hypotensive stress has not been characterized in either *O. mykiss* or *A. rostrata*.

Therefore, in the present study, using *O. mykiss* and *A. rostrata* in parallel treatments, we characterized the potential interactions between Ang II and catecholamines and their respective involvement in blood pressure regulation. Specifically, we investigated (1) the direct effects of homologous [Asn^1,Val^5]-Ang II on catecholamine release using *in situ* perfused posterior cardinal vein preparations, (2) whether exogenous Ang II injections can elicit an increase in plasma catecholamine levels, (3) the pressor effects of Ang II with and without α-adrenoceptor blockade, and (4) the relative contributions of endogenous Ang II, humoral catecholamines and neuronal catecholamines to the regulation of blood pressure during hypotensive stress. In addition to a further characterization of interactions between the renin–angiotensin and adrenergic systems in *O. mykiss* and *A. rostrata*, the goal of these experiments was to determine whether the previously described reliance of the renin–angiotensin system of the rainbow trout on the adrenergic system for blood pressure recovery during hypotensive stress (Bernier et al., 1999b) is also a general feature of cardiovascular control in other teleosts.

Materials and methods

Experimental animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) of either sex weighing between 394 and 730 g (569.4±10.2 g, mean ± S.E.M., experimental *N*=48) were obtained from Linwood Acres Trout Farm (Campellcroft, Ontario, Canada) and transported in oxygenated water to the fish-rearing facilities of the University of Ottawa. American eels *Anguilla rostrata* (LeSueur) of either sex weighing between 149 and 487 g (289.9±11.5 g, mean ± S.E.M., experimental *N*=48) were obtained from a commercial supplier (Lancaster, Ontario, Canada) and were transported on ice to the same destination. Both species were held indoors in large 1300 l fibreglass tanks supplied with flowing aerated and dechlorinated tap water. The fish were maintained at a temperature of 14 °C on a 12 h:12 h light:dark photoperiod. The trout were fed a commercial diet of Martin trout feed grower pellets, but the eels were not fed.

In situ *experiments*

To investigate the tissue-specific effects of Ang II on catecholamine release at the level of the chromaffin cells, an *in situ* posterior cardinal vein (PCV) perfusion preparation (Reid and Perry, 1994) was employed with the following modifications. Rainbow trout and American eels were killed by anaesthetic overdose using 2-phenoxy-ethanol (15 ml l^{-1}; Sigma Chemical Co., St Louis, MI, USA) and dissected ventral side up on ice. An incision was made from the vent to the pectoral girdle, and the internal organs were pushed aside to expose the head kidney and to cannulate the PCV and the bulbus arteriosus. An inflow cannula (PE 160; Clay Adams) was inserted into the PCV approximately two-thirds along the length of the kidney in the anterograde direction. The body cavity was filled with lint-free wipes, and a ligature was placed around the entire fish to secure the inflow cannula. An outflow cannula (PE 160) was inserted into the bulbus arteriosus and secured in place with a ligature around the walls of the bulbus. The head kidney of the cannulated fish was then perfused with aerated modified Cortland saline (Reid and Perry, 1995) with a final pH of 7.8 for the trout preparations and 8.0 for the eel preparations. Perfusion flow rates of 2–4 ml min^{-1} were achieved by siphon resulting from the positive pressure difference between the saline source, positioned approximately 30 cm above the preparation, and the outflow cannula.

Each preparation was perfused for 20 min before commencing an experiment. After this stabilization period, experiments were initiated by collecting a sample of outflow perfusate to assess basal catecholamine concentrations. A bolus injection (500 μl) of [Asn^1,Val^5]-Ang II (Sigma) was then administered to the preparation through a three-way valve in the inflow cannula over the course of 1 min, and five perfusate samples were collected 1, 2, 3, 4 and 5 min after the intervention. With a single dose per preparation, two doses of [Asn^1,Val^5]-Ang II, 100 and 500 pmol kg^{-1}, were assessed for their effects on catecholamine release in rainbow trout and American eels (*N*=6 for each dose). In each preparation, after

another stabilization period of 10 min, a second control perfusate sample was collected (15 min), and the preparation was given a bolus injection (500 μl) of the cholinergic agonist carbachol (10^{-5} mol kg^{-1}; Research Biochemicals International, Natick, MA, USA) over the course of 1 min. Immediately following the carbachol injection, perfusate samples were collected each minute for another 5 min (17, 18, 19, 20 and 21 min). Carbachol has previously been shown to elicit catecholamine release from *in situ* PCV perfusion preparations of both species (Reid and Perry, 1994) and was therefore used to confirm the suitability of each preparation for investigating the effects of Ang II on catecholamine release. Each perfusate sample was collected in pre-weighed vials while recording filling times, immediately placed in liquid nitrogen, and later stored at –80 °C until analysis of catecholamine concentrations. Pre- and post-sampling masses were subsequently divided by filling time to determine perfusate flow rates and catecholamine secretion rates.

In vivo *experiments*

Surgical procedures: series 1

Rainbow trout were anaesthetized in an oxygenated solution of ethyl *p*-aminobenzoate (40 mg l^{-1}; benzocaine; Sigma) 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 Ang II injections and repeated blood sampling, a lateral incision was made in the caudal peduncle below the lateral line to expose, separate from the surrounding tissue and cannulate (PE 50) the caudal vein in the anterograde direction. The incision was closed using a running stitch, and the protruding cannula was secured to the side of the trout using silk ligatures. American eels were immersed in an anaesthetic solution of ethyl *p*-amino-benzoate (1.6 g l^{-1}) for approximately 5 min and placed on a dissection tray without gill irrigation. An incision was made parallel to the lateral line in the caudal region to expose, clear away from the surrounding tissue and cannulate (PE 50) the caudal vein in the anterograde direction. The incision was closed using a running stitch, and the free end of the cannula was threaded through a small hole in the skin posterior to the incision and secured using silk ligatures. After surgery, both fish species were placed into individual flow-through opaque Perspex boxes and left to recover for 24 h prior to experimentation.

Surgical procedures: series 2 and 3

Rainbow trout and American eels were anaesthetized as described above prior to surgery. To measure dorsal aortic blood pressure (P_{DA}), both species were equipped with a dorsal aortic polyethylene cannula. In the trout, the dorsal aorta was cannulated (PE 50) according to the technique of Soivio et al. (1975). In the eel, a lateral incision was made immediately below and parallel to the lateral line approximately 3–4 cm behind the pectoral fin to expose the pneumogastric artery and to dissect it free from overlying connective tissue. A cannula (PE 10) was then occlusively inserted into the pneumogastric artery and advanced anteriorly into the dorsal aorta. In addition, to permit drug injection and/or repeated blood sampling, the caudal vein of both species was cannulated (PE 50) as outlined above for series 1. After surgery, the fish were placed into individual flow-through opaque Perspex boxes and left to recover for 24 h prior to experimentation. All cannulae were filled and flushed with heparinized (50 i.u. ml^{-1} sodium heparin; Sigma) teleost Cortland saline.

Experimental protocol

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

Experimental groups of six fish each were used to investigate the effects of bolus injections of saline or homologous [Asn^1,Val^5]-Ang II on the circulating plasma catecholamine concentrations of rainbow trout and American eels. Within a given trial, after removal of a blood sample (0.3 ml) to assess basal plasma catecholamine levels, fish were first given a bolus injection (0.3 ml) of saline over a period of 30 s, and the injection was followed by 0.25 ml of saline to clear the cannula. Four more blood samples (0.3 ml) were then taken 1, 2, 3 and 5 min after the beginning of the injection. Following a 4 h recovery period, the same injection and sampling regime was used to assess the effects of a bolus injection (0.3 ml) of Ang II (100 pmol kg^{-1}) on each fish. Finally, after a second 4 h recovery period, the effects of a 500 pmol kg^{-1} dose of Ang II were assessed. Throughout this experiment, each blood sample was replaced by an equivalent volume of saline containing 3 % bovine serum albumin (BSA). Samples were collected in a microcentrifuge tube containing 5 μl of 10 % Na_2-EDTA and centrifuged immediately at 10 000 ***g*** for 15 s. The separated plasma was quick-frozen in liquid nitrogen and stored at –80 °C for later analysis of catecholamine levels.

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

Both eels (N=6) and trout (N=6) were monitored during an initial period of 30–60 min to assess the stability of blood pressure. Upon stabilisation, control P_{DA} was recorded for 5 min, and the fish were given a bolus injection (0.3 ml) of 100 pmol kg^{-1} Ang II through the caudal vein over a period of 30 s. The vasopressor response to the injection was monitored continuously and, following recovery of P_{DA} to the control baseline level for a 1 h period, a 500 pmol kg^{-1} dose of Ang II was injected. Once the response to this second injection had been recorded and a further period of recovery had elapsed, each fish was slowly treated (over a 15 min period) with the α-adrenoceptor antagonist prazosin hydrochloride (1 mg kg^{-1}; RBI). Then, 60 min after prazosin treatment, using the same experimental protocol as above, the injections of 100 and 500 pmol kg^{-1} Ang II were repeated.

The effectiveness of the prazosin treatment in achieving α-adrenoceptor blockade was tested by comparing the pressor effects of a catecholamine cocktail given before and after the prazosin injection. The catecholamine cocktail (0.1 ml kg^{-1}) was prepared in a 0.9 % NaCl solution and consisted of

2.5×10^{-5} mol l^{-1} adrenaline and noradrenaline bitartrate (Sigma).

Series 3: contribution of the adrenergic system to blood pressure regulation following a hypotensive stress

Four separate experimental groups of rainbow trout (N=6) and American eels (N=6) were used to investigate the relative contributions of humorally and neurally derived catecholamines to blood pressure regulation during hypotensive stress. Once the stability of blood pressure had been established during an initial period of monitoring, control baseline P_{DA} was recorded for 10 min and the fish were administered one of the following intravenous injection: (a) 0.9 % NaCl (0.4 ml kg^{-1}) over a 10 min period (control treatment), (b) the smooth muscle relaxant papaverine hydrochloride (0.4 ml kg^{-1}; RBI) at 10 mg kg^{-1} over a 10 min period (papaverine treatment), (c) papaverine hydrochloride (0.4 ml kg^{-1}) at 10 mg kg^{-1} over a 10 min period after pre-treatment with the α-adrenoceptor antagonist prazosin hydrochloride (prazosin + papaverine treatment), and (d) papaverine hydrochloride (0.4 ml kg^{-1}) at 10 mg kg^{-1} over a 10 min period after pre-treatment with the adrenergic-neurone-blocking agent bretylium tosylate (Burroughs Wellcome Inc., Kirkland, PQ, Canada; bretylium + papaverine treatment). Each injection was followed by 0.3 ml of saline to clear the caudal vein cannula, and the effects of these treatments on P_{DA} were monitored continuously over the following 90 min. In each treatment, a blood sample (0.5 ml) was taken at the end of the initial 10 min control baseline period, and 10, 20, 40, 60 and 90 min into the treatment for subsequent analysis of plasma catecholamine and Ang II concentrations. Each blood sample was replaced by an equivalent volume of saline containing 3 % BSA, collected and treated as in series 1, and stored at –80 °C for later analysis. In the prazosin + papaverine treatment, α-adrenoceptor blockade was achieved by slowly injecting (over a 15 min period) a 1 mg kg^{-1} dose of prazosin hydrochloride 60 min prior to the papaverine injection. In the bretylium + papaverine treatment, a 10 mg kg^{-1} dose of bretylium tosylate (50 mg ml^{-1}) was diluted with saline to a final volume of 5 ml and slowly infused (50 µl min^{-1}) with a syringe pump over a 100 min period 24 h prior to the papaverine injection. The effectiveness of bretylium in preventing adrenergic transmission has previously been demonstrated in trout (Campbell and Ganon, 1976) and in the eel (Hipkins et al., 1986).

Analytical techniques

Perfusate and plasma adrenaline and noradrenaline concentrations were determined on alumina-extracted samples (200 µl) using high-pressure liquid chromatography (HPLC) with electrochemical detection. The HPLC consisted of a Varian Star 9012 solvent delivery system (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to a Princeton Applied Research 400 electrochemical detector (EG&G Instruments, Princeton, NJ, USA). The extracted samples were passed through an Ultratechsphere ODS-C_{18} 5 µm column (HPLC Technology Ltd, Macclesfield, UK) and the separated amines were integrated with the Star Chromatography software program (version 4.0, Varian). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylamine hydrobromide (DHBA) as an internal standard in all determinations.

Plasma Ang II concentrations were determined on extracted plasma samples and measured by radioimmunoassay (Bernier et al., 1999b). The antiserum used in this assay was initially raised against mammalian Ang II ([Asp^1,Ile^5]-Ang II; Yamaguchi, 1981). Relative to the standard curve obtained with [Asp^1,Ile^5]-Ang II (Sigma), the antiserum exhibited 62.7 % cross-reactivity with [Asn^1,Val^5]-Ang II and negligible (0.1–0.2 %) cross-reactivity with trout [Asn^1,Val^5,Asn^9]-Ang I and eel [Asn^1,Val^5,Gly^9]-Ang I (Bernier et al., 1999b). The dilution curve of immunoreactive Ang II in extracted *A. rostrata* and *O. mykiss* plasma was parallel to the standard curve of [Asn^1,Val^5]-Ang II. The intra- and inter-assay coefficients of variation were 5.2 % and 10.9 %, respectively.

Dorsal aortic blood pressure (P_{DA}) was measured using a UFI model 1050BP (UFI, Morro Bay, CA, USA) pressure transducer calibrated against a static water column. The P_{DA} signal was recorded with a data acquisition system (Biopac System Inc., Goleta, CA, USA) and collected at 0.04 s intervals using Acknowledge III (Biopac System Inc.) data acquisition software. Mean blood pressure was calculated as (systolic pressure + diastolic pressure)/2.

Statistical analyses

Data are presented as mean values ± one standard error of the mean (S.E.M.). In general, the statistical significance of the observed effects of an injection within a group was tested using a one-way repeated-measures analysis of variance (ANOVA). However, when the criteria for parametric analysis were violated (unequal variance or lack of normal distribution), a non-parametric one-way repeated-measures ANOVA on ranks was used to determine the observed effects of an injection on these variables. Dunnett's *post-hoc* multiple-comparison test was used to compare the pre-injection control data point with values at subsequent times. Following a given injection, differences between the control and maximum responses were analysed using a Student's paired *t*-test. Among various treatment, differences at a given time were determined using a one-way ANOVA followed by a Tukey test for multiple comparisons. The statistical significance of observed differences between the means of two treatments was determined by a Student's *t*-test. Throughout this paper, percentage changes were calculated as: [100×(mean response minus mean control value)/(mean control value)]. The significance level for all statistical tests was $P<0.05$.

Results

In situ *experiments*

Bolus injections of either 100 pmol kg^{-1} (Fig. 1A) or

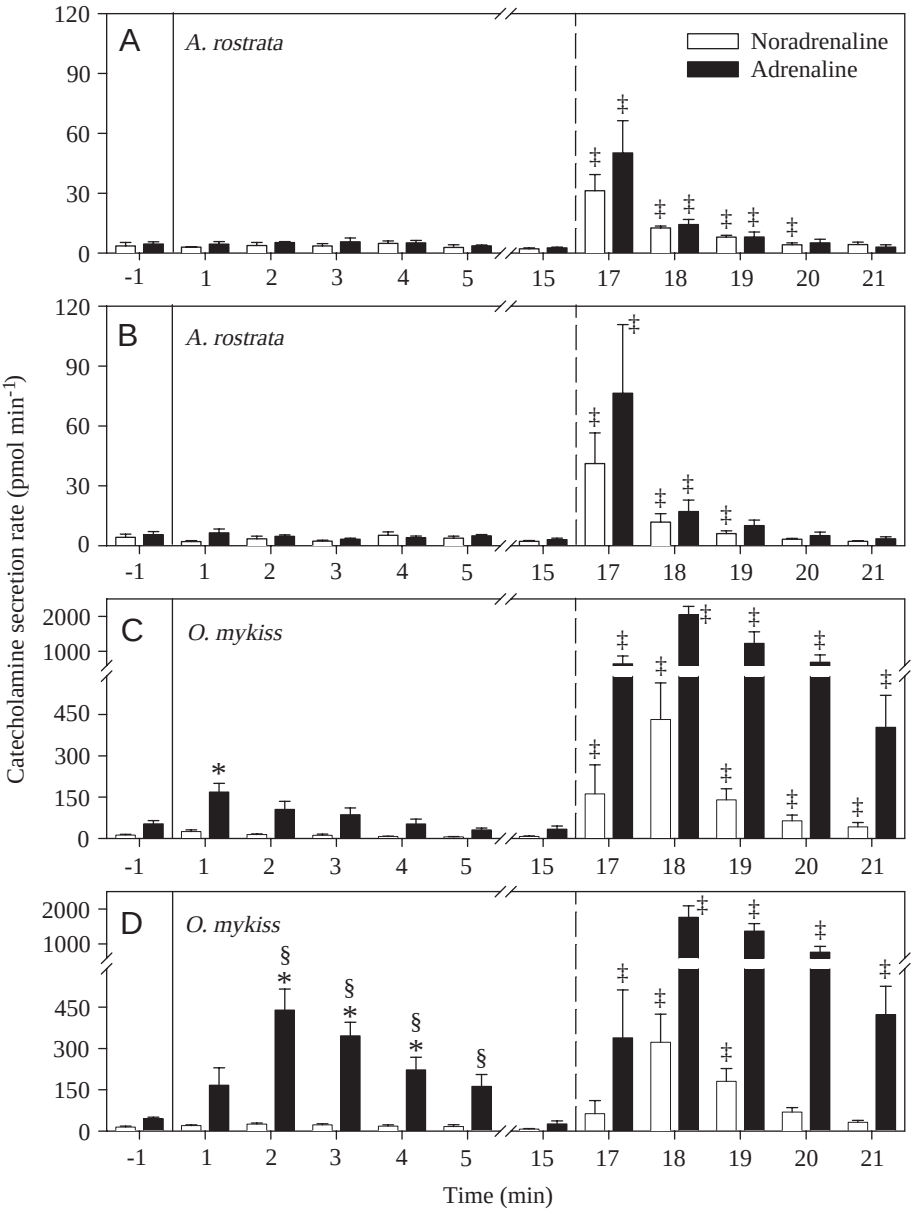

Fig. 1. Time course of changes in catecholamine secretion rates (pmol min^{-1}), noradrenaline (open columns) and adrenaline (filled columns), in *in situ* head kidney perfusion preparations of *Anguilla rostrata* (A,B) and *Oncorhynchus mykiss* (C,D) induced by either 100 pmol kg^{-1} (N=6; A,C) or 500 pmol kg^{-1} (N=6; B,D) angiotensin II followed by a bolus injection of carbachol (10^{-5} mol kg^{-1}; A–D). The solid and dashed lines indicate when the angiotensin II and carbachol injections, respectively, were given to the preparations. An asterisk denotes a significant difference from the −1 min value. A ‡ symbol denotes a significant difference from the 15 min value. A § symbol denotes a significant difference between angiotensin II doses for a given time and species (P<0.05). Values are means + 1 S.E.M. Note the different y-axis scales for A,B and C,D.

500 pmol kg^{-1} (Fig. 1B) Ang II did not affect the basal secretion rate of either noradrenaline or adrenaline from PCV preparations of *A. rostrata*. In contrast, the subsequent injection of carbachol (10^{-5} mol kg^{-1}) following either Ang II treatment elicited a temporary increase in the secretion rate of noradrenaline and adrenaline (Fig. 1A,B). While bolus injections of either 100 or 500 pmol kg^{-1} Ang II did not affect the secretion rate of noradrenaline from PCV preparations of *O. mykiss*, they elicited dose-dependent increases in the secretion rate of adrenaline (Fig. 1C,D). Subsequent injection of carbachol (10^{-5} mol kg^{-1}) in the trout PCV preparations elicited significant increases in the secretion rate of both catecholamines (Fig. 1C,D). Overall, the maximum carbachol-elicited increase in the secretion rates of noradrenaline and adrenaline from PCV preparations of *O. mykiss* (noradrenaline, 432±133 pmol min^{-1}; adrenaline, 2054±233 pmol min^{-1}) were markedly higher than those recorded from preparations of *A. rostrata* (noradrenaline, 41±15 pmol min^{-1}; adrenaline, 76±34 pmol min^{-1}).

In vivo experiments

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

In *A. rostrata*, bolus injections of either saline or 100 or 500 pmol kg^{-1} Ang II had no effect on the resting plasma adrenaline and noradrenaline concentrations (Fig. 2A,C). Similarly, injection of saline alone had no effect on the resting plasma catecholamine concentrations of *O. mykiss* (Fig. 2B,D). In contrast, bolus injections of 100 pmol kg^{-1}Ang II in *O. mykiss* elicited a significant increase in plasma adrenaline concentration, and the 500 pmol kg^{-1} dose of Ang II elicited a significant increase in the circulating concentrations of both catecholamines (Fig. 2B,D). In *O.*

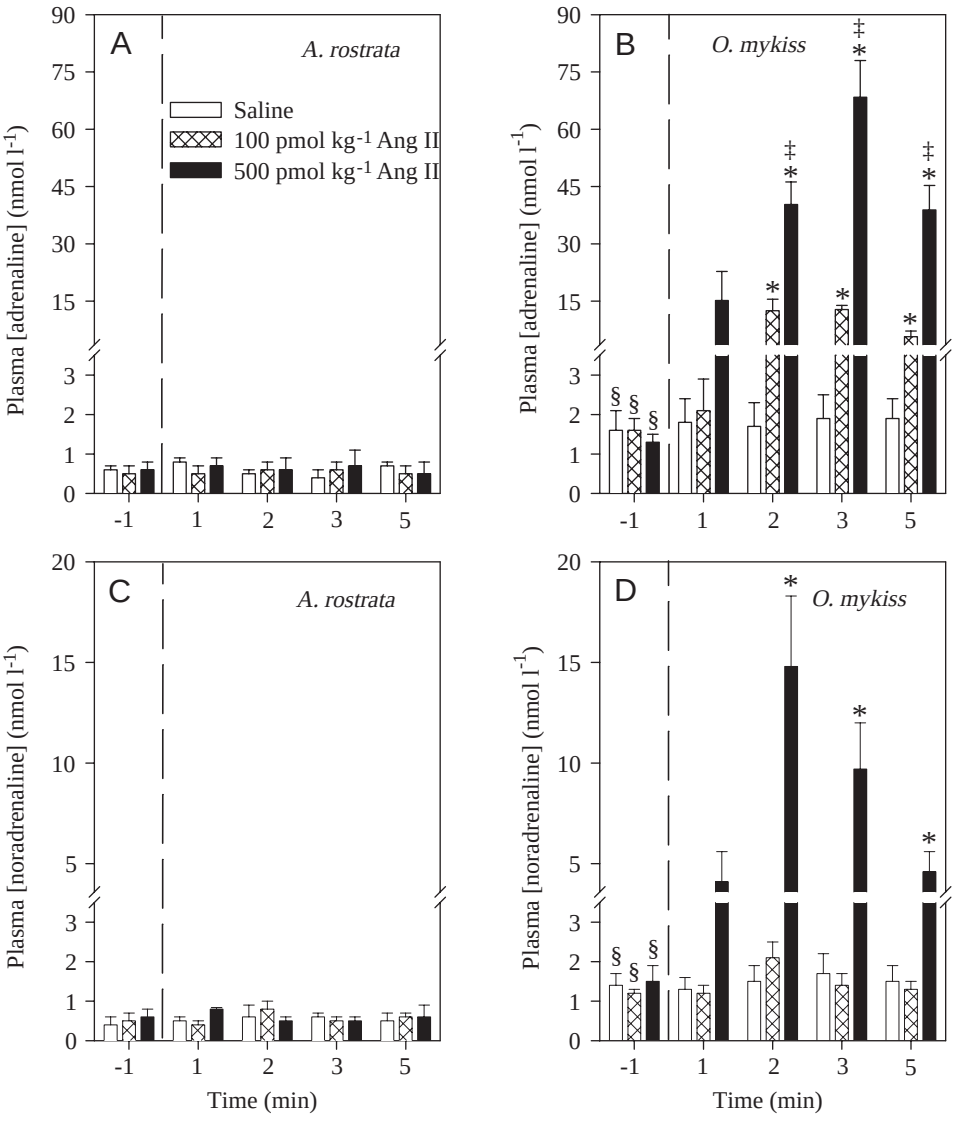

Fig. 2. Time course of changes in plasma adrenaline (A,B) and plasma noradrenaline (C,D) concentrations (nmol l^{-1}) in *Anguilla rostrata* (A,C) and *Oncorhynchus mykiss* (B,D) induced by saline (N=6; open columns) or 100 pmol kg^{-1} (N=6; cross-hatched columns) or 500 pmol kg^{-1} (N=6; filled columns) angiotensin II (Ang II). The dashed lines indicate when the injections of either saline or angiotensin II were administered. For a given treatment, an asterisk denotes a significant difference from the −1 min control value. A ‡ symbol denotes a significant difference between angiotensin II doses at a given time. A § symbol denotes a significant difference from the control value for *A. rostrata* for a given treatment (P<0.05). Values are means + 1 S.E.M.

mykiss, the Ang-II-elicited increase in plasma adrenaline concentration was dose-dependent. Overall, the resting plasma catecholamine concentrations of *A. rostrata* were significantly lower than those of *O. mykiss*.

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

Although α-adrenoceptor blockade did not significantly change the resting P_{DA} of the American eels, prazosin treatment significantly lowered resting P_{DA} in the trout (Table 1). In both species, prazosin treatment was effective in blocking α-adrenoceptors. In response to a bolus injection of catecholamines, the increase in P_{DA} was reduced by 83 % in *A. rostrata* and by 85 % in *O. mykiss* (Table 1).

Prior to prazosin treatment, bolus injections of Ang II resulted in dose-dependent increases in mean P_{DA} in both *A. rostrata* and *O. mykiss* (Fig. 3A–D). In *A. rostrata*, the Ang-II-elicited increase in P_{DA} peaked approximately 4–5 min after injection of the vasoactive hormone and remained elevated for 12.5 min (100 pmol kg^{-1} Ang II) to 20 min (500 pmol kg^{-1} Ang II) (Fig. 3A,B). In contrast, peak P_{DA} occurred approximately 2–2.5 min after the injection of Ang II in *O. mykiss*, and P_{DA} remained elevated for only 5 min before returning to resting levels (Fig. 3C,D). For a given Ang II dose, the increase in P_{DA} was significantly greater in the trout than in the eel. In comparison with the control condition prior to α-adrenoceptor blockade, the Ang-II-elicited increases in P_{DA} after prazosin treatment in *A. rostrata* were significantly reduced by 32 % following the 100 pmol kg^{-1} Ang II dose and by 31 % following the 500 pmol kg^{-1} Ang II dose. In *O. mykiss*, while prazosin treatment did not significantly affect the increase in P_{DA} following the 100 pmol kg^{-1} Ang II dose, it reduced the Ang-II-elicited increase in P_{DA} by 44 % in the 500 pmol kg^{-1} treatment.

Series 3: contribution of the adrenergic system to blood pressure regulation following a hypotensive stress

In the control treatments, intravenous injection of saline and repeated blood sampling had no effect on resting P_{DA} (Fig. 4),

Table 1. *The effects of a bolus injection of catecholamines on the dorsal aortic blood pressure of intact control and prazosin-treated* Anguilla rostrata *and* Oncorhynchus mykiss

		P_{DA} (kPa)	
Treatment	*N*	*A. rostrata*	*O. mykiss*
Control	6		
Basal value		2.91±0.13	3.50±0.17
Post-injection value		4.17±0.15*	5.26±0.15*
Change from basal value		1.26±0.07	1.76±0.19¶
Prazosin-treated	6		
Basal value		2.58±0.17	2.75±0.26‡
Post-injection value		2.79±0.19*	3.02±0.28*
Change from basal value		0.22±0.07§	0.26±0.05§

The catecholamine injection consisted of a 0.1 ml kg^{-1} dose of 2.5×10^{-5} mol l^{-1} noradrenaline bitartrate and adrenaline bitartrate diluted to a final volume of 100 μl with saline.

Values are means ± 1 S.E.M.

*Significantly different from basal value for a given treatment; ‡significantly different from basal value for the control treatment; §significantly different from the change from the basal value of the control treatment; ¶significantly different from the change from the basal value for *A. rostrata* for a given treatment (P<0.05).

plasma adrenaline, noradrenaline (Fig. 5) and Ang II (Fig. 6) concentrations in either *A. rostrata* or *O. mykiss*.

In the American eel, the smooth muscle relaxant papaverine elicited a rapid decrease in P_{DA} (from 2.82±0.10 to 1.20±0.07 kPa after 22.5 min) followed by a gradual recovery back to baseline conditions 85 min after the treatment (Fig. 4A). In rainbow trout, although papaverine also elicited a rapid decrease in blood pressure (from 3.16±0.29 to 1.20±0.41 kPa after 12 min), P_{DA} had recovered fully after 20 min and was then significantly increased above baseline conditions for a further 20 min before gradually returning to baseline values (Fig. 4B). Papaverine treatment elicited increases in plasma adrenaline concentration in both species (Fig. 5A,C). However, the increase in plasma adrenaline concentration observed in the eel (from 0.9±0.2 to 9.5±1.9 nmol l^{-1} after 20 min; Fig. 5A) in response to the hypotensive stress was markedly smaller than the increase recorded in the trout (from 1.6±0.6 to 546.5±138.6 nmol l^{-1} after 20 min; Fig. 5C). In addition, while papaverine treatment had no effect on the circulating concentrations of plasma noradrenaline in *A. rostrata* (Fig. 5B), this treatment was accompanied by a significant increase in plasma noradrenaline concentration in *O. mykiss* (from 1.7±0.7 to 101.2±27.5 nmol l^{-1} after 10 min; Fig. 5D). Administering papaverine also induced increases in plasma Ang II concentrations in both species (Fig. 6). In the trout, the increase in plasma Ang II concentration occurred earlier and was greater (from 126±29 to 4279±1207 pmol l^{-1} after 20 min; Fig. 6B) than the increase observed in the eel (from 82±16 to 1040±289 pmol l^{-1} after 60 min; Fig. 6A).

Intravenous injection of papaverine in prazosin-treated eels elicited a rapid decrease in P_{DA} (from 2.81±0.11 to 1.26±0.05 kPa after 11.5 min) followed by a gradual recovery back to baseline conditions 75 min after the treatment (Fig. 4C). In prazosin-treated rainbow trout, papaverine injection elicited a rapid decrease in blood pressure (from 3.02±0.20 to 0.95±0.12 kPa after 11 min; Fig. 4D) and, although gradual, an incomplete recovery in P_{DA} over the following 90 min (Fig. 4D). In both fish species, the combined prazosin + papaverine treatment was also associated with increases in plasma adrenaline, noradrenaline and Ang II concentrations (Figs 5, 6). Overall, as observed in the papaverine treatment described above, the increases in the concentrations of circulating plasma catecholamines and Ang II were much more pronounced in *O. mykiss* than in *A. rostrata*.

Over a 24 h period, bretylium treatment alone did not significantly change the resting P_{DA} of *A. rostrata* (Fig. 4E), but this treatment significantly lowered the resting blood pressure of *O. mykiss* (from 3.10±0.17 to 2.54±0.17 kPa; Fig. 4F). In addition, relative to the basal values of the control treatments, bretylium-treated eel and trout had slightly higher plasma adrenaline and noradrenaline concentrations under resting conditions (Fig. 5).

Papaverine treatment in bretylium-treated eels produced a rapid decrease in P_{DA} (from 2.87±0.15 to 1.05±0.12 kPa after 20.5 min) followed by a gradual recovery back to baseline conditions 85 min after the treatment (Fig. 4E). In bretylium-treated trout, administering papaverine also induced a rapid decrease in blood pressure (from 2.54±0.16 to 1.01±0.20 kPa after 11 min). However, in trout, P_{DA} had recovered fully after 25 min and was significantly increased transiently above baseline values (60 min) before returning to basal values (Fig. 4F). As with the other papaverine treatments, the combined bretylium + papaverine treatment was characterized by significant increases in plasma catecholamine and Ang II concentrations, and these were more pronounced in the rainbow trout than in the American eel (Figs 5, 6).

Overall, in *A. rostrata*, the three papaverine treatments resulted in similar decreases in P_{DA} and were characterized by similar recovery profiles back to baseline values (Fig. 4A,C,E). In *O. mykiss*, the different papaverine treatments also elicited hypotensions of equivalent magnitude, but the rate and extent to which blood pressure returned to control values varied significantly according to the different treatments (Fig. 4B,D,F). Also, in both species and with very few exceptions (at 60 and 90 min; Fig. 5B), at any given time during recovery from the hypotensive stress, there was no significant difference between the three papaverine treatments in the plasma adrenaline and noradrenaline concentrations (Fig. 5). In *A. rostrata*, with the exception of a single sampling time (at 10 min; Fig. 6A), there were no significant differences in the plasma Ang II concentrations between the three papaverine treatments. Finally, in the three groups of rainbow trout treated with the smooth muscle relaxant, although the increases in plasma Ang II were similar during the first 20 min after the papaverine injection, Ang II concentrations were subsequently highest in the prazosin + papaverine treatment,

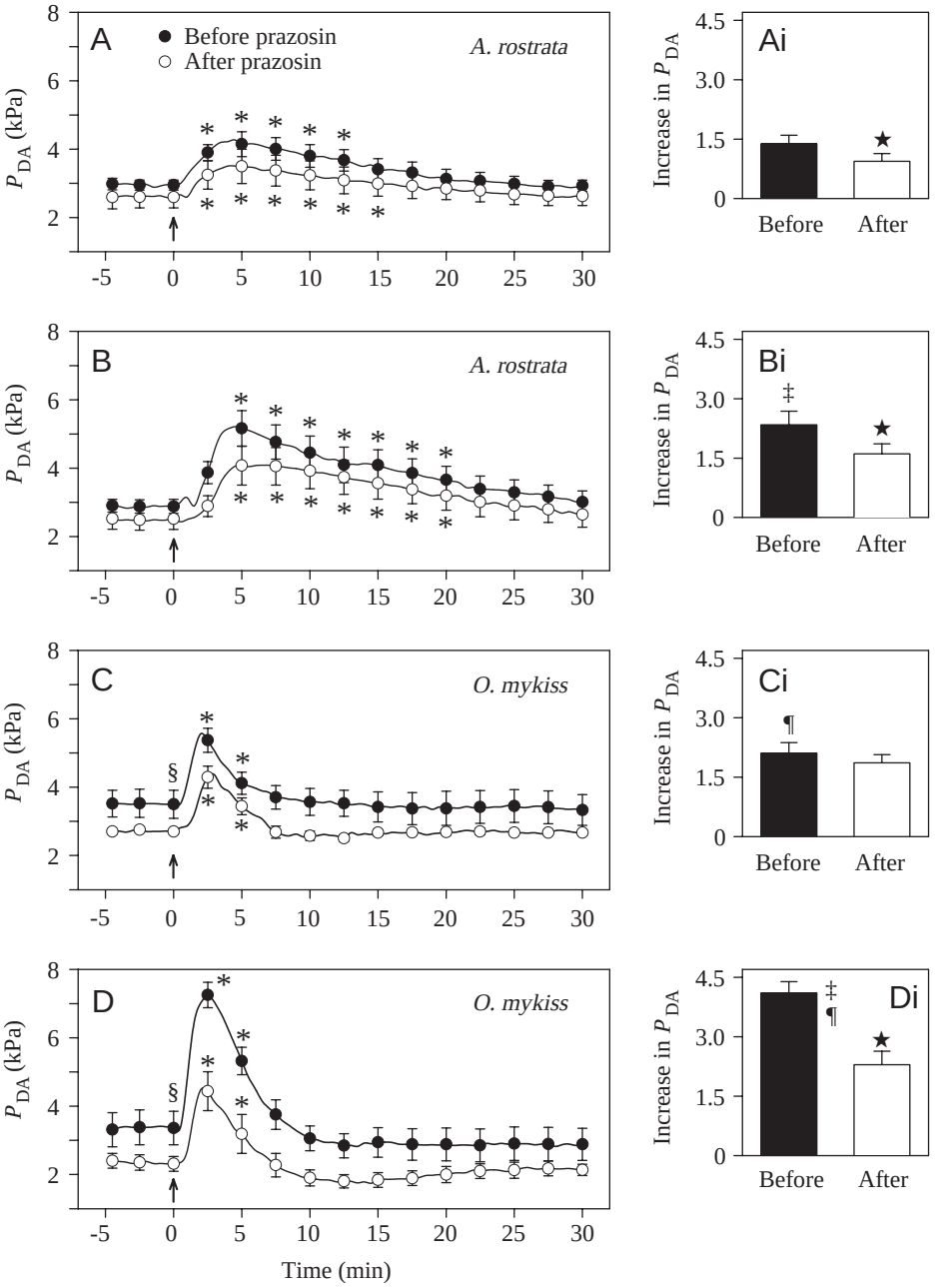

Fig. 3. Time course of changes in mean dorsal aortic pressure (P_{DA}, kPa) in *Anguilla rostrata* (A,B) and *Oncorhynchus mykiss* (C,D) induced by either 100 pmol kg^{-1} (N=6; A,C) or 500 pmol kg^{-1} (N=6; B,D) angiotensin II before (filled circles) and after (open circles) treatment with the α-adrenoceptor antagonist prazosin. The arrow at 0 min indicates when the bolus injection of angiotensin II was administered. Insets Ai–Di give the angiotensin-II-elicited maximum mean increases in P_{DA} before (filled columns) and after (open columns) prazosin treatment. An asterisk denotes a significant difference from the control 0 min P_{DA} value for a given treatment. A § symbol denotes a significant difference between the control 0 min P_{DA} value for the 'after prazosin' treatment and the 'before prazosin' treatment. The ‡ and ¶ symbols denote significant differences in the increase in P_{DA} between the two Ang II doses for a given species and between the two fish species for a given dose, respectively. A star symbol denotes a significant difference from the increase in P_{DA} before prazosin treatment (P<0.05). Values are means + 1 S.E.M.

intermediate in the bretylium + papaverine treatment, and lowest in the papaverine treatment (Fig. 6B).

Discussion

The present study demonstrates that homologous [Asn^1,Val^5]-Ang II does not influence the release of catecholamines from the chromaffin tissue of *A. rostrata* and confirms that Ang II is a potent secretagogue of humoral catecholamines in *O. mykiss*. Moreover, while a portion of the pressor responses elicited by exogenous Ang II injections can be mediated by the adrenergic system in both *O. mykiss* and *A. rostrata*, interactions between endogenous Ang II and the adrenergic system during hypotensive stress are only apparent in *O. mykiss*. Although both circulating catecholamines and adrenergic nerves play important roles in the compensatory response to hypotension in *O. mykiss*, adrenergic nerves and the α-adrenoceptor-mediated effects of humoral catecholamines are not essential contributors to the regulation of blood pressure in hypotensive *A. rostrata*.

In situ perfused PCV preparations have previously been used to investigate the control of catecholamine release from the chromaffin tissue of *A. rostrata* and *O. mykiss* (Reid and Perry, 1994, 1995; Reid et al., 1996; Al-Kharrat et al., 1997; Bernier and Perry, 1997; Abele et al., 1998; McKendry et al., 1999). In both species, the primary source of circulating catecholamines is the chromaffin cells located in the walls of the anterior region of the PCV and in the surrounding head

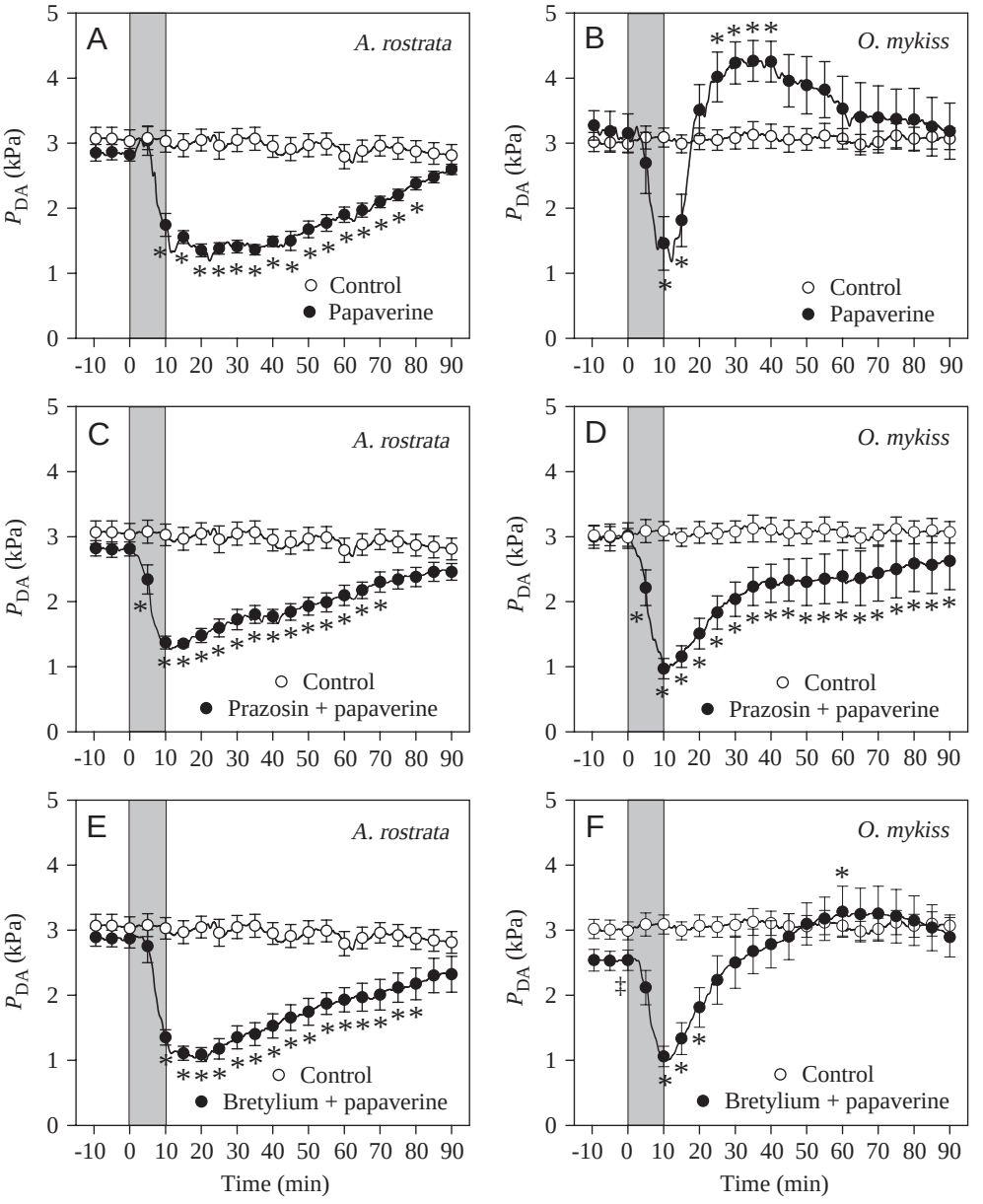

Fig. 4. Time course of changes in mean dorsal aortic pressure (P_{DA}, kPa) in *Anguilla rostrata* (A,C,E) and *Oncorhynchus mykiss* (B,D,F) induced by (A–F) saline (N=6; open circles), the smooth muscle relaxant papaverine (10 mg kg^{-1}; N=6; filled circles) (A,B), papaverine (10 mg kg^{-1}) after pre-treatment with the α-adrenoceptor antagonist prazosin (N=6; filled circles) (C,D) or papaverine (10 mg kg^{-1}) after pre-treatment with the adrenergic-neurone-blocking agent bretylium (N=6; filled circles) (E,F). The time during which papaverine was injected is shown by a shaded box in A–F. For a given treatment, an asterisk denotes a significant difference from the 0 min control value. A ‡ symbol denotes a significant difference from the control treatment for a given species and time (P<0.05). Values are means ± 1 S.E.M.

kidney tissue (Hathaway and Epple, 1989; Reid and Perry, 1994). Although Ang II did not affect the secretion rate of either adrenaline or noradrenaline in *A. rostrata*, the suitability of the eel preparations for investigating the effects of potential secretagogues was confirmed by the stimulatory effects of carbachol on catecholamine secretion. Relative to the catecholamine secretion rates of *O. mykiss*, the significantly lower carbachol-elicited catecholamine secretion rates in *A. rostrata* are characteristic of the previously described differential ability of these two species to respond to cholinergic stimulation (Reid and Perry, 1994; McKendry et al., 1999). In *O. mykiss*, the dose-dependent stimulatory effect of Ang II on adrenaline secretion confirms the results of previous *in situ* experiments (Bernier and Perry, 1997). However, and in contrast to previous results (Bernier and Perry, 1997), Ang II did not affect the secretion rate of noradrenaline in the *in situ* PCV preparations of rainbow trout. Because Ang II has previously been shown preferentially to stimulate adrenaline secretion over noradrenaline secretion in rainbow trout (Bernier and Perry, 1997; Bernier et al., 1999b), the above results may simply reflect intrinsic differences in the sensitivity of the chromaffin tissue to Ang II.

Exogenous injections of homologous Ang II were also without any effect on the circulating concentrations of plasma catecholamines in chronically cannulated and free-swimming *A. rostrata*. In contrast, and as previously observed (Bernier and Perry, 1999), identical injections of Ang II in the trout were associated with a dose-dependent increase in plasma adrenaline concentration and with a much smaller increase in plasma noradrenaline concentration. Taken together, the results from the *in situ* and *in vivo* Ang II injections suggest that the chromaffin tissue of *A. rostrata*, unlike that of *O.*

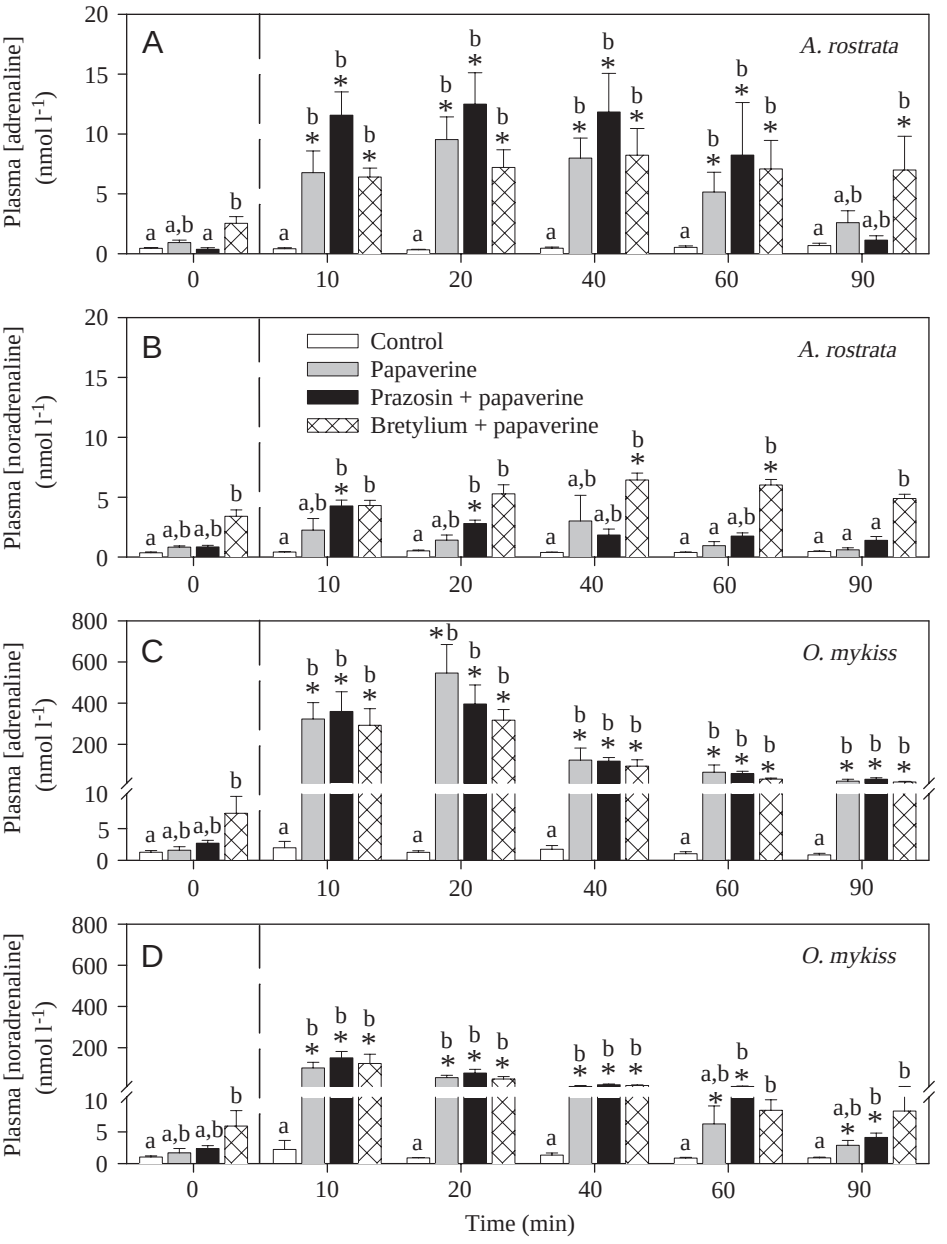

Fig. 5. Time course of changes in plasma adrenaline (A,C) and noradrenaline (B,D) concentrations (nmol l^{-1}) in *Anguilla rostrata* (A,B) and *Oncorhynchus mykiss* (C,D) induced by saline (N=6; open columns), the smooth muscle relaxant papaverine (10 mg kg^{-1}; N=6; grey column), papaverine (10 mg kg^{-1}) after pre-treatment with the α-adrenoceptor antagonist prazosin (N=6; black columns) or papaverine (10 mg kg^{-1}) after pre-treatment with the adrenergic-neurone-blocking agent bretylium (N=6; cross-hatched columns). The dashed lines indicate when the injections of either saline or papaverine were administered. For a given treatment, an asterisk denotes a significant difference from the 0 min control value. Treatments that do not share a common letter for a given time and variable are significantly different from each other (P<0.05). Values are means + S.E.M. Note the different y-axis scales for A,B and C,D.

mykiss, may be unresponsive to physiological concentrations of Ang II. Similar differences have been noted for mammals and involve species differences for the presence of Ang II receptors in the adrenal medulla (Livett and Marley, 1993).

Although exogenous injections of Ang II elicit a pressor response in both species, there are significant differences in the cardiovascular effects of Ang II between *A. rostrata* and *O. mykiss*. Relative to the response in *O. mykiss*, the smaller Ang-II-mediated vasopressor response in *A. rostrata* has a slower onset and is longer lasting. While rapid changes in systemic vascular resistance (R_S) and slower longer-lasting changes in cardiac output ($\dot{Q}$) contribute to the Ang-II-elicited vasopressor response in both species, changes in R_S are a more important contributor in *O. mykiss* (Bernier and Perry, 1999) than in *A. rostrata* (Oudit and Bulter, 1995b). Similarly, although a portion of the Ang-II-elicited vasopressor response is mediated indirectly *via* the adrenergic system in both *O. mykiss* and *A. rostrata* (the present study; Chan and Chow, 1976; Nishimura et al., 1978; Nishimura, 1985; Olson et al., 1994; Oudit and Butler, 1995b; Bernier and Perry, 1999), the nature of the interaction between Ang II and the adrenergic system differs between the two species. In *A. rostrata*, because Ang II does not stimulate humoral catecholamine release, the indirect vasopressor action of Ang II mediated by the adrenergic system must take place *via* an interaction with adrenergic nerves. In contrast, there is evidence that at least some of the cardiovascular effects of exogenous Ang II in trout can be attributed to increased levels of plasma adrenaline (Bernier and Perry, 1999). Moreover, whereas α-adrenoceptor blockade has no effect on the Ang-II-mediated increase in R_S in *A. rostrata* (Oudit and Butler, 1995b), the Ang-II-mediated increase in R_S is significantly reduced by a similar treatment

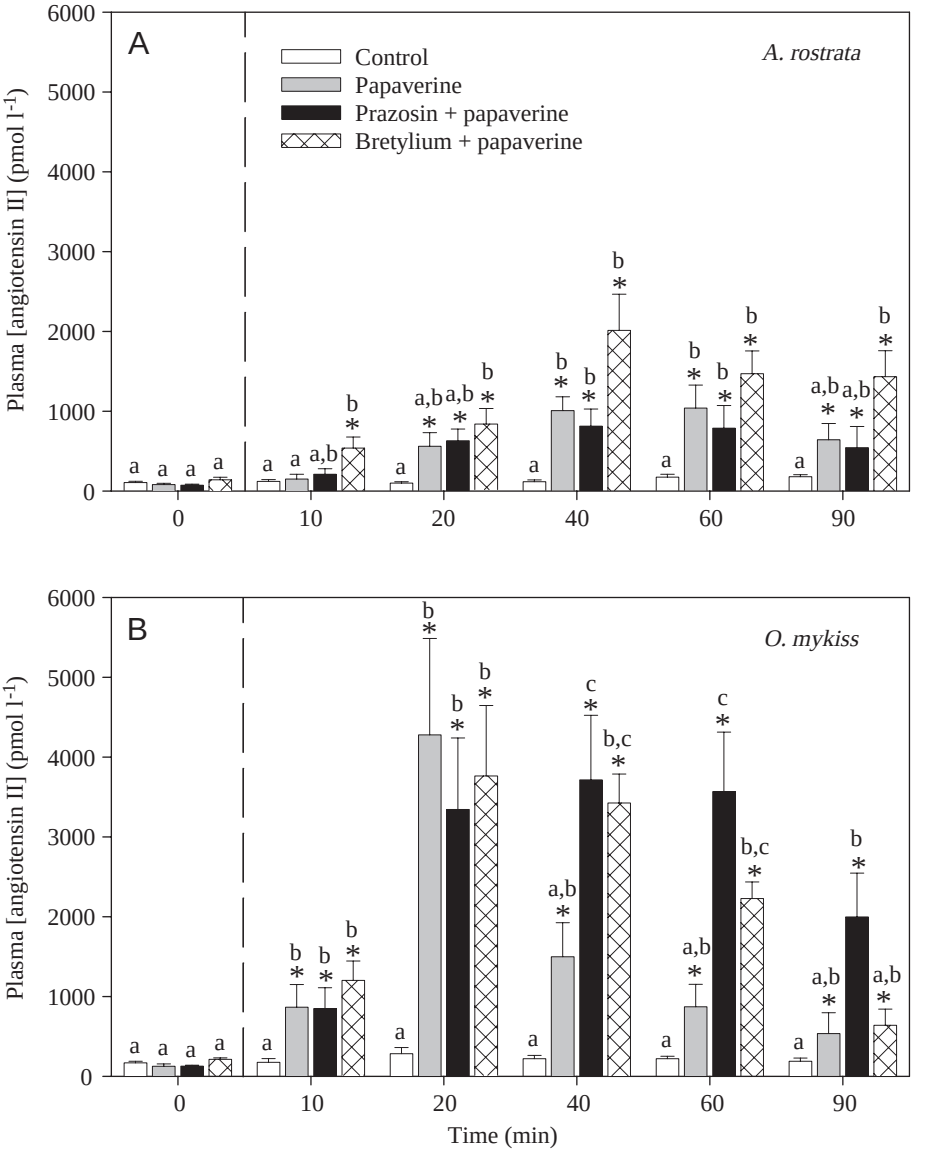

Fig. 6. Time course of changes in plasma angiotensin II concentrations (pmol l^{-1}) in *Anguilla rostrata* (A) and *Oncorhynchus mykiss* (B) induced by saline (*N*=6; open columns), the smooth muscle relaxant papaverine (10 mg kg^{-1}; *N*=6; grey columns), papaverine (10 mg kg^{-1}) after pre-treatment with the α-adrenoceptor antagonist prazosin (*N*=6; black columns) or papaverine (10 mg kg^{-1}) after pre-treatment with the adrenergic-neurone-blocking agent bretylium (*N*=6; cross-hatched columns). The dashed lines indicate when the injections of either saline or papaverine were administered. For a given treatment, an asterisk denotes a significant difference from the 0 min control value. Treatments that do not share a common letter for a given time are significantly different from each other (P<0.05). Values are means + 1 S.E.M.

in *O. mykiss* (Bernier and Perry, 1999). In fact, in *A. rostrata*, it appears that changes in $\dot{Q}$ are responsible for the indirect vasopressor action of Ang II mediated by the adrenergic system (Oudit and Butler, 1995b).

Administration of the smooth muscle relaxant papaverine elicited an acute reduction in blood pressure and, as shown by the increases in plasma Ang II levels, a recruitment of the renin–angiotensin system in both *A. rostrata* and *O. mykiss*. While these results are confirmatory (Tierney et al., 1995; Bernier et al., 1999b), the parallel design of this experiment revealed significant differences between eel and trout in blood pressure recovery from hypotensive stress. Following papaverine treatment, even though P_{DA} fell by approximately 60 % in both species, recovery of blood pressure in *O. mykiss* took less than one-quarter of the time required by *A. rostrata* and was characterized by a significant elevation of P_{DA} above resting values. Previous experiments have provided evidence for the importance of a recruitment of the renin–angiotensin system to blood pressure recovery in hypotensive teleosts (Perrott and Balment, 1990; Tierney et al., 1995; Bernier et al., 1999b). In *A. anguilla* (Tierney et al., 1995) and *O. mykiss* (Bernier et al., 1999b), preventing the formation of Ang II with an angiotensin-converting enzyme (ACE) inhibitor significantly hinders recovery from hypotensive stress. However, given a similar degree of hypotension, our results show that recruitment of the renin–angiotensin system in *O. mykiss* is faster and more pronounced than in *A. rostrata*. Under normotensive conditions, although blockade of the renin–angiotensin system with an ACE inhibitor lowers resting blood pressure in *O. mykiss* (Olson et al., 1997; Bernier et al., 1999b), a similar treatment has no effect on P_{DA} in freshwater *A. anguilla* (Tierney et al., 1995). Hence, while the renin–angiotensin system plays a tonic role in maintaining resting blood pressure in *O. mykiss*, this may not be the case in freshwater *A. rostrata*. Differences in the role that the renin–angiotensin system plays in regulating resting P_{DA}

between the two species presumably reflect on the basal activity of their renin–angiotensin system. Differences in the basal activity of the renin–angiotensin system may explain, at least in part, the differential recruitment of this system in hypotensive *A. rostrata* and *O. mykiss* and their marked differences in blood pressure recovery.

The hypotensive properties of papaverine, in addition to stimulating the renin–angiotensin system, resulted in an increase in the circulating concentrations of plasma catecholamines in both *O. mykiss* and *A. rostrata*. However, as with the recruitment of the renin–angiotensin system, there was a marked difference in the magnitude of the papaverine-elicited increase in plasma catecholamine levels between the two teleost species. In rainbow trout, since ACE blockade prior to papaverine treatment significantly attenuates the hypotension-associated increase in plasma catecholamine levels (Bernier et al., 1999b), Ang II is thought to play an important role in mediating the pronounced recruitment of humoral catecholamines during hypotensive stress. In contrast, because we found no evidence that exogenous Ang II can stimulate catecholamine release in *A. rostrata*, the renin–angiotensin system, an important mediator of catecholamine release in hypotensive trout, is unlikely to play a role in the American eel. Because bretylium treatment prior to papaverine injection did not attenuate the hypotension-associated increase in plasma catecholamine levels, it is also improbable that adrenergic nerves were the source of the relatively small increase in plasma catecholamine levels observed in the papaverine-treated eels. In *A. rostrata*, in response to hypotensive stress, while a reflexive neuronal stimulation of the chromaffin tissue *via* preganglionic cholinergic fibres may stimulate catecholamine release, a variety of other mechanisms may also contribute to the control of humoral catecholamine release (Reid et al., 1995; Abele et al., 1998). However, given that the fish from the prazosin + papaverine and papaverine treatments had similar catecholamine levels throughout the hypotensive stress, α-adrenoceptor mediation of catecholamine release (Abele et al., 1998) is unlikely to be a contributing mechanism in either the eel or the trout.

Although plasma catecholamine levels increased in both fish species following papaverine treatment, our results suggest that, while the α-adrenoceptor-mediated effects of humoral catecholamines contribute significantly to blood pressure recovery in *O. mykiss*, they do not play an essential role in the eel. In *A. rostrata*, relative to fish treated solely with papaverine, α-adrenoceptor blockade prior to papaverine treatment did not impede blood pressure recovery. Hence, since changes in $\dot{Q}$ are the primary contributor to the vasopressor response elicited by exogenous catecholamines in *A. rostrata* (Chan and Chow, 1976; Oudit and Butler, 1995a), our results suggest that the increases in plasma catecholamine levels in papaverine-treated eels did not enhance cardiac performance *via* α-adrenoceptor-mediated mechanisms; at least not to the extent that such α-adrenoceptor-mediated cardiac changes could not be compensated for by other cardiovascular adjustments in the α-adrenoceptor-treated eels. Meanwhile, although there is some controversy about whether the direct cardiac actions of catecholamines are mediated by α-adrenoceptors (Chan and Chow, 1976; Oudit and Butler, 1995a) or β-adrenoceptors (Yasuda et al., 1996) in eels, it is possible that the hypotension-elicited increases in plasma catecholamine levels had significant physiological actions *via* cardiac β-adrenoceptors (Yasuda et al., 1996). In contrast to the eel, blockade of α-adrenoceptors prior to papaverine treatment prevented P_{DA} recovery in *O. mykiss* (the present study; Bernier et al., 1999b). The observation that blockade of the adrenergic nerves with bretylium did not impede P_{DA} recovery to the same extent as α-adrenoceptor blockade suggests that humoral catecholamines contribute significantly to cardiovascular control during hypotensive stress in rainbow trout. This finding is corroborated by the previous observation that exogenous supplementation of plasma catecholamine concentrations prevents the chronic hypotensive effects of renin–angiotensin system blockade in papaverine-treated trout (Bernier et al., 1999b). Furthermore, plasma catecholamine levels of equivalent or smaller magnitude than those achieved in the papaverine-treated trout in the present study have previously been shown to contribute to cardiovascular control in *O. mykiss* by elevating P_{DA}, R_S and $\dot{Q}$ (Gamperl et al., 1994; Bernier and Perry, 1999).

The compensatory response to hypotension in *A. rostrata* and *O. mykiss* also involves a differential contribution from the adrenergic nerves. In *A. rostrata*, P_{DA} recovery following the hypotensive stress was identical between the bretylium + papaverine and the papaverine treatments. Hence, despite the observations that a portion of the vasopressor response to exogenous Ang II injections may be mediated by adrenergic nerves (the present study; Nishimura et al., 1978; Nishimura, 1985; Oudit and Butler, 1995b) and that the renin–angiotensin system is recruited following papaverine treatment (the present study; Tierney et al., 1995), there is no evidence that adrenergic nerves play an essential role in the compensatory response to hypotension in the American eel. In *O. mykiss*, treatment with bretylium prior to papaverine injection did not prevent full P_{DA} recovery as the α-adrenoceptor treatment did. However, it annulled the rapid overshoot in blood pressure that characterized recovery in the papaverine treatment. Therefore, in *O. mykiss*, adrenergic nerves appear to be primarily involved in the short-term response to blood pressure regulation that immediately follows the hypotensive insult. Whether a portion of the involvement of the adrenergic system in P_{DA} recovery results from the stimulatory effects of Ang II on adrenergic nerve endings (Reid, 1992) cannot be ascertained in the present study. However, because α-adrenoceptor blockade or bretylium treatment inhibit approximately 40 % of the vasoconstrictory effect of Ang II in a perfused dorsal aortic preparation of *O. mykiss* (Olson et al., 1994), it appears likely that endogenous Ang II mediates a portion of its cardiovascular effects *via* an enhancement of adrenergic neurotransmission. Finally, under normotensive conditions, as previously observed by Hipkins et al. (1986) in *A. australis*, bretylium treatment did not decrease resting blood pressure in *A. rostrata*.

In contrast, we corroborated the observation that chronic bretylium treatment significantly lowers resting P_{DA} in *O. mykiss* (Smith, 1978). Therefore, although the adrenergic system plays an important role in maintaining resting blood pressure in the trout (Wood and Shelton, 1975, 1980; Xu and Olson, 1993), there is no evidence for an adrenergic vasoconstrictor tone to the systemic vasculature of resting eel.

In summary, we have observed marked differences between *A. rostrata* and *O. mykiss* in their regulation of blood pressure during hypotensive stress. These species-specific responses to hypotension can be explained, at least in part, by differential involvement of the renin–angiotensin system, the adrenergic system and their interactions in the homeostatic regulation of blood pressure. In *O. mykiss*, the compensatory response to hypotension involved an acute recruitment of the renin–angiotensin system and a significant involvement of the adrenergic nerves and humoral catecholamines in blood pressure restoration. The contribution of humoral catecholamines to blood pressure regulation in the trout is mediated primarily by the stimulatory effect of Ang II on catecholamine release from the chromaffin tissue. In contrast, although the compensatory response to hypotension in *A. rostrata* also involves a recruitment of the renin–angiotensin system, Ang II does not elicit catecholamine release from the chromaffin tissue, and α-adrenoceptor-mediated catecholamine effects or adrenergic nerves do not play an essential role in blood pressure recovery.

It is difficult to extrapolate the results of the present study to other teleosts. Thus, it is unclear whether the individual and interactive contributions of the renin–angiotensin and adrenergic systems to cardiovascular homeostasis in other teleosts more closely resemble the pattern in *O. mykiss* or in *A. rostrata*. However, the present results do suggest that the capacity of a given fish species to compensate for a reduction in blood pressure is in part coupled to the involvement of its renin–angiotensin and adrenergic systems. Given the importance of prompt cardiovascular adjustments for meeting the metabolic needs of oxidative tissues and thereby maintaining homeostasis, the differential involvement of the renin–angiotensin and adrenergic systems in blood pressure regulation between *O. mykiss* and *A. rostrata* may reflect the different metabolic scopes of these two teleosts.

This study was supported by the NSERC of Canada operating and equipment grants to S.F.P. N.J.B. was the recipient of an Ontario Graduate Scholarship.

References

Abele, B., Hathaway, C. B., Nibbio, B. and Epple, A. (1998). Electrostimulation of catecholamine release in the eel: modulation by antagonists and autocrine agonists. *Gen. Comp. Endocr*. **109**, 366–374.

Al-Kharrat, H., Weiss, U., Tran, Q., Nibbio, B., Scholz, S. and Epple, A. (1997). Cholinergic control of catecholamine release in the eel. *Gen. Comp. Endocr*. **108**, 102–108.

Bernier, N. J., Gilmour, K. M., Takei, Y. and Perry, S. F. (1999a). Cardiovascular control *via* angiotensin II and circulating catecholamines in the spiny dogfish, *Squalus acanthias*. *J. Comp. Physiol.* B (in press).

Bernier, N. J., Kaiya, H., Takei, Y. and Perry, S. F. (1999b). Mediation of humoral catecholamine secretion by the renin–angiotensin system in hypotensive rainbow trout (*Oncorhynchus mykiss*). *J. Endocr*. **160**, 351–363.

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.

Bernier, N. J. and Perry, S. F. (1999). Cardiovascular effects of angiotensin-II-mediated adrenaline release in rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **202**, 55–66.

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. Endocr*. **98**, 289–302.

Campbell, G. and Gannon, B. J. (1976). The splanchnic nerve supply to the stomach of the trout, *Salmo trutta* and *S. gairdneri*. *Comp. Biochem. Physiol.* **55**C, 51–53.

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

Chan, D. K. O. and Chow, P. H. (1976). The effects of acetylcholine, biogenic amines and other vasoactive agents on the cardiovasculature functions of the eel *Anguilla japonica*. *J. Exp. Zool.* **196**, 13–26.

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. (1994). Effect of coronary ablation and adrenergic stimulation on *in vivo* cardiac performance in trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **186**, 127–143.

Hathaway, C. B. and Epple, A. (1989). The sources of plasma catecholamines in the American eel, *Anguilla rostrata*. *Gen. Comp. Endocr*. **74**, 418–430.

Hipkins, S. F., Smith, D. G. and Evans, B. K. (1986). Lack of adrenergic control of dorsal aortic blood pressure in the resting eel, *Anguilla australis*. *J. Exp. Zool*. **238**, 155–166.

Julio, A. E., Montpetit, C. J. and Perry, S. F. (1998). Does blood acid–base status modulate catecholamine secretion in the rainbow trout (*Oncorhynchus mykiss*)? *J. Exp. Biol*. **201**, 3085–3095.

Livett, B. G. and Marley, P. D. (1993). Noncholinergic control of adrenal catecholamine secretion. *J. Anat*. **183**, 277–289.

McKendry, J. E., Bernier, N. J., Takei, Y., Duff, D. W., Olson, K. R. and Perry, S. F. (1999). Natriuretic peptides and the control of catecholamine release in two freshwater teleosts and a marine elasmobranch. *Fish Physiol. Biochem*. **20**, 61–77.

Nilsson, S. (1976). Fluorescent histochemistry and cholinesterase staining of sympathetic ganglia in a teleost, *Gadus morhua*. *Acta Zool*. **57**, 69–77.

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

Nilsson, S., Abrahamsson, T. and Grove, D. J. (1976). Sympathetic nervous control of adrenaline release from the head kidney of the cod, *Gadus morhua*. *Comp. Biochem. Physiol.* **55**C, 123–127.

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.

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 (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., Conklin, D. J., Farrell, A. P., Keen, J. E., Takei, Y., Weaver, L., Jr, Smith, M. P. and Zhang, Y. (1997). Effects of natriuretic peptides and nitroprusside on venous function in trout. *Am. J. Physiol*. **273**, R527–R539.

Opdyke, D. F., Carrol, R. G., Keller, N. E. and Taylor, A. A. (1981). Angiotensin II releases catecholamines in dogfish. *Comp. Biochem. Physiol*. **70**C, 131–134.

Oudit, G. Y. and Butler, D. G. (1995a). Cardiovascular effects of arginine vasotocin, atrial natriuretic peptide and epinephrine in freshwater eels. *Am. J. Physiol*. **268**, R1273–R1280.

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

Perrott, M. N. and Balment, R. J. (1990). The renin–angiotensin system and the regulation of plasma cortisol in the flounder, *Platichthys flesus*. *Gen. Comp. Endocr*. **78**, 414–420.

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.

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*. **120**C, 1–27.

Reid, S. G., Fritsche, R. and Jonsson, A.-C. (1995). Immunohistochemical localization of bioactive peptides and amines associated with the chromaffin tissue of five species of fish. *Cell Tissue Res*. **280**, 499–512.

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. and Perry, S. F. (1995). Cholinoceptor-mediated control of catecholamine release from chromaffin cells in the American eel, *Anguilla rostrata*. *J. Comp. Physiol.* B **165**, 464–470.

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.

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.

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

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. (1993). Significance of circulating catecholamines in regulation of trout splanchnic vascular resistance. *J. Exp. Zool*. **267**, 92–96.

Yamaguchi, K. (1981). Effects of water deprivation on immunoreactive angiotensin II levels in plasma, cerebroventricular perfusate and hypothalamus of the rat. *Acta Endocr*. **97**, 137–144.

Yasuda, M., Uesaka, T., Furukawa, Y. and Ando, M. (1996). Regulation of atrial contraction in the seawater-adapted eel, *Anguilla japonica*. *Comp. Biochem. Physiol.* **113**A, 165–172.