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CARDIOVASCULAR RESPONSES *IN VIVO* TO ANGIOTENSIN II AND THE PEPTIDE ANTAGONIST SARALASIN IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

The effects of [Asn^1,Val^5]-angiotensin II (AngII) and [Sar^1,Val^5,Ala^8]-angiotensin II (saralasin) on dorsal aortic blood pressure, pulse pressure and heart rate were examined in rainbow trout *in vivo*. AngII when administered as a single dose of 25 μg kg^{-1} induced a biphasic response in blood pressure, with a significant hypertensive response during the initial 10 min, followed by a significant hypotension of 70–75 % compared with the initial blood pressure after 50 min and continuing until approximately 80 min post-injection. The co-administration of AngII (25 μg kg^{-1}) and saralasin (50 μg kg^{-1}) resulted in the same hypertensive response during the initial phase, but abolished the hypotensive effect of AngII. Heart rate was significantly increased in response to AngII, but the administration of AngII and saralasin together attenuated the increase by approximately 44 %. Stimulation of the endogenous renin–angiotensin system using a vasodilator, sodium nitroprusside, significantly increased drinking rate in rainbow trout fry, a response inhibited by saralasin, indicating a role for AngII-induced hypotension in drinking. For the first time, a decrease in blood pressure in response to AngII *in vivo* has been demonstrated in fish, and this is discussed in relation to homeostasis of blood pressure and a possible role in the control of drinking.

Key words: trout, *Oncorhynchus mykiss*, renin–angiotensin system, cardiovascular, blood pressure, hypotension, drinking.

Introduction

In vertebrates, the renin–angiotensin system (RAS) plays an important role in the control of hydromineral balance and blood pressure regulation (Robertson, 1993). The main vasoactive component of the RAS is angiotensin II (AngII), although angiotensin I (AngI) and truncated forms may also have biological effects in mammals (Head and Williams, 1992). AngII is cleaved from AngI by the action of angiotensin-converting enzyme (ACE) and exerts its biological action by means of specific membrane receptors; two main subtypes, AT_1 and AT_2, have been identified so far in mammals (Wright and Harding, 1994). In mammals, saralasin specifically antagonises the action of AngII at the receptor level, although it may have a partial agonist effect in some models. *In vivo* it causes lowering of blood pressure only when the RAS is pre-activated (Moore and Fulton, 1984).

All the components of the RAS have been identified in fish (Olson, 1992; Takei, 1993) but, despite attempts to understand its role, the physiological action of the RAS in fish is far from fully understood. The stimulation of the endogenous RAS by increases in external salinity, elevation of blood plasma ionic concentration, dehydration, blood volume depletion or lowered blood pressure is the major stimulus for the development of the drinking response, which compensates for osmotic loss of water from fish in sea water (Balment and Carrick, 1985; Takei, 1993; Tierney *et al.* 1995; Fuentes *et al.* 1996). The exogenous administration of components of the RAS, e.g. AngI and AngII, also results in increased drinking rates in both freshwater- and seawater-adapted fish (Perrot *et al.* 1992; Fuentes and Eddy, 1996), a response antagonised by saralasin (Fuentes and Eddy, 1996); however, saralasin was without effect on the blood pressure response to AngII in eel (*Anguilla rostrata*) and sculpin (*Myoxocephalus octodecimspinosus*) in sea water (Nishimura *et al.* 1978; Carroll, 1981) and in freshwater rainbow trout *O. mykiss* (Conklin and Olson, 1994*b*).

The contribution of the RAS to blood pressure regulation has recently been demonstrated in a series of elegant studies based on cardiovascular preparations, and can be summarised as follows: (i) although AngII is a potent vasoconstrictor in perfused systemic tissues in trout, there is little contractile activity in large blood vessels *in vitro* (Conklin and Olson, 1994*a*); (ii) there is a triphasic response to AngII in precontracted large blood vessels, with an initial small and transient contraction followed by a large relaxation, before recovery (Conklin and Olson, 1994*b*); and (iii) the microcirculation is the most important target for AngII pressor responses (Olson *et al.* 1994), and the venous system is not an important target of the RAS (Zhang *et al.* 1995).

There have been few *in vivo* cardiovascular studies involving AngII in fish and, although the response of heart rate

among species was variable, all studies demonstrated a short-term hypertensive effect, lasting approximately 10 min (cod *Gadus morhua*, Platzack *et al.* 1993; *Pagothenia borchgrevinki*, Axelsson *et al.*1994; rainbow trout *O. mykiss*, Olson *et al.* 1994; Le Mevel *et al.* 1994; eel *Anguilla anguilla*, Oudit and Butler, 1995*b*). The aim of the present work was to examine cardiovascular responses to exogenous AngII and saralasin in trout in order to explore the possibility that changes in blood pressure are involved in the drinking response.

Materials and methods

Animals

Adult and fry rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex were obtained from College Mill Trout Farm in Perthshire, Scotland, UK, and reared at Dundee University Aquarium for at least 2 weeks before experiments. Mean values of freshwater quality (mmol l^{-1}) were: Na^+, 0.19; K^+, 0.02; Ca^{2+}, 0.24; Mg^{2+}, 0.07; Cl^-, 0.03; free CO_2, 0.02; alkalinity as $CaCO_3$, 20.5 mg l^{-1}; total hardness as $CaCO_3$, 31.3 mg l^{-1}; non-bicarbonate hardness as $CaCO_3$, 10.6 mg l^{-1}; pH 8.2; the temperature was 9–11 °C, the temperature for all procedures unless otherwise stated.

Surgery

Rainbow trout (250–350 g) were anaesthetised in 3-aminobenzoic acid ethyl ester (Sigma, 1:25 000 neutralised with $NaHCO_3$), weighed and transferred to an operating table. The gills were perfused constantly with aerated water containing a neutralised anaesthetic solution (1:50 000) and then chronically fitted with an indwelling cannula (Portex, i.d. 0.58 mm, o.d. 0.96 mm) in the dorsal aorta according to previous methods (Soivio *et al.* 1972). After surgery, the cannulae were filled with heparinized Cortland fish saline, and the fish were transferred to 5 l darkened plastic boxes with flowing aerated water (0.5 l min^{-1}). Fish were allowed to recover for 72 h before performing any procedure, and the cannulae were flushed daily with heparinized fish saline (30 i.u. ml^{-1}, sodium heparin, Sigma).

Chemicals

[Asn^1,Val^5]-angiotensin II (acetate salt, Sigma) was diluted in fish saline to give final doses of 25 μg kg^{-1}. [Sar^1,Val^5,Ala^8]-angiotensin II (saralasin, Sigma) was prepared as above to give a final dose of 50 μg kg^{-1}. The doses of AngII and saralasin were selected according to previous studies on drinking responses (Fuentes and Eddy, 1996), according to preliminary experiments (data not shown) and according to the range used previously *in vitro* (Conklin and Olson, 1994*b*; Olson *et al.* 1994) and *in vivo* (Axelsson *et al.* 1994; Olson *et al.* 1994). Saline composition was as follows (mmol l^{-1}): NaCl, 124.1; $CaCl_2{\cdot}2H_2O$, 1.4; KCl, 5.1; $Na_2HPO_4{\cdot}H_2O$, 2.9; $NaHCO_3$, 11.9; $MgSO_4{\cdot}7H_2O$, 1.9 and glucose, 5.6, pH 7.5 (Houston *et al.* 1985), administered at the experimental temperature. The peptides, freshly prepared in 200–300 μl of fish saline, were slowly delivered over a period of 3 min *via* the cannula without physical disturbance to the fish, and dorsal aortic pressure recordings were taken initially at 5 min and then at 10 min intervals over a period of 90 min.

Cardiovascular responses

Blood pressure, pulse pressure and frequency were monitored using a Washington PT 400 pressure transducer attached to a Washington oscillograph 400 MD/211, using a static column of water as a standard. Recordings were taken for at least 30 min before and 90 min after administration of the peptides. Recorded traces were used to calculate heart rate (beats min^{-1}), mean arterial blood pressure [(systolic+diastolic)/2] (Olson *et al.* 1994) and pulse pressure (systolic minus diastolic). Blood pressure and pulse pressure were expressed in kPa, and all values are presented as mean ±1 S.E.M.

Drinking measurements

Drinking rate was measured as described previously (Fuentes and Eddy, 1996). In brief, groups of rainbow trout fry (2–3 g) were placed in 400 ml of aerated fresh water in darkened plastic boxes (13–15 °C) with approximately 30–40 kBq ^{51}Cr-labelled EDTA (Amersham) to give an activity of approximately 2000 cts min^{-1} ml^{-1} (Wallac gamma counter). At the end of the experiments (3 h), fish were killed by an overdose of benzocaine (400 mg l^{-1}), gently blotted dry and kept at −20 °C until frozen (1 h), then weighed and quickly dissected before thawing. The guts were transferred to a tube for radioactivity counting. Drinking rates were expressed as ml kg^{-1} h^{-1}.

To establish whether changes in blood pressure induced by AngII were involved in the drinking response, drinking rates were measured, following the methods of Fuentes and Eddy (1996), in trout fry (2–3 g) in response to (1) a 10 μl intramuscular injection of fish saline; (2) a saline injection plus the addition of 1.5 mmol l^{-1} of the vasodilator sodium nitroprusside to the water (McGeer and Eddy, 1996; Fuentes *et al.* 1996); (3) the addition of 1.5 mmol l^{-1} sodium nitroprusside in the water plus an intramuscular injection of 3 μg g^{-1} saralasin (Sigma); and (4) intramuscular injection of 3 μg g^{-1} saralasin.

Statistics

Comparisons between means were performed using analysis of variance (ANOVA) (two-way and one-way followed by Dunnet's test) after testing for normality (Kolmogorov–Smirnov's test) and homogeneity of variances (Cochran's test). Differences between means were considered significant at $P<0.05$, unless stated otherwise.

Results

Blood pressure

Measurement of dorsal aortic blood pressure for at least 30 min in untreated conscious rainbow trout gave steady values of approximately 3.7 kPa, and administration of AngII resulted

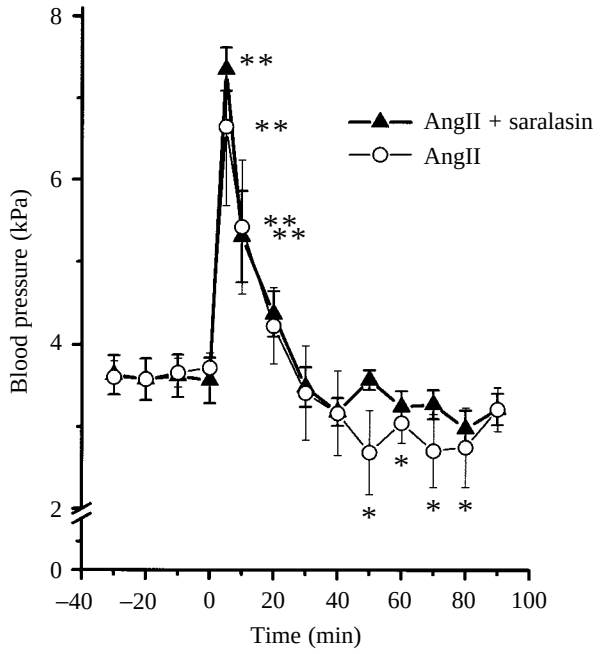

Fig. 1. Dorsal aortic blood pressure (kPa) in rainbow trout in response to a bolus injection of AngII (25 μg kg^{-1}) (open circles). The response to a bolus injection of AngII (25 μg kg^{-1}) plus saralasin (50 μg kg^{-1}) (filled triangles) is also shown. Values for at least 30 min preceding injection are shown, and the peptide was administered at time 0. Significant increases or decreases are shown with respect to pre-injection values (**P<0.01, *P<0.05). Values are means ± S.E.M. of six fish for each treatment.

in a biphasic response in blood pressure (Fig. 1). After the administration of AngII (25 μg kg^{-1}), hypertension occurred, reaching a maximum after 5 min, an increase of approximately 80 % (Fig. 1) with respect to pre-injection values, followed by a rapid decrease towards control levels in the next 10 min. Subsequently, blood pressure showed a slower, but continuous, decrease until 50 min following injection, when it was significantly (P<0.05) lower than pre-injection values (Fig. 1). This period of hypotension lasted between 50 and 80 min post-injection, with values of approximately 2.7 kPa, about 70 % of the initial blood pressure.

The hypertensive response to AngII administration was not inhibited by the co-administration of saralasin (50 μg kg^{-1}), and the increase in blood pressure resembled the response to AngII alone (Fig. 1). However, the hypotensive response to the administration of AngII was not obtained in presence of saralasin (Fig. 1). Blood pressure decreased to pre-injection levels after 10 min, then declined to approximately 90 % of control values, and reached a minimum value of approximately 85 % at 80 min (Fig. 1). These decreases were not significantly different from pre-injection values.

Pulse pressure

Table 1 shows pulse pressure dynamics in response to AngII and to co-administration of AngII and saralasin. Both treatments produced the same response with a significant

Table 1. *Pulse pressure in rainbow trout before (pre-injection) and after the administration of angiotensin II (AngII) or AngII and saralasin*

Time after injection (min)	Pulse pressure (kPa)	
	AngII	Saralasin+AngII
Pre-injection	0.048±0.005	0.84±0.081
5	1.98±0.53**	2.00±0.62**
10	1.14±0.28	0.93±0.49
20	1.07±0.16	0.92±0.22
30	0.83±0.21	0.63±0.11
40	0.77±0.20	0.48±0.06
50	0.72±0.21	0.57±0.09
60	0.62±0.11	0.54±0.11
70	0.60±0.10	0.54±0.10
80	0.65±0.21	0.57±0.09
90	0.57±0.13	0.56±0.07

AngII (25 μg kg^{-1}) or AngII (25 μg kg^{-1}) plus saralasin (50 μg kg^{-1}). Values are shown as means ± 1 S.E.M. (N=6 for each treatment); **significant (P<0.01) difference from pre-injection values (one-way ANOVA).

(P<0.01) increase 5 min after administration of the peptide(s), followed immediately by a return to pre-injection values which continued for the duration of the measurements.

Heart rate

As shown in Fig. 2, AngII induced a significant (P<0.001) increase in heart rate of approximately 60 % (between 40 % and

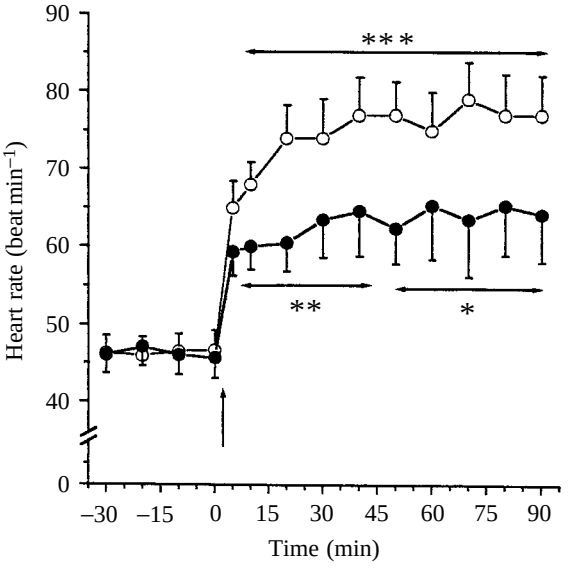

Fig. 2. Heart rate (beats min^{-1}) in response to a bolus injection of AngII (25 μg kg^{-1}) (open circles) or AngII (25 μg kg^{-1}) plus saralasin (50 μg kg^{-1}) (filled circles). The administration of the peptides is represented by the arrow. Significant differences are shown with respect to pre-injection values (***P<0.001, **P<0.01, *P<0.05). Values are represented as means ± S.E.M. of six fish for each treatment. Treatments are significantly different (P<0.001, F=8.357, two-way ANOVA).

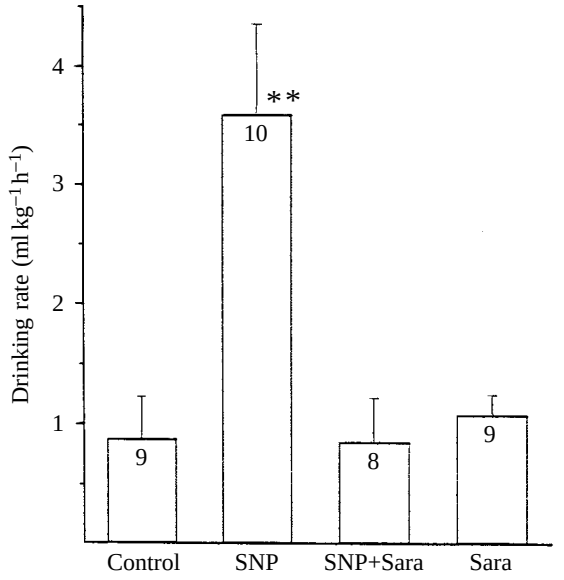

Fig. 3. Drinking rates (ml kg^{-1} h^{-1}) in rainbow trout fry (mass 2–3 g) in response to an intramuscular injection of saline (control), an intramuscular injection of 3 μg g^{-1} saralasin (Sara), exposure to 1.5 mmol l^{-1} sodium nitroprusside (SNP) added to the water and exposure to 1.5 mmol l^{-1} water-borne sodium nitroprusside plus an intramuscular injection of 3 μg g^{-1} saralasin (SNP+Sara). **Significant difference (P<0.01) from the control value (one-way ANOVA). Results are as mean + S.E.M. of 8–10 fish (values of N are given within bars).

70 %) throughout the experimental period. The co-administration of saralasin and AngII resulted in a smaller increase in heart rate, of approximately 44 % (between 35 % and 48 %) compared with pre-administration levels, which was significantly different from both control values and AngII values (P<0.001, F=8.357, two-way ANOVA).

Drinking rates

Rainbow trout fry drank at a rate of approximately 0.9 ml kg^{-1} h^{-1} in fresh water, a value that was significantly (P<0.01) increased to 3.6 ml kg^{-1} h^{-1} by exposure to 1.5 mmol l^{-1} sodium nitroprusside added to the water (Fig. 3). The response to sodium nitroprusside was totally abolished by administration of 3 μg g^{-1} saralasin. Saralasin alone did not result in alteration of drinking rate in trout fry.

Discussion

Effects of angiotensin and saralasin on blood pressure

Hypertension in response to AngII and related peptides has been described in a number of studies in rainbow trout and other fish species (Platzack *et al.* 1993; Axelsson *et al.* 1994; Olson *et al.* 1994; Le Mevel *et al.* 1994; Oudit and Butler, 1995*b*). The present results agree with previous *in vivo* work which has focused on the hypertensive response to AngII, occurring immediately and continuing for 20–30 min post-administration (Fig. 1; Platzack *et al.* 1993; Axelsson *et al.* 1994; Olson *et al.* 1994; Le Mevel *et al.* 1994; Oudit and Butler, 1995*b*). A response not reported previously is that, after approximately 50 min, a significant hypotension develops, lasting for at least 30 min (Fig. 1). Conklin and Olson (1994*b*) showed that the epibranchial artery and anterior cardinal vein from rainbow trout responded to AngII with a transitory contraction followed by a 5 min relaxation, a similar response but on a much shorter time scale than noted here *in vivo* (Fig. 1). Relaxation of large blood vessels in response to AngII (Conklin and Olson, 1994*b*) could account for the hypotension noted here *in vivo*, but this point requires further study.

The hypotension observed *in vivo* in response to AngII was unexpected. It could result from a direct effect of AngII, which presumably overrides any homeostatic hypertensive mechanisms. Other possibilities for this response include a cardiovascular reflex response to the initial hypertension or that the hypotension may arise as a consequence of release of other vasodilators (e.g. vasodilator prostaglandins, Olson *et al.* 1997); however, this is an area requiring further study.

Saralasin failed to inhibit the angiotensin-dependent hypertension in eel *Anguilla rostrata*, sculpin *Myoxocephalus octodecimspinosus* and dogfish *Squalus acanthias* (Nishimura *et al.* 1978; Carroll, 1981), a result confirmed in the present study in trout (Fig. 1). However, the hypotensive effects of AngII in rainbow trout were substantially attenuated by co-administration with saralasin, with a return to normal blood pressure within 20 min (Fig. 1). Some possible reasons for this response are (a) that the hypertensive and hypotensive responses to AngII involve two populations of receptors (Conklin and Olson, 1994*a*,*b*) with different saralasin sensitivities, (b) that AngII metabolites, e.g. the heptapeptide AngIII and the hexapeptide AngIV, may be involved since these peptides elicit circulatory responses mediated by AngII receptors in mammals (Head and Williams, 1992; Wright and Harding, 1994) and (c) that the hypotensive effect of AngII is indirect and mediated by other vasodilators, e.g. bradykinin and vasodilator prostaglandins. However, further work is required to explore these possibilities.

A doubling of pulse pressure during the 5 min hypertensive phase (Table 1; Fig. 1) could indicate decreased compliance in the arterial system; however, normal values were rapidly regained even during the hypotensive phase (Table 1). The mechanisms involved are not understood and merit further study.

Effects of angiotensin and saralasin on heart rate

Previous studies in mammals have shown that the chronotropic effects of AngII may be direct (Baker *et al.* 1992) or caused by modification of vagal tone (Reid, 1992), but in fish the responses of heart rate to AngII are variable. In the Antarctic fish *Pagothenia borchrevinki*, heart rate decreased in response to AngII (Axelsson *et al.* 1994), while it was unchanged in cod *Gadus morhua* after administration of AngI (Platzack *et al.* 1993). The heart rate of rainbow trout increased following intracerebroventricular administration of AngII (Le Mevel *et al.* 1994). In eels *Anguilla anguilla*, there was a small

but significant increase in heart rate in response to intravenous injections of AngII, with the possible involvement of catecholamines (Oudit and Butler, 1995*a*). In the present study, AngII has a direct chronotropic effect on trout heart, since co-administration of saralasin (an inhibitor specific to the RAS; Moore and Fulton, 1984) significantly attenuated the response (Fig. 2). The reason that the heart rate recorded *in vivo* increased in response to AngII, whereas the perfused heart *in vitro* did not (Olson *et al.* 1994), is unknown but could be related to the tissue specificity of AngII cardiovascular effects, as suggested previously (Olson *et al.* 1994; Conklin and Olson, 1994*a*,*b*).

Physiological relevance

The physiological significance of the hypotensive effects of AngII recorded here *in vivo* is unknown, but it may be linked with regulation of blood pressure. Although the control of contraction/relaxation in large vessels is thought to be of neuronal origin, the microcirculation is believed to be a major effector of the actions of the RAS in fish (Olson *et al.* 1994). However, the presence of specific receptors for AngII in the ventral and dorsal aortas has been demonstrated in rainbow trout (Cobb and Brown, 1992), making them accessible targets for changes in humoral AngII levels.

In fish, hypovolaemia and hypotension are major determinants of the dipsogenic response (Takei, 1993), and the involvement of the RAS in the control of drinking has been demonstrated on many occasions. The drinking response may be initiated either by administration of exogenous AngI or AngII (Perrot *et al.* 1992; Fuentes and Eddy, 1996) or by hypotensive agents such as papaverine (Balment and Carrick, 1985). Similar results are obtained using the vasodilator, sodium nitroprusside (Fuentes *et al.* 1996) and this response is inhibited by saralasin (Fig. 3). The short-lived hypertensive response to AngII which has been observed on many occasions (Platzack *et al.* 1993; Axelsson *et al.* 1994; Olson *et al.* 1994; Le Mevel *et al.* 1994; Oudit and Butler, 1995*b*; and Fig. 1) seems unlikely to be a major stimulus for sustained drinking since, in Japanese eels *Anguilla japonica*, administration of AngII resulted in simultaneous hypertension and inhibition of drinking (Hirano and Hasegawa, 1984).

Hypotensive agents such as papaverine induced drinking in freshwater and seawater European eels *Anguilla anguilla* (Tierney *et al.* 1995) by stimulating the endogenous RAS, a response inhibited by the angiotensin-converting enzyme inhibitor captopril. Thus, hypotension alone was insufficient to produce the drinking response, although any hypotensive effects of AngII in eels (Tierney *et al.* 1995) may have been masked by the effects of the hypotensive agent itself. The present study shows that AngII treatment, in the form of a single bolus, causes a prolonged hypotension, which is in keeping with the time course for sustained drinking (Perrot *et al.* 1992; Fuentes and Eddy, 1996). Responses to hypovolaemia or other appropriate stimuli could involve the release of AngII, resulting in a period of drinking that continues until sufficient water has been absorbed to restore normal electrolyte balance. The cycle could be repeated once dehydration again reaches a critical level. However, how AngII is involved in the coordination of hypotension and in the initiation, continuation and termination of drinking remains unknown.

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References

Axelsson, M., Davisson, B., Foster, M. and Nilsson, S. (1994). Blood pressure control in the Antarctic fish *Pagothenia borchgrevinki*. *J. exp. Biol.* **190**, 265–279.

Baker, K. M., Booz, G. W. and Dostal, D. E. (1992). Cardiac actions of angiotensin II: Role of an intracardiac renin–angiotensin system. *A. Rev. Physiol.* **54**, 227–241.

Balment, R. J. and Carrick, S. (1985). Endogenous renin–angiotensin system and drinking behavior in flounder. *Am. J. Physiol.* **248**, H295–H300.

Carroll, R. G. (1981). Vascular response of the dogfish and sculpin to angiotensin II. *Am. J. Physiol.* **240**, R139–R143.

Cobb, C. S. and Brown, J. A. (1992). Angiotensin II binding to tissues of the rainbow trout *Oncorhynchus mykiss*, studied by autoradiography. *J. comp. Physiol.* B **162**, 197–202.

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

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

Fuentes, J. and Eddy, F. B. (1996). Drinking in freshwater adapted rainbow trout fry *Oncorhynchus mykiss* (Walbaum) in response to angiotensin I, angiotensin II, angiotensin converting enzyme inhibition and receptor blockade. *Physiol. Zool.* **69**, 1555–1569.

Fuentes, J., McGeer, J. C. and Eddy, F. B. (1996). Drinking rate in juvenile Atlantic salmon, *Salmo salar* L. fry in response to a nitric oxide donor, sodium nitroprusside and angiotensin converting enzyme, enalapril. *Fish Physiol. Biochem.* **15**, 65–69.

Head, G. A. and Williams, N. S. (1992). Haemodynamic effect of central angiotensin I, II and III in conscious rabbits. *Am. J. Physiol.* **263**, R845–R851.

Hirano, T. and Hasegawa, S. (1984). Effects of angiotensins and other vasoactive substance on drinking in the eel, *Anguilla japonica*. *Zool. Sci*. **1**, 106–113.

Houston, A. H., McCulloch, A. M., Keen, C., Maddalena, C. and Edwards, J. (1985). Rainbow trout red cells *in vitro*. *Comp. Biochem. Physiol.* A **81**, 555–565.

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.

McGeer, J. C. and Eddy, F. B. (1996). Effects of sodium nitroprusside on blood circulation, acid–base balance and ionic balance in rainbow trout: indications for nitric oxide induced vasodilation. *Can. J. Zool*. **74**, 1211–1219.

Moore, A. F. and Fulton, F. W. (1984). Angiotensin II antagonists – Saralasin. *Drug Dev. Res*. **4**, 331–349.

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 volume regulation: role of the renin–angiotensin system, kallikrein–kinin system and atrial natriuretic peptides. In *Fish Physiology. The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 136–232. San Diego: Academic Press.

OLSON, K. R., CHAVEZ, A., CONKLIN, D. J., COUSINS, K. L., FARREL, A. P., FERLIC, R., KEEN, J. E., KNE, T., KOWALSKI, K. A. AND VELDMAN, T. (1994). Localisation of angiotensin II responses in the trout cardiovascular system. *J. exp. Biol*. **194**, 117–138.

OLSON, K. R., CONKLIN, D. J., WEAVER, L. R. JR, DUFF, D. W., HERMAN, C. A. WANG, X. AND CONLON, J. M. (1997). Cardiovascular effects of homologous bradykinin in rainbow trout. *Am. J. Physiol*. **41**, R1112–R1120.

OUDIT, G. Y. AND BUTLER, D. G. (1995*a*). 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. (1995*b*). Angiotensin II and cardiovascular regulation in a freshwater teleost, *Anguilla anguilla* LeSuer. *Am. J. Physiol*. **269**, R726–R735.

PERROT, M. N., GRIERSON, C. E., HAZON, N. AND BALMENT, R. (1992). Drinking behaviour in sea water and fresh water teleosts, the role of the renin–angiotensin system. *Fish Physiol. Biochem.* **10**, 161–168.

PLATZACK, B., AXELSSON, M. AND NILSSON, S. (1993). The renin–angiotensin system in blood pressure control 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.

ROBERTSON, J. I. S. (1993). Renin and angiotensin: a historical review. In *The Renin–Angiotensin System: Biochemistry, Physiology, Pathophysiology, Therapeutics*, vol. 1 (ed. J. I. S. Robertson and M. G. Nichols), pp. 1–18. London: Gower Medical Publishers.

SOIVIO, A., WESTMAN, K. AND NYHOLM, K. (1972). Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout. *Finnish Fish. Res.* **1**, 11–21.

TAKEI, Y. (1993). Role of peptide hormones in fish osmoregulation. In *Fish Ecophysiology* (ed. J. C. Rankin and F. B. Jensen), pp. 136–160. London: Chapman & Hall.

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.

WRIGHT, J. W. AND HARDING, J. W. (1994). Brain angiotensin receptor subtypes in the control of physiological and behavioural responses. *Neurosci. Behav. Rev.* **18**, 21–53.

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.