

**RENIN, ATRIAL NATRIURETIC PEPTIDE AND BLOOD PLASMA IONS IN PARR AND SMOLTS OF ATLANTIC SALMON *SALMO SALAR* L. AND RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM) IN FRESH WATER AND AFTER SHORT-TERM EXPOSURE TO SEA WATER**

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**Summary**

Freshwater Atlantic salmon (*Salmo salar* L.) smolts were abruptly transferred to sea water in May and over 3 days blood plasma ion concentrations were determined together with atrial natriuretic peptide (ANP) and plasma renin activity (PRA) using antibodies raised against human ANP and angiotensin I. Blood plasma  $\text{Na}^+$  and  $\text{Cl}^-$  levels in smolts increased and, between 24 and 72 h, PRA increased significantly to  $0.9 \text{ ng ml}^{-1} \text{ h}^{-1}$ , while there was a gradual rise in ANP levels to  $10 \text{ pmol l}^{-1}$  at 72 h. Similar measurements were made on parr transferred to sea water in September; in these fish  $\text{Na}^+$  and  $\text{Cl}^-$  levels increased as in smolts, PRA remained unchanged at about  $0.6 \text{ ng ml}^{-1} \text{ h}^{-1}$  and ANP levels increased significantly to about  $20 \text{ pmol l}^{-1}$  at 24 and 72 h. After 2 h in sea water parr showed wide variability in ANP levels, in keeping with circulatory stress, hypoxia and increased atrial stretching. Parr transferred to sea water in December showed low drinking rates of  $1.95 \text{ ml kg}^{-1} \text{ h}^{-1}$ , even after 20 days, compared to a high drinking rate of about  $7 \text{ ml kg}^{-1} \text{ h}^{-1}$  reported for smolts transferred in May.

Rainbow trout acclimated to sea water for 3 weeks showed elevated levels of both ANP and PRA, similar to values reported for marine species. Freshwater rainbow trout fed a high-salt diet (12% NaCl) showed significantly elevated levels of ANP compared to fish fed a normal diet (1.5% NaCl), while PRA levels were depressed, though not significantly.

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It is concluded that, in fish acclimating to sea water, ANP is involved in excretion of salt loads, while the response to dehydration is activation of the renin-angiotensin system, which is required to initiate drinking. It is suggested that, in freshwater salmonids, as is the case in mammals, ANP and the renin-angiotensin system are antagonistic, but successful acclimation to sea water requires the systems, in alternation with the renin-angiotensin system, to 'break through' ANP suppression.

### Introduction

Ionic regulation of freshwater and marine fish has been extensively studied (Eddy, 1982; Evans, 1984) and in euryhaline species, such as salmon, the parr/smolt transformation prior to seawater migration has received considerable attention (Hoar, 1988). However, the mechanisms controlling branchial transport and excretion of salt acquired by drinking, ionic diffusion or through the diet are little understood.

Salt excretion in mammals is controlled in part by atrial natriuretic peptide (ANP) (Laragh, 1986; Struthers, 1986; Brenner *et al.* 1990) and although ANP-like peptides have been shown to be present in fish (Chapeau *et al.* 1985; Reinecke *et al.* 1985), their role in teleosts is not well defined (Duff and Olson, 1986; Eddy *et al.* 1989, 1990; Evans *et al.* 1989). Circulating levels of ANP in rainbow trout (*Oncorhynchus mykiss*), the euryhaline *Gilia atraia* and various marine fish increase upon exposure to sea water (Galli *et al.* 1988; Westenfelder *et al.* 1988, 1989; Evans *et al.* 1989), suggesting a role for this peptide in salt excretion. The structure of ANP has been strongly conserved amongst vertebrates (Evans *et al.* 1989; Takei *et al.* 1989) and all measurements have been based on antibodies raised against human ANP, because assays for fish ANP have yet to be developed.

Fish entering sea water experience dehydration and respond, *via* stimulation of the renin-angiotensin system (Henderson *et al.* 1976; Hazon *et al.* 1989; Sokabe *et al.* 1973; Carrick and Balment, 1983; Balment and Carrick, 1985; Takei *et al.* 1979), by drinking (Usher *et al.* 1988; Shehadeh and Gordon, 1969; Evans, 1979; Hirano, 1974; Holstein, 1979). However, Stagg *et al.* (1989) noted a marked loss of osmoregulatory capacity in parr transferred to sea water in December compared to almost immediate adaptation by smolts transferred in May. Hence, the purpose of these experiments was to investigate the role of the renin-angiotensin system and of ANP in the control of salt balance in salmon parr and smolts and rainbow trout following transfer to sea water and in rainbow trout fed a high-salt diet.

### Materials and methods

Atlantic salmon (*Salmo salar* L.) were obtained from the Department of Agriculture Fisheries for Scotland salmon hatchery at Almondbank, Perthshire, Scotland. The fish had first smolted in their second spring (S2 smolts) and were subsequently retained in fresh water for an additional 2 years. On the basis of external appearance they were considered to be smolt-like in April/May, with

silvering and an elongate body (low condition factor), but they reverted to parr-like condition in September. Fish weighed  $190.0 \pm 30$  g in May and  $365.0 \pm 10$  g in September ( $N$ =at least 7 for each season).

During May and September batches of fish were transported to the aquarium at the University of Dundee, where they were held in 200 l tanks supplied with running fresh water, at  $10.5 \pm 1.0^\circ\text{C}$  in May and  $13.0 \pm 1.0^\circ\text{C}$  in September, with ionic content (in  $\text{mmol l}^{-1}$ ):  $\text{Na}^+$ , 0.19;  $\text{K}^+$ , 0.02;  $\text{Ca}^{2+}$ , 0.15;  $\text{Mg}^{2+}$ , 0.05;  $\text{Cl}^{-}$ , 0.13; pH 8.2. Fish were acclimated to these conditions for at least 2 weeks and pelleted diet was offered at 2% body mass per day.

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), weighing approximately 200 g were obtained from a local hatchery (College Mill Trout Farm, Almond-bank) and held in fresh water as described, one group was held in sea water (33%) for at least 3 weeks. A population of freshwater trout was fed a diet containing 1.5% NaCl while a similar group received a diet containing 12% NaCl for at least 6 months before use in experiments (for details, see Salman and Eddy, 1988).

Fish were anaesthetised in  $50 \text{ mg l}^{-1}$  benzocaine (Sigma), placed on an operating table, and blood was withdrawn *via* the dorsal aorta using a technique based on that of Smith and Bell (1964). This procedure, which lasted 3 min, was relatively stress-free compared to many other blood sampling techniques and each fish yielded about 5 ml, which was divided between three tubes at ice temperature for the following determinations. (1) Renin activity was measured in 1 ml of blood placed in a tube with 1% EDTA. (2) ANP measurements were made in 3 ml of blood added to 0.14 g of aprotinin proteinase inhibitor (Trasylol, Bayer). (3) Electrolyte analyses were performed on 0.5 ml of blood. All samples were kept on ice for the few minutes prior to high-speed centrifugation ( $12\,000 \text{ revs min}^{-1}$ ) for 30 s, and the plasma was removed and stored at  $-30^\circ\text{C}$ .

Renin activity was equated with angiotensin II concentration and was measured by radioimmunoassay of angiotensin I generated during a 1.5 h incubation at  $37^\circ\text{C}$  using a commercially available kit (CIS, UK Ltd, High Wycombe, Buckinghamshire). Angiotensin I antibodies were raised in rabbits to conjugates of angiotensin I. The standard was ileu-5-angiotensin I and the intra- and inter-assay coefficients of variation were 6% and 11%, respectively, with a lower limit of detection of  $0.2 \text{ ng ml}^{-1} \text{ h}^{-1}$ .

ANP was determined by radioimmunoassay (Amersham International Ltd, Little Chalfont, Buckinghamshire, UK), after extraction from plasma, by the method of Richards *et al.* (1987). ANP antibodies were raised in rabbits to conjugates of alpha-human ANP. The standard used was alpha-human ANP and the intra- and inter-assay variabilities were 9.8% and 7.1%, respectively. The assay is sensitive to  $4 \text{ pmol l}^{-1}$  and this method measures 'ANP-like' substances in fish blood (for a discussion, see Evans *et al.* 1989). Full details of both methods are reported in McMurray and Struthers (1989).

Sodium and magnesium were analysed by atomic absorption spectrophotometry (Pye Unicam SP900) and chloride by amperometric titration (Radiometer CMT10).

Groups of fish were carefully transferred to well-aerated and filtered sea water in a 200 l tank ( $\text{Na}^+$   $470 \text{ mmol l}^{-1}$ ) and individually removed for blood sampling (as described earlier) at 2, 24 or 72 h. Seawater transfer of salmon parr and smolts and rainbow trout resulted in no mortalities.

Drinking rates in a group of salmon parr, weighing  $22.6 \pm 0.6 \text{ g}$ , in fresh water and 2 and 24 h and 3, 7 and 21 days after transfer to sea water were estimated by the accumulation of [ $^{51}\text{Cr}$ ]EDTA in the gut (Usher *et al.* 1988). Fish were placed in a 6 l chamber ( $29 \text{ cm} \times 20 \text{ cm} \times 13 \text{ cm}$ ) of well-aerated sea water, radioisotope ( $0.3 \text{ KBq ml}^{-1}$ ) was added and water samples were removed at appropriate times. After a known period (usually 2 h), fish were rapidly killed by an overdose of anaesthetic ( $500 \text{ mg l}^{-1}$  Benzocaine, Sigma), rinsed, blotted dry and weighed. After tying off the oesophagus and rectum the whole gut was removed and placed in a 10 ml tube. Gut and water sample activities were measured using an LKB gamma counter. Drinking rate (in  $\text{ml kg}^{-1} \text{ h}^{-1}$ ) was calculated as:

$$\frac{C_g \times 1000}{C_w \times T \times W}$$

where  $C_g$  is counts in the gut,  $C_w$  is the counts per millilitre of medium,  $T$  is the duration of the drinking period (h) and  $W$  is fish wet mass (g).

Statistical analysis was by Student's *t*-test or one-way analysis of variance.

### Results

Following transfer to sea water, smolts and parr showed progressive increases in plasma  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations; after 3 days values were similar in the two groups (Fig. 1A,B). Over this period plasma  $\text{Mg}^{2+}$  values increased significantly in both groups and, although smolts showed higher values after 3 days, the difference was insignificant (Fig. 2).

Smolt ANP values rose gradually to  $10 \text{ pmol l}^{-1}$  (Fig. 3), whilst in parr there were major fluctuations at 2 h before values around  $20 \text{ pmol l}^{-1}$  were recorded at 24 h and 3 days. After 24 h in sea water plasma renin activity in smolts remained unchanged; this was followed by a significant increase to  $0.89 \text{ ng ml}^{-1} \text{ h}^{-1}$  at 3 days. In contrast, values for parr remained steady at about  $0.6 \text{ ng ml}^{-1} \text{ h}^{-1}$  throughout the experimental period (Fig. 4).

Drinking rates for salmon parr transferred to sea water in December increased significantly after 3 days, but by 20 days the rate was only  $1.95 \text{ ml kg}^{-1} \text{ h}^{-1}$ , significantly lower than that of seawater smolts in May (Fig. 5).

Compared with freshwater rainbow trout, acclimation to sea water for 3 weeks resulted in significantly elevated ANP and renin levels. Freshwater trout fed a 12% NaCl diet showed significantly higher ANP levels but insignificantly lower renin levels compared to those fed the 1.5% NaCl normal diet (Table 1).

### Discussion

In man ANP acts on vasculature, kidneys and adrenals to reduce blood pressure

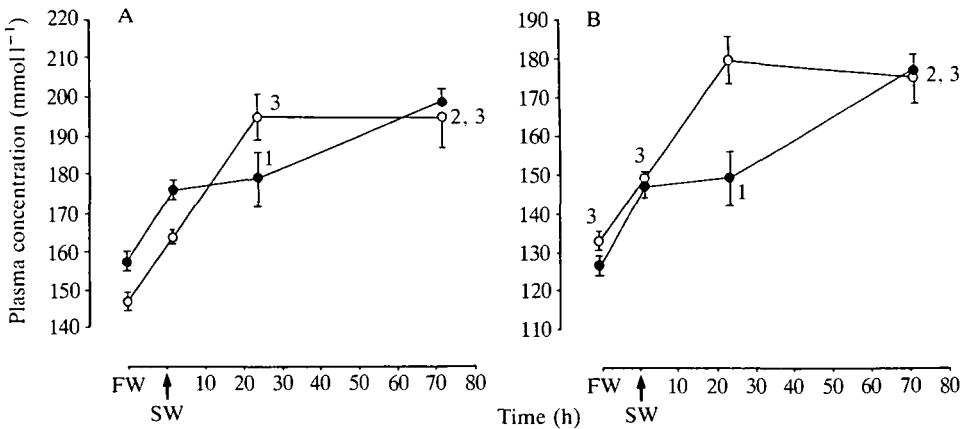


Fig. 1. Plasma sodium (A) and chloride (B) concentrations in freshwater-adapted (FW) salmon parr (○) and smolts (●) following abrupt transfer to sea water (SW). Values are mean  $\pm$  s.e. for at least seven parr and at least six smolts, except for freshwater smolts, where  $N=12$ . For both sodium and chloride all points are significantly different from each other at  $P<0.05$  or greater unless marked. Smolts: 1, insignificantly different from 2 h value. Parr: 2, insignificantly different from 24 h value. Smolts and parr: 3, insignificantly different from one another.

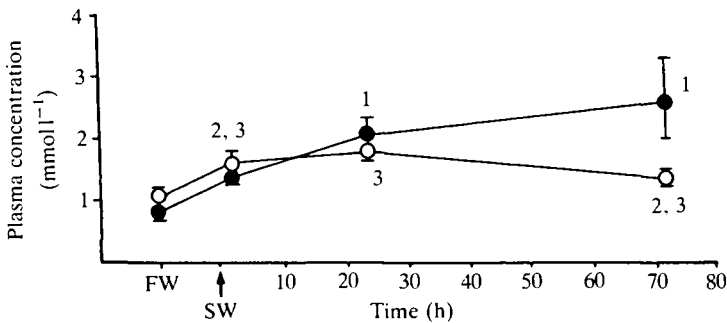


Fig. 2. Plasma magnesium concentrations in freshwater-adapted (FW) salmon parr (○) in September and smolts (●) in May following abrupt transfer to sea water (SW). Values are mean  $\pm$  s.e. for at least six fish. All points are significantly different from each other at  $P<0.05$  or greater unless marked. Smolts: 1, insignificantly different from 2 h value. Parr: 2, insignificantly different from 24 h value. Smolts and parr: 3, insignificantly different from one another.

and intravascular volume. In the kidney, natriuresis is promoted through hyperfiltration, reduced tubular reabsorption and suppression of renin and aldosterone release, while intravascular volume is also reduced by ANP through facilitated transduction of plasma water to interstitium (Cantin and Genest, 1985; Laragh, 1986; Struthers, 1986; Brenner *et al.* 1990). Whilst in mammals control of salt balance by ANP and the renin-angiotensin-aldosterone system (RAAS) is

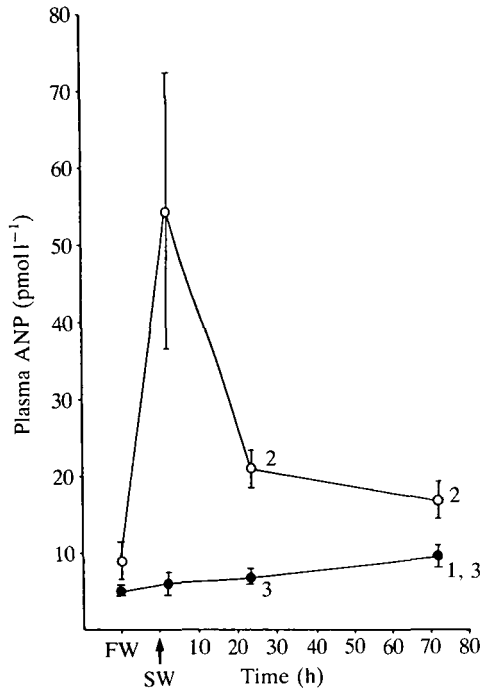


Fig. 3. Plasma atrial natriuretic peptide (ANP) levels in freshwater-adapted (FW) salmon parr (○) and smolts (●) and following abrupt transfer to sea water (SW). Values are mean  $\pm$  s.e. for at least seven parr and at least six smolts, except for freshwater smolts, where  $N=12$ . Key to differences significant at  $P<0.05$ : 1 (smolt) and 2 (parr), different from freshwater value only; 3, smolt values different from parr values. For details of the ANP determinations see Materials and methods section.

Table 1. Blood plasma ANP ( $\text{pmol l}^{-1}$ ), renin activity ( $\text{ng ml}^{-1} \text{h}^{-1}$ ) and sodium and chloride levels ( $\text{mmol l}^{-1}$ ) for freshwater rainbow trout fed a normal (1.5% NaCl) or a high-salt (12% NaCl) diet and adapted to sea water for 3 weeks

	Freshwater-adapted		Seawater-adapted
	1.5% NaCl diet	12% NaCl diet	
[Na <sup>+</sup> ]	158.6 $\pm$ 2.0 (7)*	150.1 $\pm$ 3.6 (7)**	176.4 $\pm$ 5.2 (5)
[Cl <sup>-</sup> ]	129.7 $\pm$ 2.7 (7)*	121.6 $\pm$ 3.0 (7)**	149.6 $\pm$ 5.7 (5)
[ANP]	7.1 $\pm$ 0.64 (7)**	9.5 $\pm$ 0.87 (8)*†	24.4 $\pm$ 2.2 (3)
Renin	0.25 $\pm$ 0.014 (7)*	0.18 $\pm$ 0.03 (8)**	0.63 $\pm$ 0.15 (5)

Values are means  $\pm$  s.e. ( $N$ =no. of fish).

Values significantly different from those for seawater fish: \* $P<0.05$ , \*\* $P<0.01$ . † Values significantly different between fish fed 1.5% and 12% NaCl diets,  $P<0.05$ .

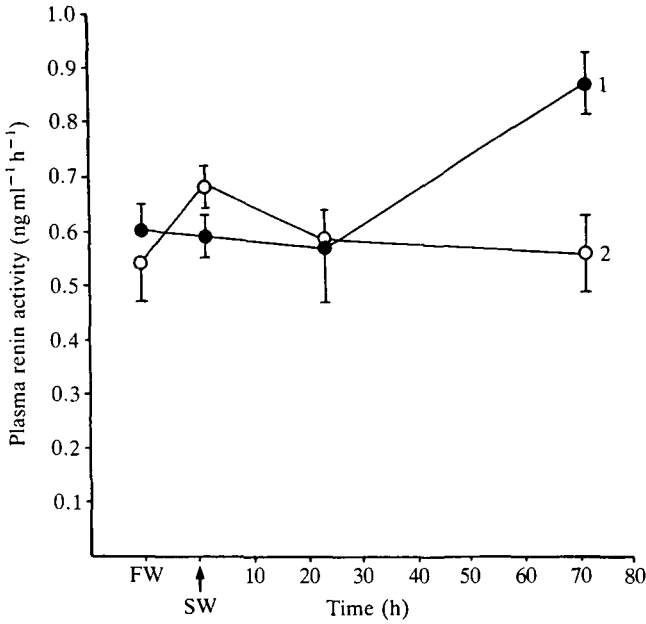


Fig. 4. Plasma renin activity in freshwater-adapted (FW) salmon parr (○) and smolts (●), following abrupt transfer to sea water (SW). Values are mean  $\pm$  s.e. For numbers of fish see Fig. 3. Key to significant differences at  $P < 0.05$ : 1, (smolt) different from all other values; 2, smolt and parr values different.

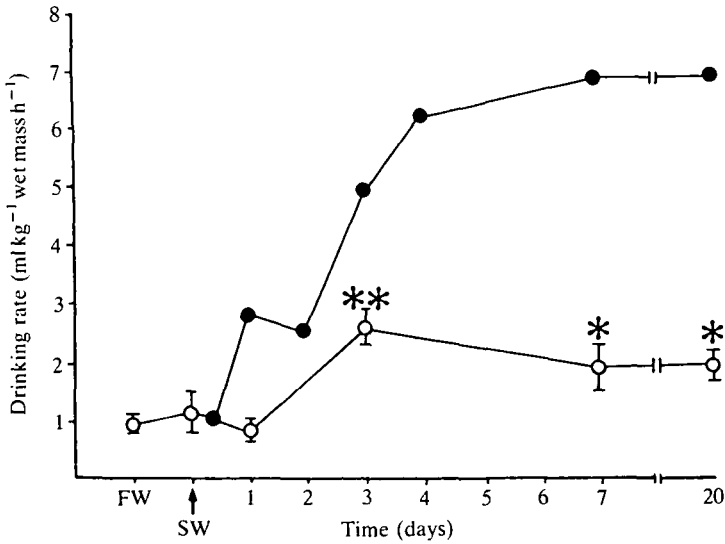


Fig. 5. Drinking rate of freshwater-adapted (FW) salmon parr (○) during short-term adaptation to sea water (SW). Values are mean  $\pm$  s.e. for at least seven fish. \* $P < 0.05$ , \*\* $P < 0.01$  compared to freshwater values. Drinking rate of salmon smolts (●) over a similar period is shown for comparison (Usher *et al.* 1988).

relatively well studied (Brenner *et al.* 1990), the situation in fish is less well understood and although ANP-like substances are present (Chapeau *et al.* 1985; Reinecke *et al.* 1985) their role is less well defined (Duff and Olson, 1986; Evans *et al.* 1989; Eddy *et al.* 1990). The role of the renin–angiotensin system in drinking by seawater fish is well established (Hirano *et al.* 1978; Henderson *et al.* 1976; Balment and Carrick, 1985; Hazon *et al.* 1989), but the interactions between ANP and the renin–angiotensin system in migrating fish entering sea water are unknown.

#### *ANP in parr and smolts*

Parr and smolts transferred to sea water experienced an increased salt intake resulting in elevated blood plasma  $\text{Na}^+$  and  $\text{Cl}^-$  levels (Fig. 1A,B), an expanded  $\text{Na}^+$  space (Bath and Eddy, 1979) and a salt load. Thus, in parr and smolts there is likely to be a correlation between salt load, plasma ANP levels and salt excretion, as has been demonstrated in mammals (Smith *et al.* 1986), though in mammals blood  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations are more closely controlled than in fish. This agrees with the proposed role for ANP in salt excretion by seawater-adapted fish, which have higher levels of the peptide compared with freshwater-adapted fish (Westendfelder *et al.* 1988, 1989; Evans *et al.* 1989), and it was suggested that the stimulus for ANP release was salt loading rather than dehydration (Evans *et al.* 1989). However, the mechanisms linking ANP release and salt excretion in fish remain unknown.

After transfer to sea water, ANP levels were consistently higher in parr than in smolts (Fig. 3); possible reasons will be discussed in relation to drinking.

It is interesting that 2 h after transfer to sea water some parr exhibited ANP values of more than  $100 \text{ pmol l}^{-1}$ , though lower values were noted subsequently (Fig. 3). Increased ANP levels were noted in hypoxic rabbits (Baertschi *et al.* 1988), in which distension of the atria stimulated ANP release (see also Brenner *et al.* 1990). Severe hypoxia was noted in parr 30 min after transfer to sea water (Stagg *et al.* 1989), coinciding with a 78% increase in dorsal aortic blood pressure to  $7.24 \pm 0.067 \text{ kPa}$  ( $N=4$ ), presumably associated with cardiac distension and release of ANP. Such responses were not shown by smolts, in which blood pressure (similar to that for freshwater parr) remained unchanged after transfer to sea water (R. M. Stagg, C. Talbot, F. B. Eddy and M. Williams, unpublished observations).

#### *Control of drinking*

In smolts the response to dehydration was an elevation in renin levels (Fig. 4), which is the major stimulus for increased drinking rates (Usher *et al.* 1988; Fig. 5), a system poorly developed in parr (Fig. 4). The role of the renin–angiotensin system in drinking by marine fish is well established (Henderson *et al.* 1976; Hazon *et al.* 1989), while in mammals centrally administered angiotensin II stimulates thirst, a response suppressed by simultaneous administration of ANP (Antunes-Rodrigues *et al.* 1985; Nakamura *et al.* 1985).



In parr the response to dehydration was a minimal change in drinking rate (even after 20 days in sea water, Fig. 5) associated with relatively low and unchanged renin levels (Fig. 4). This may be because renin-producing cells either lack appropriate receptors or are inactive, although, in the longer term, if ANP and renin are antagonistic (see below), high levels of ANP (Fig. 3) could be the reason for suppressed renin activity and hence explain the minimal change in drinking rate observed in parr (Fig. 5).

Some 20% of the ingested magnesium is absorbed (Hickman, 1968; Parmelee and Renfro, 1983; Kirsch *et al.* 1985) and higher plasma  $Mg^{2+}$  levels for smolts (Fig. 2 and Stagg *et al.* 1989) suggest substantial drinking compared to parr, which show low  $Mg^{2+}$  levels and low drinking rates.

It is interesting to note that 3 days after transfer to sea water smolts exhibited high levels of both ANP and renin (Figs 3, 4), which may be a requirement for seawater adaptation. This was also the case for seawater-adapted trout, which had high levels of ANP as well as increased renin activity compared to freshwater trout (Table 1), apparently related to requirements for simultaneous drinking and salt excretion. Such is presumably the prevailing relationship between renin and ANP in most marine fish, e.g. the renin activity of seawater-acclimated trout is comparable to that of seawater-adapted eels (Henderson *et al.* 1976), while ANP levels in seawater trout are comparable to those for a variety of marine fish (Evans *et al.* 1989).

#### *Relationship between ANP and renin*

In mammals salt balance is controlled by interactions between ANP and the renin-angiotensin-aldosterone system (RAAS) where ANP (natriuretic and vasodilatory) is antagonistic to the RAAS (antinatriuretic and vasoconstrictory). Hence, in mammals increased dietary salt intake or saline infusion results in elevated ANP levels and depressed renin activity (Sagnella *et al.* 1986; Iwao *et al.* 1988). A similar system probably exists in freshwater trout fed a high-salt diet, which have significantly elevated ANP values though insignificantly lower renin activity compared to control values (Table 1). This aspect warrants further investigation.

Smolts and seawater-adapted trout show high levels of ANP which would normally inhibit the renin-angiotensin system, but the need to relieve dehydration eventually suppresses the influence of ANP, thus stimulating renin release and initiation of drinking. The accumulated salt load is, in turn, excreted under the influence of ANP.

In conclusion, the achievement of salt balance by salmon smolts within a few days of transfer to sea water involves initiation of drinking through increased activity of the renin-angiotensin system and excretion of the salt load through increased levels of ANP. A similar response was noted in rainbow trout acclimated to sea water for 3 weeks, while freshwater trout receiving 12% dietary salt exhibited a response similar to that of salt-loaded mammals, that is an elevation of ANP levels, though renin activity was not significantly depressed. Salmon parr

transferred to sea water out of season were also capable of regulating salt balance for several weeks mainly by excretion of excess salt through increased ANP levels (significantly higher than those in smolts), which presumably suppress the renin-angiotensin system, so leading to minimal drinking rates.

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