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Differential status of the renin–angiotensin system of silver sea bream (*Sparus sarba*) in different salinities

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Abstract

Silver sea bream (*Sparus sarba*) is extremely euryhaline and can survive in a wide range of salinities (0–70‰). The status of the renin–angiotensin system (RAS) in sea bream adapted to different salinities was studied. As indicated by plasma Ang II levels, a suppressed status of the RAS was found to occur under brackish water conditions; while under hypersaline conditions, an activated RAS prevailed, especially in fish adapted to double strength seawater (70‰). Captopril successfully blocked the conversion of Ang I to Ang II, causing a dramatic drop in plasma Ang II levels, and such decrease was accompanied by lowered plasma cortisol levels. The pattern of changes in branchial Na–K-ATPase activity in different salinities was similar to those of plasma Ang II and cortisol, suggesting a causal regulatory role of Ang II on branchial Na–K-ATPase activity. Intraperitoneal injection of Ang II elicited a dose-dependent increase in branchial Na–K-ATPase activity in both 33- and 6‰-adapted sea bream, but a relatively more intense stimulation of enzyme activity occurred in hyposmotic-adapted fish. Abrupt hyposmotic transfer rapidly lowered plasma Ang II level but elevated branchial Na–K-ATPase and transiently elevated plasma cortisol, indicating that these parameters are not solely controlled by Ang II but are also influenced by other hormonal factors that change during salinity transfer. Blood volumes of both 33- and 6‰-adapted sea bream exhibited high stability during short-term salinity transfers and after long-term salinity adaptation. Captopril significantly reduced resting blood pressure in both 33- and 6‰-adapted sea bream, indicating that the RAS was involved in maintenance of resting blood pressure in both hyperosmotic and hyposmotic environments. Blood pressure was highly stable during abrupt salinity transfer and captopril blockade did not alter such stability. The vasopressive effect of angiotensins was more potent in 6‰-adapted sea bream. These results showed that the RAS is involved in the maintenance of fluid and pressure homeostasis in sea bream and hyposmotic-adapted sea bream has an abated RAS status.

Keywords: Renin–angiotensin system; Sea bream; Blood volume; Blood pressure; Na–K-ATPase; Salinity adaptation

1. Introduction

The renin–angiotensin system (RAS) is one of the major endocrine systems modulating osmoregulatory processes in vertebrates. It is involved in the regulation of blood volume and pressure, drinking rate, and electrolyte homeostasis (Kobayashi and Takei, 1996). Angiotensinogen, which is the precursor protein, is cleaved into angiotensin I (Ang I) by renin. Ang I is basically biologically inactive and is cleaved into active angiotensin II (Ang II) at the carboxyl end by angiotensin converting enzyme (ACE) which in fish, is mainly located in the gills (Olson et al., 1989). The angiotensins are structurally conserved throughout phylogeny, especially for the active peptide sequence (Balment et al., 2003; Rankin et al., 2004; Takei et al., 2004). Ang II causes vasoconstriction to increase blood pressure and acts as an antidrop regulator during episodes of decline in blood pressure and volume (Olson, 1992). Teleostean fish that live in various osmotic media often encounter osmotic stress so that rapid volume and pressure regulation is vital to survival.

In eels, freshwater-adapted animals generally have higher resting blood pressure than their seawater counterparts

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(Chan et al., 1978; Tierney et al., 1995), and when being transferred from freshwater to seawater, blood pressure drops rapidly, often within periods of 1–2 h (Chester Jones et al., 1969). Perturbation of blood volume that would produce hypotension or hypovolemia, such as hemorrhage and salinity transfer, usually activates the RAS (Sokabe et al., 1973; Nishimura et al., 1979; Kobayashi and Takei, 1996). Increased renin activity has been observed in toadfish (Nishimura and Madey, 1989) and trout after hemorrhage (Bailey and Randall, 1981). Transfer of seawater eel to freshwater rapidly lowers plasma renin activity (Henderson et al., 1976) and plasma Ang II levels are higher in seawater eels (Henderson et al., 1985). It therefore seems that the RAS is activated whenever fish encounter a hyperosmotic medium, a strategy for counteracting the imminent drop in blood pressure (Olson, 1992). In contrast, captopril, an ACE inhibitor, which acts to prevent the formation of Ang I to Ang II, abolishes the pressor effect of Ang I in the toadfish (Qin et al., 1999) and the eel (Nishimura et al., 1978). Infusion of captopril in freshwater eel did not alter the resting blood pressure but the same treatment lowered resting blood pressure in seawater eel (Tierney et al., 1995). These data suggested that the RAS could be more important in regulating blood pressure in seawater fishes such as the seawater-adapted eel. However, it is impossible to generalize such a contention since there is little information available for other euryhaline or marine teleosts that have been adapted to freshwater or hypoosmotic conditions. In order to assess more fully whether the RAS is more important in regulating blood pressure in seawater-adapted fish, we studied the effect of captopril on blood pressure control in a euryhaline marine species (*Sparus sarba*) adapted to hyposmotic and hyperosmotic media.

Na–K-ATPase is an important enzyme for ion regulation in teleosts and it provides the primary driving force for the extrusion and absorption of ions across the gill epithelia in both seawater and freshwater conditions (Marshall, 1995). It has been recently shown that in some osmoregulatory tissues such as the eel intestinal enterocytes, Na–K-ATPase activity can be modulated by Ang II (Marsigliante et al., 2001). Although it is known for some time that activation of the RAS stimulates the whole ACTH-cortisol axis and steroidogenesis in fish (Weld and Fryer, 1987), a direct stimulatory effect of Ang II on Na–K-ATPase activity has only been shown in the branchial and renal tissues of the eel (Marsigliante et al., 1997, 2000). Furthermore, work on the stimulatory effects of Ang II on Na–K-ATPase activity has only involved in vitro systems, evidence of such a relationship has not been demonstrated in vivo. Here we report a stimulatory effect of Ang II on branchial Na–K-ATPase in vivo.

Silver sea bream (*S. sarba*) respond rapidly to salinity changes by alteration of chloride cell morphology and reorganization of stored metabolites (Kelly and Woo, 1999a). Although it is a predominantly marine fish, sea bream is markedly euryhaline and can be adapted to a wide range of salinities (0–70‰). Moreover, the role of the RAS in sea bream during salinity adaptation is not fully understood, especially regarding its role on the modulation of blood pressure, blood volume, and Na–K-ATPase activity. We report in the present study that the status of the RAS is intricately correlated with changes in environmental salinity and such a differential RAS status contributes to the marked euryhaline characteristics of the sea bream.

2. Materials and methods

2.1. Fish and culture conditions

Silver sea bream (100–200 g) were purchased from a local fish farm. They were kept in a recirculating seawater system in the Simon S.F. Li Marine Science Laboratory, the Chinese University of Hong Kong. Fish were fed daily with a formulated diet according to Woo and Kelly (1995). Fish were then divided randomly into six groups in separate seawater tanks. Water in the tanks was gradually adjusted to the desired salinity by flushing with dechlorinated tap water (0‰) or hypersaline water (50 and 70‰) as appropriate, until the final salinity was reached. The entire flushing process was completed within 7 days, after which, fish were further adapted to the final salinities (0, 6, 12, 33, 50, and 70‰) for four more weeks before sampling. In a separate experiment, various groups of 33‰-adapted sea bream were abruptly transferred to 6‰ and these fish were sampled at 0.5, 2, 6, 24, 72, and 120 h after transfer. Pre-transfer fish were taken as fish sampled at 0 h.

2.2. Blood and tissue sampling

Fish from various treatments were sampled without using any anesthetics. Blood (1 ml) was withdrawn from the caudal vessels using heparinized syringe pre-cooled on ice. Blood samples were centrifuged immediately and the plasma obtained was frozen in liquid N_2. Blood withdrawal was accomplished within 20 s and the centrifuged plasma was plunged into liquid N_2 within 1.5 min after blood withdrawal. Fish were then killed by spinal transection and the gills were removed and frozen in liquid N_2. Groups of sea bream that were adapted to 33 and 6‰ were injected with saline (0.8% NaCl), Ang II (1, 10, and 100 nmol/100 g body weight) or captopril (500 nmol/100 g body weight) intraperitoneally, and these fish were sampled 30 min after injection. Plasma and gill tissues were sampled as described and quick frozen in liquid N_2. All tissue samples were kept at −80 °C until analysis.

2.3. Radioimmunoassay procedures for angiotensins

Frozen plasma samples (0.5 ml) were partially thawed on ice and equal volumes of chilled acidic acetone (acetone:water:1 M HCl = 40:5:1) were added to extract peptides. The mixtures were vortexed vigorously and incubated at −20 °C for 20 min. They were then centrifuged at 12,000 rpm for 5 min at 4 °C and the supernatants were freeze-dried using a rotary evaporator. The dried plasma samples were stored at −20 °C until immunoassay was performed.

Plasma Ang II level in sea bream was determined using a standard radioimmunoassay developed for eel plasma according to Tsuchida and Takei (1998). To validate the suitability of the assay for measuring sea bream angiotensins, the parallelism of serially diluted sea bream plasma extract was compared with a standard curve for synthetic [Asn^1,Val^5]-Ang II. Since sea bream plasma was collected without adding inhibitors for angiotensin degradation, it is necessary to show that sampling without inhibitor had no effect on peptide integrity. To achieve this, a comparison of angiotensin levels was made for plasma samples obtained using heparin only and samples obtained with the addition of an inhibitor cocktail (0.1% ammonium EDTA and 0.1 TIU aprotinin in final concentration). The antibody used for the immunoassay was raised against [Asp^1,Ile^5]-Ang II and is cross-reactive with standard [Asn^1,Val^5]-Ang II and sea bream-Ang II (Tierney et al., 1998). The cross-reactivity between [Asn^1,Val^5]-Ang II

and sea bream-Ang II was 93.6%. The intra-assay and inter-assay coefficients of variation were 3.3 and 10.7%, respectively.

2.4. Branchial Na–K-ATPase activity

Gill filaments were homogenized in ice-cold 4:1 sucrose–EDTA–imidazole (SEI)/sucrose–EDTA–imidazole–deoxycholic acid (SEID) buffer and Na–K-ATPase activity was determined according to McCormick (1993). ATPase activity was measured by the rate of disappearance of reduced NADH in the assay solution. Na–K-ATPase activity was measured from the difference between total ATPase activity with and without ouabain. Protein concentration in the homogenate was determined according to Bradford (1976). Na–K-ATPase activity was expressed as μmol NADH/min/g protein.

2.5. Plasma cortisol measurement

Plasma cortisol level was measured using a commercial enzyme immunoassay kit (IBL, Hamburg, Germany), which has been validated for measuring cortisol in sea bream plasma by using serially diluted and spiked silver sea bream serum samples (Deane et al., 2001; Deane and Woo, 2003). The assay conditions were adopted from the instructions of the manufacturer and color developed was measured at 450 nm using a microplate reader (Dynatech 5000). Cortisol level was expressed in ng/ml plasma.

2.6. Blood volume and pressure measurements

Sea bream (150–250 g) were anesthetized by 3-aminobenzoic acid ethyl ester (MS-222, Sigma, 1:10,000) until breathing movement stopped. Fish were then transferred to an operation table and the gills were continuously flushed with dilute MS-222 solution (1:100,000) during operation using a peristaltic pump. The branchial artery was cannulated using a method modified from Ames and Condie (1966). The gills were exposed using a pair of retractors and the entire fourth gill arch on the left was tied off distally using silk suture. A sharpened PE-50 polyethylene cannula (Clay-Adams, USA) filled with heparinized physiological saline [composition (in g/L) modified from Rankin and Maetz (1971): NaCl, 8.36; KCl, 0.25; Na_2HPO_4, 0.20; $MgSO_4 \cdot 7H_2O$, 1.01; $CaCl_2$, 0.14; $(NH_4)_2SO_4$, 0.04; KH_2PO_4, 0.04; glucose, 1.00] was directly inserted into the afferent branchial artery. The cannula was further secured using silk suture. The fish was then allowed to recover in a dark chamber containing aerated water of appropriate salinity for 24 h before any experiment was done.

Blood volume was estimated using an indicator-dilution method according to Conte et al. (1964). Evans Blue (T-1824, 2 mg/ml in physiological saline) was injected through the cannula and flushed with heparinized saline until all the blue dye had entered the circulation. Serial blood samples were collected from the same fish at 5, 10, 15, 25, and 35 min post-injection through the cannula into heparinized micro-hematocrit tubes and centrifuged immediately. Hematocrit values were determined and the dye concentration in the plasma was determined spectrophotometrically at 605 nm. A graphical extrapolation of plasma dye concentration at time zero was used to calculate the plasma volume. Volume disturbance was minimized by infusion of same amount of physiological saline after each blood sampling. Blood volume was measured for 33- and 6‰-adapted fish and also for fish abruptly transferred from 33 to 6‰, and vice versa for 3 h.

Some fish were cannulated for blood pressure measurements. The afferent branchial cannula was connected to a pressure transducer coupled to a Harvard Biograph System (Harvard Apparatus, 2120). A three-way stopcock was connected between the transducer and the cannula to facilitate flushing the cannula with heparinized saline. All drugs were administered as bolus injections through the cannula in less than 0.2 ml physiological saline. Changes in blood pressure were measured in fish that had been abruptly transferred from 33 to 6‰ and vice versa with or without captopril blockade (50 nmol/100 g). The vasopressive effect of synthetic salmon-Ang I (Sigma) in 33- and 6‰-adapted sea bream was also measured before and after captopril inhibition.

2.7. Statistical analysis

Data were expressed as means ± S.E.M. For multiple groups, comparisons were carried out using one-way analysis of variance (ANOVA) followed by Tukey's test with $p < 0.05$ to delineate significance. For comparisons between two groups, unpaired Student's t test was used with $p < 0.05$ to delineate significance. In paired comparisons, the paired t test was used with $p < 0.05$ to indicate significance between pairs.

3. Results

3.1. Plasma levels of angiotensins

Serially diluted sea bream plasma extract showed displacement curves that exhibited strong parallelism to that of standard [Asn^1,Val^5]-Ang II. There was no significant difference in Ang II levels between plasma sampled with inhibitor and plasma sampled with heparin only, thus validating the use of plasma sampled without inhibitor for subsequent experiments. The antibody used in the present study recognizes the carboxyl terminal of Ang II, which can cross react with Ang II, Ang III, and Ang IV, so the assay was a collective measurement of different Ang species. However, the antibody does not cross react with Ang I or Ang (1–7), which has a different carboxyl terminal than that of Ang II, Ang III or Ang IV.

Circulating levels of angiotensins in sea bream adapted to different salinities (0–70‰) are shown in Fig. 1. A general U-shape pattern was observed with increasing salinity and the lowest and highest plasma Ang II levels were found at 6 and 70‰, respectively. The time course of changes in plasma Ang II levels of sea bream subjected to an abrupt hyposmotic challenge (33–6‰) is shown in Fig. 2. Within 2 h after transfer to 6‰, plasma Ang II dropped to a level similar to that of sea bream fully adapted to 6‰. Captopril injection potently decreased levels of circulating Ang II of both 33- or 6‰-adapted sea bream (Fig. 3).

3.2. Branchial Na–K-ATPase activity

Branchial Na–K-ATPase activity of sea bream adapted to different salinities is shown in Fig. 4. On adaptation to

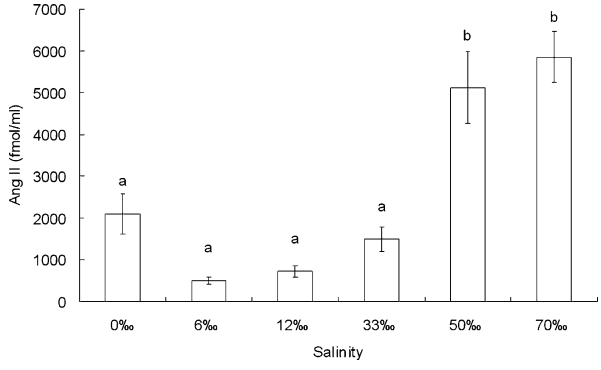

Fig. 1. Plasma Ang II concentration of silver sea bream adapted to different salinities ($n = 6$). A significant increase in plasma Ang II was found in double-strength seawater condition. Different letters denote significant difference among groups (one way ANOVA, Tukey's test, $p < 0.05$).

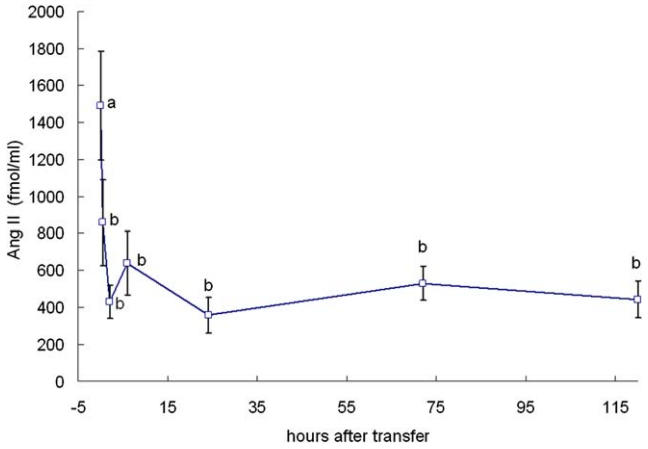

Fig. 2. Time course changes in plasma Ang II concentration of silver sea bream subjected to abrupt hyposmotic transfer from 33 to 6‰ ($n = 6$). Significant decrease in plasma Ang II started at 0.5 h after transfer and remained significantly depressed throughout the experiment. Different letters denote significant difference among groups (one-way ANOVA, Tukey's test, $p < 0.05$).

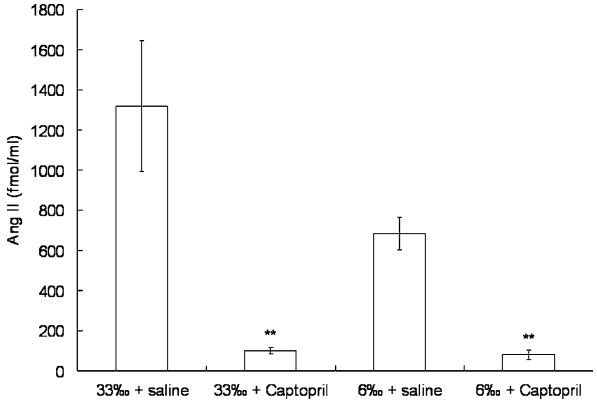

Fig. 3. Plasma Ang II levels of 33- and 6‰-adapted sea bream injected with saline and captopril (500 nmol/100 g) ($n = 6$). Double asterisk (**) indicates significant difference between saline-injected and captopril-injected groups using Student's t test ($p < 0.01$).

double-strength seawater (70‰), there was an over 10-fold increase in Na–K-ATPase activity when compared with those adapted to intermediate salinities (6–33‰) (Fig. 4). Although enzyme activities were not significantly different among fish adapted to 0–50‰, the pattern of branchial Na–K-ATPase activities exhibited a general U-shape pattern across the salinity axis, with Na–K-ATPase activity tending to be higher in salinity extremes while lower activities prevailed under brackish water conditions. Sea bream that were abruptly transferred from 33 to 6‰ developed a 10-fold elevation in branchial Na–K-ATPase activity 1 day after transfer, and high Na–K-ATPase activity was maintained throughout the entire transfer experiment (Fig. 5). Ang II injection significantly increased branchial Na–K-ATPase activity in sea bream adapted to both 33 and 6‰ (Fig. 6) in a dose-dependent manner. Ang II injection at low concentration (1 nmol/100 g) elicited a significant increase in branchial Na–K-ATPase in the 6‰-adapted sea bream but not in fish adapted to 33‰ (Fig. 6).

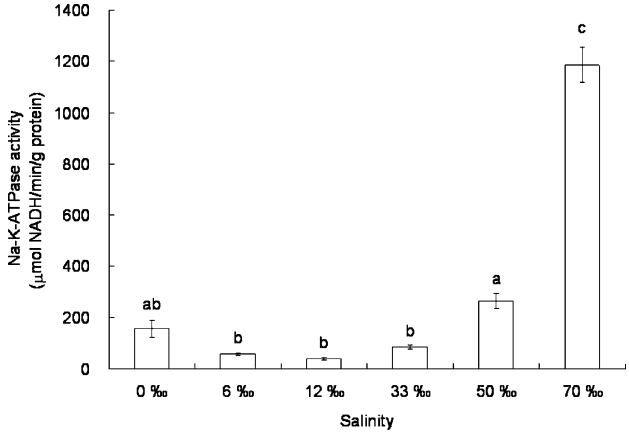

Fig. 4. Branchial Na–K-ATPase activity of silver sea bream adapted to different salinities ($n = 6$). ATPase activity was expressed as μmol NADH/min/g protein. A significant increase was found in double-strength seawater condition. Different letters denote significant difference among groups (one-way ANOVA, Tukey's test, $p < 0.05$).

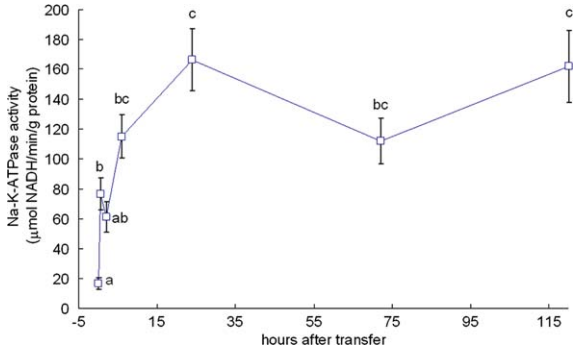

Fig. 5. Time course of changes in branchial Na–K-ATPase activity of silver sea bream subjected to abrupt hyposmotic transfer from 33 to 6‰ ($n = 6$). A significant increasing trend was found after abrupt hyposmotic transfer. Different letters denote significant difference among groups (one-way ANOVA, Tukey's test, $p < 0.05$).

3.3. Plasma cortisol

A general U-shape pattern was observed for plasma cortisol levels of sea bream adapted to different salinities (Fig. 7). Significantly lower cortisol levels were observed in brackish water environments and higher concentrations prevailed in freshwater and hyperosmotic conditions. Abrupt hyposmotic transfer resulted in generally higher plasma cortisol levels for the initial 6 h, but cortisol levels fell to significantly lowered levels 5 days after transfer when compared with the peak value at 6 h after transfer (Fig. 8). Captopril injection significantly reduced plasma cortisol levels in 33- and 6‰-adapted fish (Fig. 9).

3.4. Blood volume and blood pressure

Sea bream adapted to 33‰ had a resting blood pressure similar to that of 6‰-adapted fish (Table 1). Blood volumes of both 33- and 6‰-adapted sea bream were approximately 20 ml/kg (Table 2). Abrupt hypo- or hyper-osmotic transfer

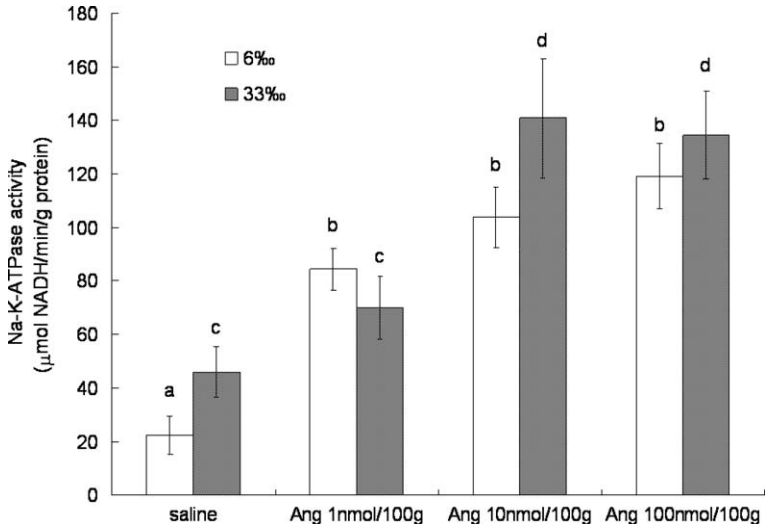

Fig. 6. Branchial Na–K-ATPase activity of 33‰-adapted and 6‰-adapted silver sea bream with intraperitoneal injection of various concentrations of Ang II ($n = 6$). Different letters denote significant difference among groups adapted to the same salinity (one-way ANOVA, Tukey's test, $p < 0.05$).

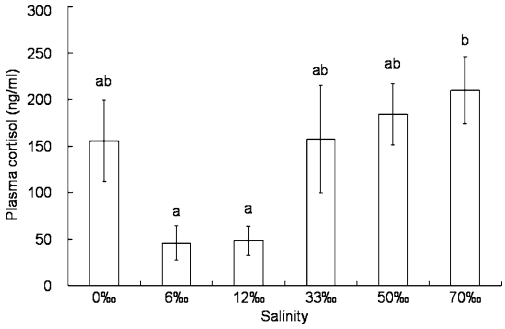

Fig. 7. Plasma cortisol levels of silver sea bream adapted to different salinities ($n = 6$). Cortisol concentration was expressed as ng/ml plasma. A significant decrease was found in brackish water environment (6 and 12‰). Different letters denote significant difference among groups (one-way ANOVA, Tukey's test, $p < 0.05$).

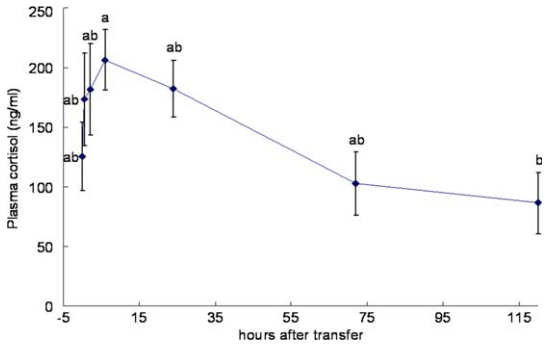

Fig. 8. Plasma cortisol levels of silver sea bream subjected to abrupt hyposmotic transfer from 33 to 6‰ ($n = 6$). Cortisol concentration was expressed as ng/ml plasma. Different letters denote significant difference among groups (one-way ANOVA, Tukey's test, $p < 0.05$). A significant decreasing trend was found after abrupt hyposmotic transfer for 5 days.

did not affect blood volume and blood pressure stability of sea bream. Intra-arterial injection of captopril lowered blood pressure significantly, an effect that was more pronounced in 33‰-adapted fish (Table 1). Ang I injection elicited a dose-dependent increase in blood pressure in 33- and 6‰-adapted sea bream, and the response to Ang I injection was generally more pronounced in 6‰-adapted fish, particularly in those which received low doses of Ang I (Fig. 10).

4. Discussion

Different species in the family Sparidae have become increasingly popular as models for research on teleostean osmoregulatory physiology (Kelly and Woo, 1999a,b; Kelly et al., 1999; Mancera et al., 1995; Woo and Fung, 1981). The sparid model used in our laboratory, the silver sea bream, *S. sarba*, has the advantage of possessing excellent osmoregulatory capabilities in terms of survival in a wide range of salinities (Kelly and Woo, 1999b) and tolerance of abrupt salinity transfers (Kelly and Woo, 1999a). The status of the RAS in teleosts has been shown to be related to several osmoregulatory responses including blood volume and pressure maintenance and drinking rate regulation (Olson, 1992; Kobayashi and Takei, 1996). Here we provide data to support the presence of an active RAS in silver sea bream and to outline the significance of a differential RAS status in salinity adaptation.

The circulating concentration of Ang II in silver sea bream was exceptionally high (500–6000 fmol/ml) compared with other experimental models such as seawater eel (50–200 fmol/ml) (Takei and Tsuchinda, 2000). Such discrepancy may be a consequence of species variation, but the other contributing factor may be activation of the RAS by the extremely high salinity conditions (50–70‰) used in the present experiments. There is a general trend that increasing environmental salinity would elevate plasma angiotensins in sea bream (6–70‰). Abrupt hyposmotic transfer (33–6‰) rapidly decreased plasma Ang II from a high level at 33‰ (~1500 fmol/ml) to a low level at 6‰ (~450 fmol/ml) within several hours, probably representing

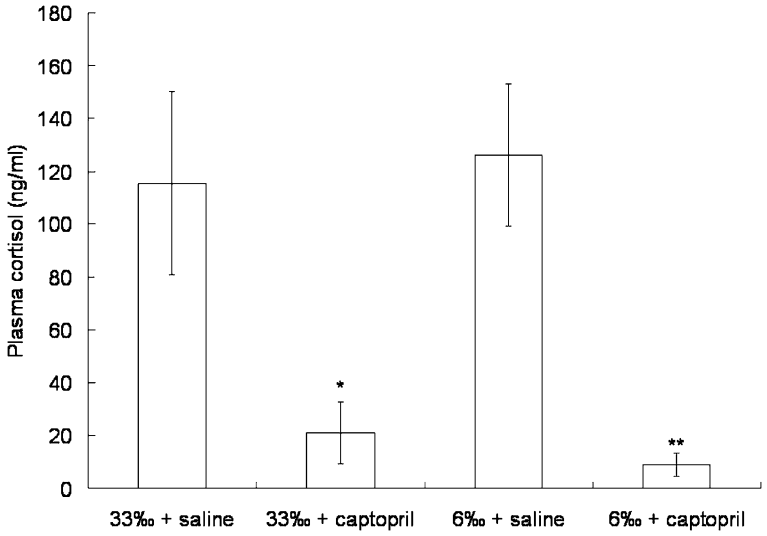

Fig. 9. Plasma cortisol levels of 33- and 6‰-adapted sea bream injected with saline and captopril (50 nmol/100 g) (n = 6). Asterisk (∗) and double asterisk (∗∗) indicate significant difference between saline-injected and captopril-injected groups using Student's *t* test ($p < 0.05$ and $p < 0.01$). Captopril significantly decreased plasma cortisol in both 33- and 6‰-adapted sea bream.

an adaptive phenomenon that acts against water influx and subsequent hypervolemia. Administration of captopril resulted in a significant decrease in plasma Ang II, an observation that indicates an angiotensin converting enzyme system similar to that reported for mammals and other vertebrates is present in sea bream, and such a system acts to limit the conversion of Ang I to Ang II.

Branchial Na–K-ATPase activities were generally low among fish groups in intermediate salinities (6–33‰), and an increasing trend was observed upon adaptation to hypersaline seawater (50–70‰). Our previous study showed that 6‰, as a salinity treatment, was already effective to stimulate the osmoregulatory organs of the sea bream to change from a seawater form to a "freshwater" form (Kelly and Woo, 1999b). The increase in branchial Na–K-ATPase in 0‰ compared to 6‰-adapted fish, though statistically insignificant, may imply that sea bream experience little difficulty in conserving ions through active branchial ion uptake at 6‰ but the process becomes increasingly difficult under freshwater conditions (0‰). Such contention was supported by the fact that mitochondria-rich cells increased in number and surface area in freshwater-adapted black sea bream (Kelly et al., 1999). The markedly higher Na–K-ATPase activities observed in gills of fish adapted to hypersaline seawater (50–70‰) probably reflect the response required for survival under the extremely steep ionic gradients that exist in such extreme environments.

Table 2
Mean changes in blood volume in 33- and 6‰-adapted silver sea bream subjected to abrupt osmotic transfer

Treatment	Blood volume (ml/kg body weight)
33–33‰ ($n = 7$)	21.2 ± 1.6
6–6‰ ($n = 7$)	20.8 ± 1.2
33–6‰ ($n = 8$)	21.0 ± 1.4
6–33‰ ($n = 6$)	20.1 ± 1.2

Measurements were taken 3 h after transfer. Blood volume was estimated using an indicator dilution method (T-1824 space). Blood volumes from various groups were not significantly different from each other (Student's *t* test, $p > 0.05$).

The pattern of changes in plasma cortisol was similar to that of plasma Ang II in sea bream adapted to different salinities. Captopril blockade drastically suppressed plasma cortisol level, implying a possible regulatory role of Ang II on cortisol secretion. In flounder, administration of Ang II and papaverine caused an increase in cortisol level which was abolished by captopril (Perrott and Balment, 1990). Plasma cortisol level also exhibited a similar rise following induced hypotension and hemorrhage in flounder (Carrick and Balment, 1984). When eels were transferred from

Table 1
Mean changes in blood pressure (BP) in 33- and 6‰-adapted silver sea bream subjected to abrupt osmotic transfer before and after captopril blockade

	Resting BP (mmHg)	BP after captopril blockade (mmHg)	BP after abrupt transfer to 6‰ (mmHg)	BP after abrupt transfer to 33‰ (mmHg)	BP after abrupt transfer to 6‰ (with captopril blockade) (mmHg)	BP after abrupt transfer to 33‰ (with captopril blockade) (mmHg)
33‰ ($n = 6$)	35.8 ± 1.7	NA	38.0 ± 2.5	NA	NA	NA
6‰ ($n = 7$)	31.4 ± 2.3	NA	NA	30.1 ± 2.3	NA	NA
33‰ ($n = 6$)	35.0 ± 2.4	$24.4 \pm 3.2^*$	NA	NA	25.6 ± 3.6	NA
6‰ ($n = 6$)	34.3 ± 1.7	$29.1 \pm 1.3^*$	NA	NA	NA	30.7 ± 1.8

* Significant difference (paired *t* test, $p < 0.05$) between blood pressure before and after captopril administration.

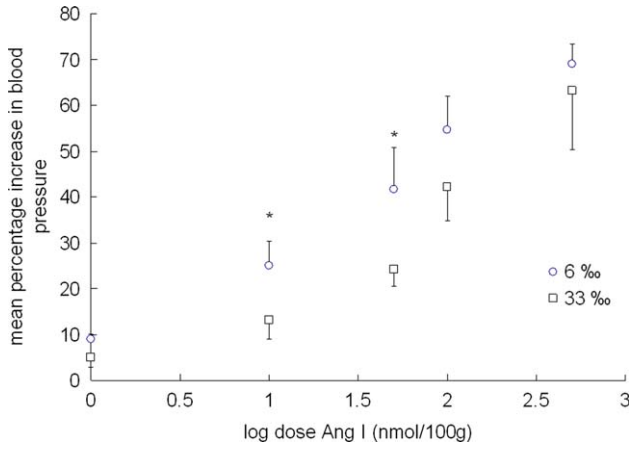

Fig. 10. Mean percentage increase in blood pressure in 6- and 33‰-adapted silver sea bream with bolus injections of various doses of Ang I ($n = 5$). Asterisk (*) indicates significant difference (paired t test, $p < 0.05$) in vasopressive response between two salinity groups receiving the same dose.

freshwater to seawater, the elevation in plasma cortisol level was blocked by administration of captopril (Kenyon et al., 1985). In a goldfish pituitary cell column, both Ang I and Ang II stimulated the release of ACTH (Weld and Fryer, 1987), implying a regulatory role of the RAS on the ACTH-cortisol axis and steroidogenesis in fish. However, during abrupt hyposmotic transfer, the patterns of changes in plasma Ang II and plasma cortisol are different, suggesting that changes in plasma Ang II and cortisol occur independently of each other during hyposmotic transfer. The influence of Ang II on plasma cortisol, as demonstrated by change following captopril administration, may be overridden by other endocrine factors during rapid changes in environmental salinity.

It is generally accepted that the RAS plays a more important role in hyperosmotic media to maintain blood pressure, blood volume and copious drinking (Olson, 1992). Apart from these effects, the peptide system was also found to be involved in regulation of Na–K-ATPase activity in the osmoregulatory organs of the eel including gill (Marsigliante et al., 1997), kidney (Marsigliante et al., 2000), and intestine (Marsigliante et al., 2001). Therefore, it is possible that the high circulating Ang II level observed in sea bream is related to the maintenance of high branchial Na–K-ATPase activity. Furthermore, intraperitoneal injection of Ang II to sea bream increased branchial Na–K-ATPase activity in a dose-dependent manner. The increase in branchial Na–K-ATPase activity by Ang II injection is unlikely to be mediated through the action of cortisol, since perfusing the isolated gill preparation with Ang II yielded similar stimulation of branchial Na–K-ATPase activity (Wong, M.K.S.; Woo, N.Y.S., unpublished data). Moreover, it appears that 6‰-adapted sea bream were relatively more sensitive to Ang II as a low dose of Ang II (1 nmol/100 g body weight) provoked significant elevation in branchial Na–K-ATPase activity in 6‰-adapted fish only. However, these results appeared to contradictory to that involving abrupt hyposmotic transfer, in that hyposmotic transfer was followed by a decrease in plasma angiotensins along with an increase in branchial Na–K-ATPase activity. This may indicate that some other hormonal systems may be involved in the regulation of gill reformation under hyposmotic challenge (Mancera et al., 1995). In the sea bream, the chloride cell was found to transform from a seawater (sunken) to a freshwater (protruding) type within 3 h after abrupt hyposmotic transfer (Kelly and Woo, 1999a). A decline in plasma Ang II during abrupt hyposmotic exposure may lower blood pressure, tending to stabilize blood volume, and ease the effect of sudden water influx which would otherwise cause hypervolemia. However, under such conditions, a high rate of ion loss across the gill epithelium is expected and therefore an increase in Na–K-ATPase activity may facilitate ion conservation (McCormick, 1995). Such a response could be triggered by heightened cortisol levels as significantly higher circulating cortisol level was found after hyposmotic transfer, which may contribute to stimulation of branchial Na–K-ATPase activity, which in turn might override the effect of Ang II (Perrott and Balment, 1990). The detailed mechanism is yet to be elucidated but it is clear that a subdued RAS status is prevalent following abrupt hyposmotic transfer.

The blood volume of sea bream was approximately 20 ml/kg body weight in both hyperosmotic (33‰) and hyposmotic (6‰) media. These values were comparable to that of the trout (Conte et al., 1963) but considerably lower than the blood volumes reported for high-energy demand teleosts such as the yellowfin tuna (Brill et al., 1998). When sea bream were abruptly transferred from 33 to 6‰ or vice versa, the blood volume remained unchanged. This finding is similar to the situation in the eel where blood volume remained constant upon transfer from seawater to freshwater (Nishimura et al., 1976). Blood pressure of silver sea bream was around 30–35 mmHg which is close to the ventral aortic pressure reported in other experimental fish models (Chester Jones et al., 1969; Platzack et al., 1993). Our previous experiment has shown that sham-salinity transfer had no effect on the blood pressure of silver sea bream adapted to hyper- or hyposmotic salinities (Wong, unpublished data). Blood pressure remained highly stable following abrupt hyposmotic (33–6‰) and hyperosmotic (6–33‰) transfers. This is in contrast to the situation in the eel where a higher resting blood pressure occurred in freshwater than in seawater (Chester Jones et al., 1969; Tierney et al., 1995). Taken together, the results of these abrupt hyposmotic transfer experiments showed that sea bream may counteract blood pressure and volume changes by decreasing circulating Ang II, and therefore Ang II may be the contributing factor behind the extreme stability of blood volume and pressure exhibited by sea bream during abrupt salinity changes.

Blood pressure of silver sea bream injected with physiological saline showed no significant difference with the pre-injection value. Blood pressure of 33- and 6‰-adapted sea bream was significantly lowered by intra-arterial administration of captopril. The hypotensive effect of

captopril in 6‰-adapted sea bream differed from the results obtained in freshwater eels, in which captopril did not have any effect on resting blood pressure (Tierney et al., 1995). However, captopril administration elicited significant hypotension in seawater-adapted toadfish (Madey et al., 1984; Nishimura and Bailey, 1982) and freshwater-adapted trout (Galardy et al., 1984; Lipke and Olson, 1990). The existence of a hypotensive effect of captopril in sea bream indicated that the RAS was involved in regulating resting blood pressure. However, when captopril-treated sea bream were abruptly transferred from 33 to 6‰ or vice versa, blood pressure did not change significantly, indicating that the RAS was not the sole hormonal system regulating blood pressure of sea bream and when the RAS is "shut off", compensatory events may occur in sea bream that would tend to stabilize blood pressure rapidly.

Intra-arterial injection of Ang I elicited a dose-dependent increase in blood pressure in both 33- and 6‰-adapted sea bream. However, the same dose of Ang I induced a greater increase in blood pressure in 6‰-adapted sea bream than those adapted to 33‰. The explanation for such a discrepancy may reside in the low circulating level of Ang II in 6‰-adapted fish, as injection of the same dose of Ang II would tend to cause a greater percentage increase in the circulating peptide in hyposmotic sea bream. The higher sensitivity of 6‰-adapted sea bream to angiotensin could be related to receptor upregulation or sensitization, but further experiments are needed to confirm such hypothesis. Captopril treatment abolished the vasopressive effect of Ang I but not that of Ang II, confirming that Ang II is the biologically active form. From a functional perspective, such discrepant response to angiotensins may serve as a preparatory tool that allows sea bream to re-enter hyperosmotic medium rapidly. Although sea bream is a predominantly marine fish, such euryhalinity corroborates the suggestion of a freshwater or brackish water origin of the species, and the retention of rapidly adjustable osmoregulatory machinery capable of a return to the dilute medium from which they have evolved. An active RAS is likely to be one arm of the osmoregulatory machinery in view of a high correlation of its status with the external salinities.

In conclusion, the status of the RAS in sea bream varies with salinity, being particularly activated in hyperosmotic media and thus allowing the system to play a more important role in maintaining volume, pressure, and other functions in a dehydrating environment. Hyperosmotic (33‰) sea bream has a more activated RAS than those in hyposmotic (6‰) medium on the grounds that there was a higher circulating level of angiotensins in 33‰-adapted sea bream and abrupt hyposmotic transfer rapidly decreased the plasma level of angiotensins. On the other hand, 6‰-adapted sea bream is more sensitive to angiotensins because administration of angiotensin induced a larger vasopressive response and stimulatory effect on branchial Na–K-ATPase activity. Inhibition of ACE by captopril lowered plasma cortisol levels, indicating a possible role of the RAS in controlling cortisol secretion or catabolism. Our results clearly demonstrate that the RAS is related to the maintenance of osmoregulatory functions in sea bream and is rapidly recruited for the regulation of body fluid homeostasis under changing osmotic conditions.

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