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ScienceDirect
General and Comparative Endocrinology 148 (2006) 348–358
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Osmoregulatory and metabolic changes in the gilthead sea bream *Sparus auratus* after arginine vasotocin (AVT) treatment

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Received 31 October 2005; revised 11 April 2006; accepted 17 April 2006
Available online 5 June 2006

Abstract

The influence of arginine vasotocin (AVT) on osmoregulation and metabolism in gilthead sea bream *Sparus auratus* was evaluated by two experimental approaches. In the first, seawater (SW, 36 ppt)-acclimatized fish were injected intraperitoneally with vehicle (vegetable oil) or two doses of AVT (0.5 and 1 μg/g body weight). Twenty-four hours later, eight fish from each group were sampled; the remaining fish were transferred to low saline water (LSW, 6 ppt, hypoosmotic test), SW (transfer control), and hypersaline water (HSW, 55 ppt, hyperosmotic test). After another 24 h (48-h post-injection), fish were sampled. The only significant effect observed was the increase of sodium levels in AVT-treated fish transferred to HSW. In the second experiment, fish were injected intraperitoneally with slow-release vegetable oil implants (mixture 1:1 of coconut oil and seeds oil) alone or containing AVT (1 μg/g body weight). After 3 days, eight fish from each group were sampled; the remaining fish were transferred to LSW, SW, and HSW as above, and sampled 3 days later (i.e. 6 days post-injection). In the AVT-treated group transferred from SW to SW, a significant increase vs. control was observed in gill Na^+,K^+-ATPase activity. Kidney Na^+,K^+-ATPase activity decreased in the AVT-treated group transferred to LSW and no changes were observed in the other groups. These osmoregulatory changes suggest a role for AVT during hyperosmotic acclimation based on changes displayed by gill Na^+,K^+-ATPase activity. AVT treatment increased plasma cortisol levels in fish transferred to LSW and HSW. In addition, AVT treatment affected parameters of carbohydrate, lipid, amino acid, and lactate metabolism in plasma and tissues (gills, kidney, liver, and brain). The most relevant effects were the increased potential of liver for glycogen mobilization and glucose release resulting in increased plasma levels of glucose in AVT-treated fish transferred to LSW and HSW. These changes may be related to the energy repartitioning process occurring during osmotic adaptation of *S. auratus* to extreme environmental salinities and could be mediated by increased levels of cortisol in plasma.

Keywords: Gilthead sea bream; *Sparus auratus*; AVT; Energy metabolism; Osmoregulation

1. Introduction

In teleost fish, the nonapeptide hormone arginine vasotocin (AVT) plays a role in the maintenance of salt and fluid balance, cardiovascular activity, reproduction, and in neurotransmission and neuromodulation processes in the central nervous system (Balment et al., 1993; Warne, 2002; Warne et al., 2002). An osmotic challenge alters plasma AVT levels (Perrot et al., 1991; Balment et al., 1993; Pierson et al., 1995; Kulczykowska, 2001; Warne et al., 2005) and pituitary content of the peptide (Perrot et al., 1991; Harding et al., 1997), suggesting an osmoregulatory role for AVT in teleosts.

In teleost fish, functional AVT receptors have been demonstrated in gills, with higher number in seawater (SW)-adapted fish than in freshwater (FW)-adapted fish

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0016-6480/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.ygcen.2006.04.005

(Guibbolini et al., 1988; Warne et al., 2002). However, whether these receptors are specifically present in mitochondrial-rich cells and the effect of AVT on function of these cells remain unclear (Guibbolini and Avella, 2003). In addition, AVT receptors have also been demonstrated in teleost fish kidney (Perrot et al., 1993; Warne, 2001). AVT has both diuretic and antidiuretic effects dependent on the dose used (Amer and Brown, 1995). However, recent studies showed that in eel and other teleosts low circulating levels of AVT are present and this indicates that the antidiuretic effect is the physiological one (Balment et al., 1993). However, the mechanisms by which AVT brings about its antidiuretic effect in kidney are not well understood, but the existence of both a decrease in glomerular filtration rate and an increase in tubular water reabsorption has been proposed (Amer and Brown, 1995; Warne, 2002; Warne et al., 2002).

Gilthead sea bream (*Sparus auratus*) is a euryhaline species in which we have demonstrated osmoregulatory and metabolic changes during acclimation to variable ambient salinities (Laiz-Carrión et al., 2005; Sangiao-Alvarellos et al., 2003, 2005), and the effects of several hormones on osmoregulatory and metabolic changes associated with these acclimations (Mancera et al., 2002; Laiz-Carrión et al., 2003).

Several studies have shown changes in AVT levels during osmotic acclimation of teleosts (see above). In addition, AVT treatment has been demonstrated to be associated in some cases, with increased glycogenolytic potential in liver (Janssens and Lowrey, 1987; Moon and Mommsen, 1990) and brain (Sangiao-Alvarellos et al., 2004). Different hormones could be involved in the interconnection between osmotic acclimation and changes in energy metabolism. In this way, one candidate to regulate energy metabolism during osmotic acclimation could be AVT. Therefore, we aimed to evaluate the possible role of AVT on osmoregulatory and metabolic performance of gilthead sea bream before and after transfer to different salinities.

2. Materials and methods

2.1. Fish and experimental salinities

Sexually immature (male) gilthead sea bream (*S. auratus* L., 56 ± 0.9 g body weight in Experiment 1 and 144 ± 3.1 g in Experiment 2) were provided by Planta de Cultivos Marinos (C.A.S.E.M., Universidad de Cádiz, Puerto Real, Cádiz, Spain) and transferred to the laboratories at Faculty of Marine Science (Puerto Real, Cádiz). They were acclimatized to SW in 500-l aquaria for, at least, 2 weeks in an open system (36 ppt salinity) before the experiments. Low-salinity water (LSW, 6 ppt salinity) was obtained by mixing SW with dechlorinated tap water in a recirculating system. Hypersaline water (HSW, 55 ppt salinity) was obtained by mixing full SW with natural marine salts (Unionsal, Cádiz, Spain) in a recirculating system. The system in the tanks containing SW was also recirculated to keep the set up comparable with that of LSW and HSW fish. Once the systems were recirculated, the common water quality criteria (hardness, and the levels of oxygen, carbon dioxide, hydrogen sulfide, nitrite, nitrate, ammonia, calcium, chlorine, and suspended solids) were assessed throughout the experiment. No major changes were observed. Water salinity was checked everyday and corrected when necessary. During the experiments (June–July 2004), fish were maintained under natural photoperiod and constant temperature (21 °C), and fed daily with a ration of 1% body weight commercial dry pellets (Dibaq-Diprotg SA, Segovia, Spain). They were fasted for 24 h before sampling. The experiments described comply with the Guidelines of the European Union Council (86/609/EU) and of the University of Cádiz (Spain) for the use of laboratory animals.

2.2. Experimental design

2.2.1. Experiment 1

SW-acclimatized fish were lightly anaesthetized with 2-phenoxyethanol (0.05%, v/v), weighed, intraperitoneally injected with 10 μl/g body weight of seeds oil alone (control, $n = 32$) or containing AVT (0.5 or 1 μg/g body weight, $n = 32$ per dose). Twenty-four hours after injection, eight fish from each group were sampled and the rest of the group ($n = 24$) was transferred to LSW (hypoosmotic test, $n = 8$), SW (transfer control, $n = 8$), and HSW (hyperosmotic test, $n = 8$). Twenty-four hours after transfer (48-h post-injection), all groups were sampled (see below). One group of uninjected fish ($n = 9$) was also sampled and served as an extra control.

2.2.2. Experiment 2

SW-acclimatized fish were lightly anaesthetized with 2-phenoxyethanol (0.05%, v/v), weighed, intraperitoneally injected with slow-release vegetable oil implants (mixture 1:1 of coconut oil and seeds oil) following procedures previously described for hormone administration (Shrimpton and McCormick, 1999; Pelis and McCormick, 2001). Thus, fish were injected with 10 μl/g body weight of slow-release vegetable oil alone (control, $n = 32$) or containing AVT (1 μg/g body weight, $n = 32$). Three days later implant, eight fish of each group were sampled and the rest of the fish ($n = 24$) was transferred to LSW (hypoosmotic test, $n = 8$), SW (transfer control, $n = 8$), and HSW (hyperosmotic test, $n = 8$). Three days after transfer (6 days post-injection), all groups were sampled (see below). One group of uninjected fish ($n = 8$) was also sampled at the same time and served as extra control.

2.3. Sampling

Fish were deeply anaesthetized with 2-phenoxyethanol (0.1%, v/v), weighed, and sampled. Blood was obtained with ammonium-heparinized syringes from the caudal vessels. Plasma samples were obtained by centrifugation of blood (30 s at 13,000*g*) and divided into two aliquots. One aliquot was immediately frozen on liquid nitrogen for the assessment of plasma osmolality, and levels of ions and protein, whereas the other aliquot was deproteinized immediately (using 6% perchloric acid) and neutralized (using 1 mol/l potassium bicarbonate) before freezing on liquid nitrogen and storage at −80 °C until further assay. To assess Na^+,K^+-ATPase activity 3–5 filaments from the second branchial arch (cut just above the septum with fine point scissors) and a small portion of the caudal kidney were taken and placed in 100 μl of ice-cold SEI buffer (150 mmol/l sucrose, 10 mmol/l EDTA, 50 mmol/l imidazole, pH 7.3) and frozen at −80 °C. Brain, liver, and the remaining kidney and branchial arches were removed in few seconds from each fish, freeze-clamped on liquid nitrogen, and stored at −80 °C until further assay.

2.4. Analytical techniques

Plasma glucose, lactate, and triglyceride levels were measured using commercial kits from Spinreact (Spain) adapted to microplates. Plasma protein was measured using the bicinchoninic acid method with BCA protein kit (Pierce, Rockford, USA) for microplates, with bovine albumin as standard. Plasma Cl^- levels were measured with the Chloride Sigma kit (No. 461-3). Plasma osmolality was measured with a vapor pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA) and expressed as mOs m/kg. Plasma Na^+ was measured using an atomic absorption spectrophotometer (Philips PU7000).

AVT levels in plasma were determined by high-performance liquid chromatography (HPLC) with fluorescence detection preceded by solid-

phase extraction (SPE). HPLC assay was performed with a Beckman modular system (Beckman Instruments, San Ramon, CA, USA) with spectrofluorometric detector RF-551 (Shimadzu, Columbia, MD). Chromatographic separations were carried out on an Ultrasphere ODS column (250 × 4.6 mm i.d., 5 μm particle diameter) preceded by a precolumn (45 × 4.6 mm i.d.) filled with the same material (both from Beckman Instruments, San Ramon, CA, USA). Fluorescence detection was carried out at 530 nm with excitation at 470 nm. SPE procedures were accomplished on Bakerbond spe™ Octadecyl C_{18} Speedisk (20 mg, 1 ml) connected to the Baker SPE 12G column Processor (J.T. Baker, Phillipsburg, NJ, USA). The method has been described in detail by Gozdowska and Kulczykowska (2004) with subsequent modification appended by Gozdowska et al. (2006).

Plasma cortisol levels were measured by indirect enzyme immunoassay (ELISA) validated for gilthead sea bream (Tintos et al., 2006).

Gill and kidney Na^+,K^+-ATPase activity were determined using the microassay method of McCormick (1993) adapted for *S. auratus* (Mancera et al., 2002).

Frozen liver, brain, kidney, and gill were finely minced on a chilled Petri dish to very small pieces that, still frozen, were mixed and divided into two different (but relatively homogeneous) aliquots to assess enzyme activities and metabolite levels, respectively. The aliquots of frozen tissue used for the assessment of metabolite levels were homogenized immediately by ultrasonic disruption with 7.5 vol of ice-cooled 6% perchloric acid, neutralized (using 1 mol/l of potassium bicarbonate), centrifuged (2 min at 13,000*g*, Eppendorf 5415R), and the supernatant used to assay tissue metabolites. Tissue lactate and triglyceride levels were determined spectrophotometrically using a commercial kit (Spinreact, Spain). Tissue glycogen levels were assessed using the method of Keppler and Decker (1974). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Biomérieux, Spain). Tissue total α-amino acids were assessed colorimetrically using the nynhidrin method of Moore (1968) with modifications to adapt the assay to a microplate format. The aliquots of tissues used for the assessment of enzyme activities were homogenized by ultrasonic disruption with 10 vol of ice-cold stopping-buffer containing: 50 mmol/l imidazole–HCl (pH 7.5), 15 mmol/l 2-mercaptoethanol, 100 mmol/l KF, 5 mmol/l EDTA, 5 mmol/l EGTA, and a protease inhibitor cocktail (Sigma, P-2714). The homogenate was centrifuged and the supernatant used in enzyme assays. In those cases, where microsomal enzymes were assessed, appropriate centrifugations were carried out to obtain the samples. Enzyme activities were determined using a Unicam UV-2 spectrophotometer (Thermo Unicam, Waltham, USA). Reaction rates of enzymes were determined by the increase or decrease in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition of homogenates (0.05 ml), at a pre-established protein concentration, omitting the substrate in control cuvettes (final volume of 1.35 ml), and allowing the reactions to proceed at 15 °C for pre-established times (5–15 min). No changes were found in tissue protein levels in any of the groups studied (data not shown), and therefore enzyme activities are expressed in terms of milligram of protein. Protein was assayed in triplicate in homogenates using microplates following the bicinchoninic acid method with bovine serum albumin (Sigma, USA) as standard. The specific conditions for enzyme assays were described previously (Laiz-Carrión et al., 2003; Sangiao-Alvarellos et al., 2003, 2005).

2.5. Statistics

In Experiment 1, the differences among groups before transfer were assessed by one-way ANOVA with treatment (control, 0.5, or 1 μg AVT/g body weight) as the independent variable. After transfer, the differences among groups were assessed by two-way ANOVA with treatment (control, 0.5, or 1 μg AVT/g body weight) and salinity as independent variables. When significant differences were obtained from the ANOVA, multiple comparisons were carried out using the Tukey test. Significance level was set at $P < 0.05$.

In Experiment 2, the differences between control and AVT-treated fish after 3 days of implantation were assessed with a Student's *t* test (significance level $P < 0.05$). The differences observed between different groups assessed after implantation followed by subsequent transfer to different salinities were analyzed using a two-way ANOVA with treatment (control and AVT) and salinity (LSW, SW, and HSW) as independent variables. When significant differences were obtained from the ANOVA, multiple comparisons were carried out using the Tukey test. Significance level was set at $P < 0.05$.

In both experiments, values of SW-control and uninjected fish were compared using a Student's *t* test (significance level $P < 0.05$).

3. Results

3.1. Experiment 1

There were no differences between SW-control and uninjected fish for all parameters assessed (data not shown). One day after injection, prior to salinity transfer, no significant differences were observed between control and AVT group in any of the parameters assessed. Because of the small volume of plasma, it was not possible to evaluate AVT plasma levels in this experiment.

Significant salinity effects were noticed for plasma osmolality, and levels of sodium, lactate, triglyceride, and protein. No effects of AVT treatment were observed in gill Na^+,K^+-ATPase activity (Fig. 1A) and plasma osmolality (Fig. 1B). The values of plasma Na^+ increased after AVT treatment in fish transferred to HSW (Fig. 1C). No significant differences were observed for plasma Cl^- levels (data not shown). No effects of AVT were also noticed for plasma levels of glucose, lactate, and protein (data not shown). Plasma triglyceride levels decreased in fish treated with the lower dose of AVT compared with controls in SW and HSW (data not shown).

3.2. Experiment 2

There were no differences between SW-control and uninjected fish for all parameters assessed (data not shown).

Because of the small amount of plasma, it was necessary to pool plasma samples of fish in each group. AVT plasma values in uninjected fish and SW-control were similar (range 2.69 and 2.79 pmol/ml). Hormonal treatment for 3 days increased AVT plasma levels (up to 5.30 pmol/ml in SW-acclimated fish before transfer). In oil-treated fish, AVT plasma levels showed a linear relationship with respect to environmental salinity (LSW: 1.69 pmol/ml, SW: 2.81 pmol/ml, HSW: 4.54 pmol/ml). However, this was not the case for AVT-treated fish (LSW: 3.7 pmol/ml, SW: 3.37 pmol/ml, HSW: 3.17 pmol/ml).

In osmoregulatory and plasma parameters, significant salinity effects were noticed for gill Na^+,K^+-ATPase activity, and plasma osmolality and levels of cortisol, glucose, lactate, and triglycerides. In addition, a significant interaction (salinity × AVT treatment) was observed for gill Na^+,K^+-ATPase activity, and plasma cortisol and glucose levels. AVT treatment increased gill Na^+,K^+-ATPase activity only in fish maintained in SW (Fig. 2A), while kidney Na^+,K^+-ATPase activity decreased in AVT-treated group transferred to LSW (Fig. 2B). Plasma osmolality (Fig. 2C),

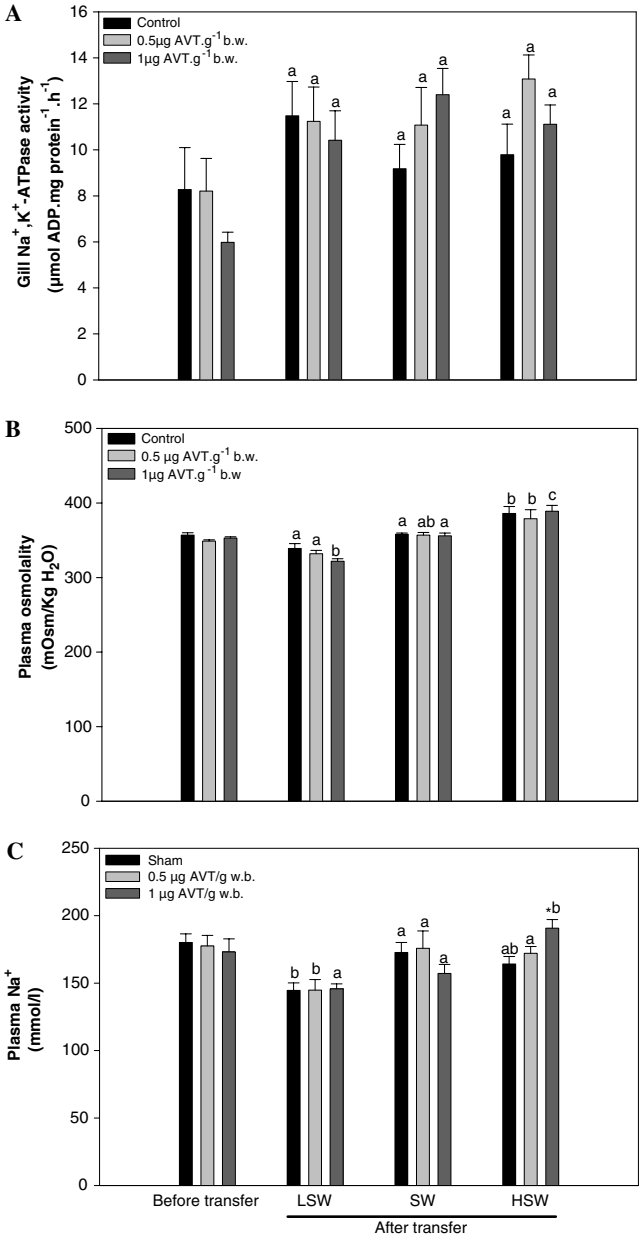

Fig. 1. Effects of an injection of seeds oil alone (control) or containing AVT (0.5 or 1 µg/g body weight) on gill Na^+,K^+-ATPase activity (A), plasma osmolality (B), and plasma Na^+ (C) before and after salinity transfer from SW (36 ppt) to LSW (6 ppt), SW (36 ppt), or HSW (55 ppt). Fish were intraperitoneally injected and kept in SW for 24 h. One group was sampled before transfer and the remaining fish from each group were transferred to new environmental salinities for 24 h and sampled. Values are the means ± S.E.M. ($n = 8$ fish per group). *Significantly different ($P < 0.05$) from fish injected with seeds oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (LSW, SW, HSW) within each treatment (control, 0.5, or 1 µg AVT/g body weight).

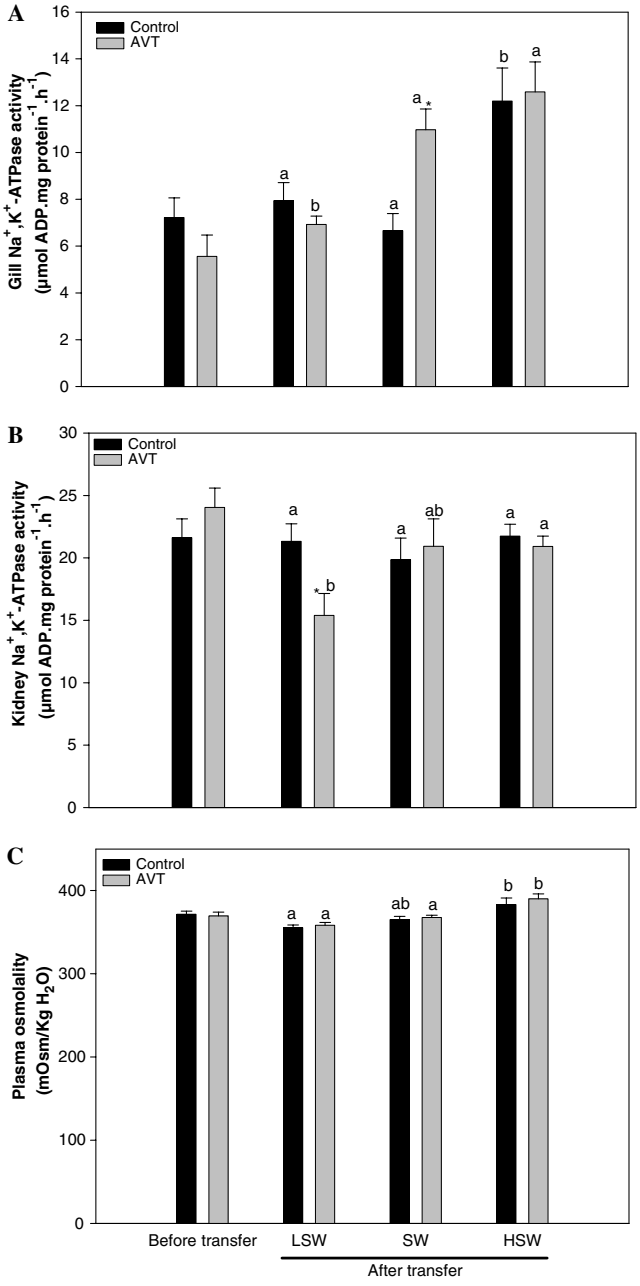

Fig. 2. Effects of intraperitoneal implantation of vegetable oil (mixture 1:1 of coconut oil and seeds oil) alone (control) or containing AVT (1 µg/g body weight) on gill Na^+,K^+-ATPase activity (A), kidney Na^+,K^+-ATPase activity (B), and plasma osmolality (C) before and after salinity transfer from SW (36 ppt) to LSW (6 ppt), SW (36 ppt), or HSW (55 ppt). Fish were intraperitoneally implanted and kept in SW for 3 days. One group was sampled before transfer and the remaining fish from each group were transferred to new environmental salinities and sampled after 3 days. Values are the means ± S.E.M. ($n = 8$ fish per group). *Significantly different ($P < 0.05$) from fish injected with oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (LSW, SW, HSW) within each treatment (control or AVT).

and Na^+ and Cl^- levels (data not shown) were not affected by hormonal treatment.

Plasma cortisol levels were enhanced by AVT treatment in fish transferred to LSW and HSW (Fig. 3), while plasma lactate levels (Fig. 4A) were not affected by AVT treatment. The values of plasma triglycerides decreased in AVT-treated fish before transfer (Fig. 4B). Plasma glucose levels increased in AVT-treated groups compared with controls after transfer to LSW and HSW (Fig. 4C). There were no significant differences in plasma protein levels (data not shown).

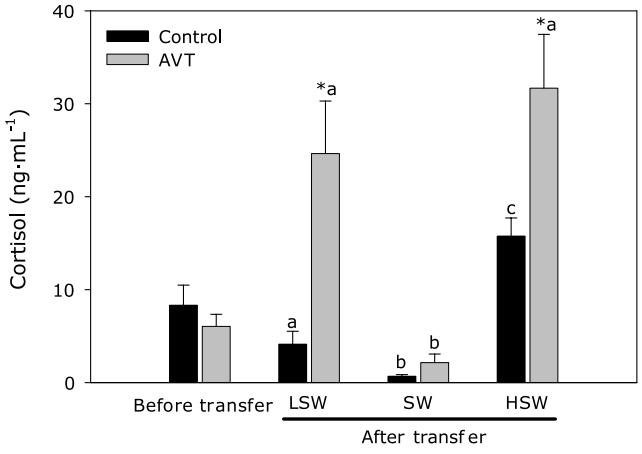

Fig. 3. Effects of intraperitoneal implantation of vegetable oil (mixture 1:1 of coconut oil and seeds oil) alone (control) or containing AVT (1 μg/g body weight) on plasma cortisol levels before and after salinity transfer from SW (36 ppt) to LSW (6 ppt), SW (36 ppt), or HSW (55 ppt). See legend of Fig. 2 for further details.

Metabolic parameters assessed in liver are displayed in Table 1. Before transfer, AVT treatment increased glucose and amino acid levels and G6Pase[1] activity. After transfer, significant salinity effects were noticed for triglyceride levels and FBPase, G6Pase, and PFK (optimal) activities. Significant effects of AVT were observed for glycogen and triglyceride levels, and GPase (total) and G6Pase activities. A significant interaction (salinity × AVT treatment) was observed for glycogen and lactate levels, and GPase (total), G6Pase, and GDH activities. No significant changes were noticed for G6PDH, GDH, and HOAD activities (data not shown).

Table 2 displayed metabolic parameters assessed in gills. Before transfer, AVT treatment increased amino acid levels, and PK (optimal) and GDH activities. After transfer, significant salinity effects were observed for glycogen, glucose, lactate, and amino acid levels, and GPase (total) and PK (optimal) activities. Significant effects of AVT treatment were noticed for glucose, and GPase (total) and HK activities. In addition, significant interactions (salinity × AVT treatment) occurred in glycogen and glucose levels as well as in HOAD and LDH-O activities. No significant changes were noticed for G6PDH activity (data not shown).

Metabolic parameters assessed in kidney are displayed in Table 3. Before transfer, AVT treatment increased glucose levels and decreased PK (activity ratio) and HK activities. After transfer, significant salinity effects were noticed for glycogen, glucose, and lactate levels and GPase (total and %GPase *a*), PK (optimal, activity ratio, and activation ratio), GDH, and LDH-O activities. Significant AVT effects were observed in glycogen, glucose, and lactate levels as well as in PK (activity and activation ratios) and LDH-O activities. A significant interaction (salinity × AVT treatment) was observed for glycogen and glucose levels, and HK and PK (activation ratio) activities. No significant changes were noticed for amino acid and triglyceride levels as well as G6PDH and GDH activities (data not shown).

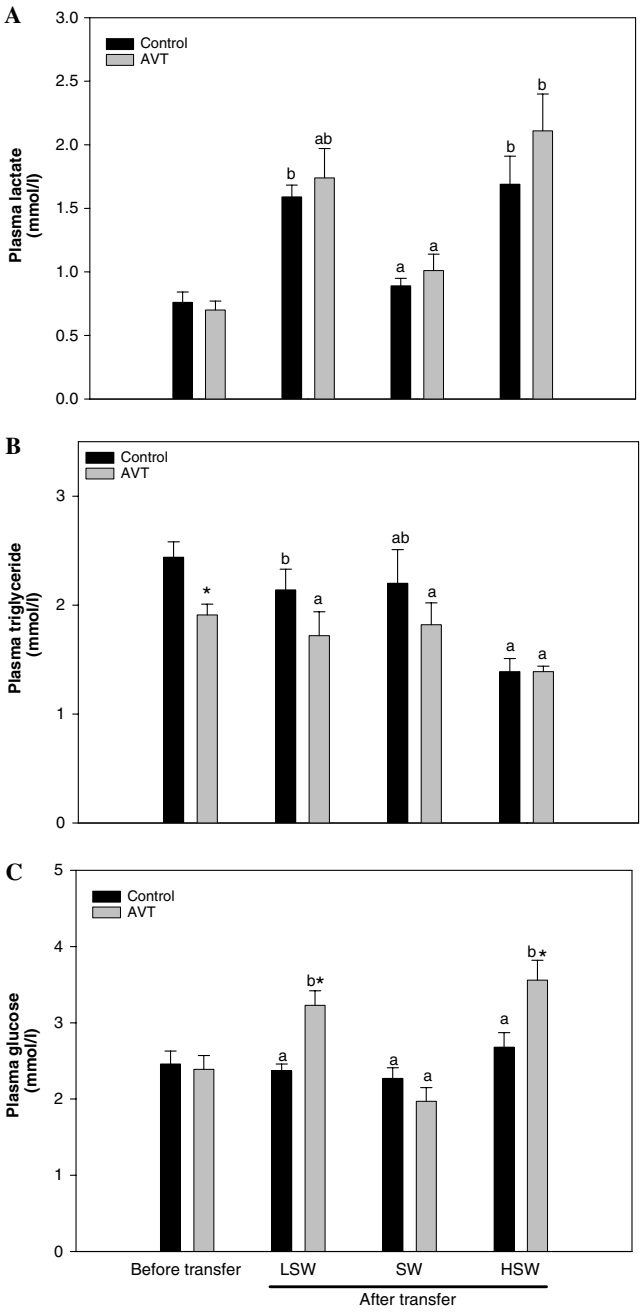

Fig. 4. Effects of intraperitoneal implantation of vegetable oil (mixture 1:1 of coconut oil and seeds oil) alone (control) or containing AVT (1 μg/g body weight) on plasma lactate (A), triglyceride (B), and glucose (C) levels before and after salinity transfer from SW (36 ppt) to LSW (6 ppt), SW (36 ppt), or HSW (55 ppt). See legend of Fig. 2 for further details.

[1] *Abbreviations used:* Asp-AT, aspartate aminotransferase (EC. 2.6.1.1.); G6Pase, glucose 6-phosphatase (EC. 3.1.3.9.); G6PDH, glucose 6-phosphate dehydrogenase (EC. 1.1.1.49.); GDH, glutamate dehydrogenase (EC. 1.4.1.2.); GPase, glycogen phosphorylase (EC. 2.4.1.1.); HK, hexokinase (EC. 2.7.1.1.); HOAD, 3-hydroxiacil-CoA-dehydrogenase (EC. 1.1.1.35.); LDH-O, lactate dehydrogenase-oxidase (EC. 1.1.1.27.); PFK, 6-phosphofructo 1-kinase (EC. 2.7.1.11.); PK, pyruvate kinase (EC. 2.7.1.40.).

Table 1
Liver changes in metabolite levels and enzyme activities in SW-acclimatized gilthead sea bream 3 days after intraperitoneal implantation of 10 μl/g body weight of vegetable oil alone (control) or containing AVT (1 μg/g body weight), followed by 3 days of acclimation to LSW (6 ppt), SW (36 ppt), or HSW (55 ppt)

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Glycogen (μmol glycosyl units/g wet wt.)	Control	494 ± 14.2	477 ± 14.8[a]	474 ± 16.5[a]	473 ± 16.9[a]
	AVT	501 ± 7.63	305 ± 21.3*,[b]	485 ± 15.2[a]	287 ± 17.7*,[b]
Glucose (μmol/g wet wt.)	Control	1.17 ± 0.02	1.34 ± 0.05[a]	1.31 ± 0.06[a]	1.25 ± 0.06[a]
	AVT	1.37 ± 0.04*	1.27 ± 0.09[a]	1.36 ± 0.05[a]	1.44 ± 0.09[a]
Lactate (μmol/g wet wt.)	Control	0.17 ± 0.03	0.17 ± 0.03[a]	0.20 ± 0.03[a]	0.22 ± 0.02[a]
	AVT	0.16 ± 0.04	0.21 ± 0.02[a]	0.18 ± 0.02[a,b]	0.12 ± 0.02*,[b]
Total α-amino acids (μmol/g wet wt.)	Control	10.1 ± 0.20	12.1 ± 0.80[a]	12.0 ± 1.01[a]	13.7 ± 1.32[a]
	AVT	12.3 ± 0.38*	11.8 ± 0.59[a]	11.3 ± 1.01[a]	10.0 ± 1.26[a]
Triglyceride (μmol/g wet wt.)	Control	34.7 ± 4.04	26.0 ± 2.27[a]	27.7 ± 2.69[a]	22.4 ± 1.90[a]
	AVT	36.5 ± 6.07	21.5 ± 0.99[b]	26.0 ± 2.24[a]	19.8 ± 1.09[b]
GPase activity					
Total activity (U/mg protein)	Control	0.74 ± 0.06	0.61 ± 0.04[a]	0.72 ± 0.05[a]	0.72 ± 0.08[a]
	AVT	0.66 ± 0.03	0.84 ± 0.05*,[a,b]	0.65 ± 0.05[a]	0.90 ± 0.06*,[b]
% GPase *a*	Control	18.8 ± 1.97	19.2 ± 1.74[a]	20.4 ± 0.86[a]	21.7 ± 2.29[a]
	AVT	20.3 ± 1.69	19.4 ± 0.80[a]	20.1 ± 1.71[a]	19.0 ± 1.30[a]
PFK activity					
Optimal activity (U/mg protein)	Control	0.86 ± 0.06	0.93 ± 0.07[a]	1.04 ± 0.09[a]	0.27 ± 0.04[b]
	AVT	0.93 ± 0.05	0.90 ± 0.09[a]	0.93 ± 0.06[a]	0.25 ± 0.02[b]
Activity ratio (%)	Control	16.1 ± 1.40	15.1 ± 3.23[a]	17.9 ± 3.85[a]	11.5 ± 1.37[a]
	AVT	14.2 ± 1.47	18.4 ± 2.39[b]	10.2 ± 0.97[a]	13.5 ± 0.63[a,b]
Fructose 2,6-P_2 activation ratio (%)	Control	62.9 ± 5.78	73.5 ± 6.82[a]	65.2 ± 6.06[a]	54.2 ± 4.67[a]
	AVT	70.1 ± 5.96	61.2 ± 4.49[a]	51.1 ± 5.95[a]	63.4 ± 7.00[a]
FBPase activity (U/mg protein)	Control	0.60 ± 0.03	0.92 ± 0.06[b]	0.82 ± 0.08[a,b]	0.60 ± 0.05[a]
	AVT	0.55 ± 0.04	1.05 ± 0.08[a]	0.80 ± 0.07[a]	0.81 ± 0.07[a]
G6Pase activity (U/mg protein)	Control	15.4 ± 2.00	20.9 ± 3.05[a]	16.6 ± 1.68[a,b]	9.18 ± 2.12[b]
	AVT	38.8 ± 6.58*	36.3 ± 0.77*,[b]	14.5 ± 1.70[a]	31.6 ± 1.66*,[b]
Asp-AT activity (U/mg protein)	Control	2.66 ± 0.25	3.67 ± 0.27[a]	3.38 ± 0.34[a]	3.19 ± 0.24[a]
	AVT	2.80 ± 0.23	3.28 ± 0.35[a,b]	2.79 ± 0.25[a]	3.74 ± 0.15[b]

Values are the means ± SE ($n = 8$ fish per group).
* Significantly different ($P < 0.05$) from fish implanted with vegetable oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (LSW, SW, and HSW) within each treatment (control and AVT).

Finally, Table 4 describes metabolic parameters assessed in brain. Before transfer, AVT treatment decreased lactate and triglyceride levels and increased HOAD activity. After transfer, significant salinity effects were observed in glycogen and amino acid levels, and HK, PK (activity and activation ratios), and HOAD activities. Significant effects of AVT treatment were also observed in lactate and amino acid levels as well as in GPase (%GPase *a*), HK, PK (activation ratio), and HOAD activities. A significant interaction (salinity × AVT treatment) was observed for glycogen levels and HK, PK (activation ratio) and HOAD activities. No significant changes were noticed for glucose levels, and LDH-O and GDH activities.

4. Discussion

Transfer of *S. auratus* from SW to LSW or HSW revealed, similar to other euryhaline teleost, two stages of response: (i) an adaptative period during the first days of acclimation (1–3 days) with important changes occurring in osmotic parameters and (ii) a chronic regulatory period (usually after 3 days of transfer) where these parameters are restored (Laiz-Carrión et al., 2005; Sangiao-Alvarellos et al., 2005). In this study, we addressed the osmoregulatory action of AVT on the hyperosmotic and hypoosmotic capacity of gilthead sea bream using short- and long-term paradigms. In the first experiment, we aimed to check the influence of AVT on the adaptative period directly after hypoosmotic and hyperosmotic transfer. In the second experiment, we aimed to test the influence of AVT on the chronic regulatory period after hypoosmotic or hyperosmotic transfer.

We have no data about plasma AVT levels in Experiment 1 (short-term treatment) because of the small amount of plasma. However, in Experiment 2 (long-term treatment), AVT plasma values in SW-acclimated fish were similar to those reported previously for gilthead sea bream (about 2.5 pmol/ml) and these values showed in oil-treated fish submitted to salinity transfer a linear relationship with respect to environmental salinity, a situation that agrees with that previously reported for this species (Kulczykowska, personal communication).

Our results showed that hormonal treatment increased relevant plasma AVT levels after 3 days (5.30 pmol/ml in SW-acclimated fish before transfer). After salinity transfer (6 days post-injection), these values still showed higher

Table 2
Gill changes in metabolite levels and enzyme activities

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Glycogen (μmol glycosyl units/g wet wt.)	Control	2.89 ± 0.23	2.40 ± 0.24[a]	2.81 ± 0.26[a]	2.63 ± 0.29[a]
	AVT	2.55 ± 0.14	3.87 ± 0.64[a]	2.39 ± 0.26[a,b]	1.79 ± 0.27[b]
Glucose (μmol/g wet wt.)	Control	0.76 ± 0.10	0.91 ± 0.10[a]	1.08 ± 0.13[a]	1.43 ± 0.22[a]
	AVT	0.88 ± 0.09	1.63 ± 0.22*[,a]	0.89 ± 0.07[a]	3.07 ± 0.30*[,b]
Lactate (μmol/g wet wt.)	Control	0.25 ± 0.02	0.37 ± 0.02[a]	0.39 ± 0.04[a]	0.51 ± 0.03[b]
	AVT	0.30 ± 0.03	0.39 ± 0.02[a]	0.26 ± 0.02*[,a]	0.44 ± 0.02[b]
Total α-amino acids (μmol/g wet wt.)	Control	5.13 ± 0.63	5.12 ± 0.54[a]	6.04 ± 0.47[a]	6.54 ± 0.74[a]
	AVT	8.66 ± 0.81*	6.19 ± 0.30[a]	5.51 ± 0.48[a]	7.80 ± 0.49[b]
GPase activity					
Total activity (U/mg protein)	Control	0.32 ± 0.03	0.20 ± 0.04[b]	0.30 ± 0.02[a]	0.36 ± 0.02[a]
	AVT	0.35 ± 0.03	0.30 ± 0.03[a]	0.34 ± 0.06[a]	0.40 ± 0.03[a]
% GPase *a*	Control	18.2 ± 3.29	16.7 ± 2.38[a]	11.7 ± 3.25[a]	15.9 ± 2.94[a]
	AVT	16.8 ± 3.09	15.5 ± 3.18[a]	18.3 ± 3.93[a]	15.9 ± 4.16[a]
PK activity					
Optimal activity (U/mg protein)	Control	2.67 ± 0.35	5.90 ± 0.32[a]	5.74 ± 0.39[a]	7.00 ± 0.38[a]
	AVT	6.26 ± 0.41*	6.60 ± 0.50[a]	5.70 ± 0.31[a]	7.20 ± 0.26[a]
Activity ratio (%)	Control	89.2 ± 2.62	89.2 ± 2.32[a]	89.3 ± 2.11[a]	87.7 ± 1.26[a]
	AVT	88.2 ± 2.11	89.8 ± 1.30[a]	88.5 ± 1.78[a]	88.7 ± 1.99[a]
Fructose 1,6-P_2 activation ratio (%)	Control	99.2 ± 1.69	98.4 ± 2.86[a]	96.3 ± 2.56[a]	93.6 ± 2.73[a]
	AVT	99.1 ± 1.97	97.8 ± 3.15[a]	99.5 ± 3.48[a]	100 ± 3.55[a]
HK activity (U/mg protein)	Control	0.86 ± 0.07	0.69 ± 0.03[a]	0.68 ± 0.06[a]	0.85 ± 0.04[b]
	AVT	0.76 ± 0.05	0.85 ± 0.92[a]	0.97 ± 0.06*[,a]	0.92 ± 0.07[a]
LDH-O activity (U/mg protein)	Control	5.99 ± 0.44	6.25 ± 0.53[a]	5.50 ± 0.53[a]	5.08 ± 0.14[a]
	AVT	5.41 ± 0.43	4.84 ± 0.35[a]	4.53 ± 0.32[a]	6.00 ± 0.27*[,b]
GDH activity (U/mg protein)	Control	2.55 ± 0.25	3.58 ± 0.28[a]	3.70 ± 0.25[a]	4.17 ± 0.18[a]
	AVT	3.52 ± 0.33*	3.40 ± 0.30[a,b]	3.21 ± 0.34[a]	4.26 ± 0.20[b]
HOAD activity (U/mg protein)	Control	0.069 ± 0.006	0.058 ± 0.005[b]	0.085 ± 0.005[a]	0.075 ± 0.006[a]
	AVT	0.066 ± 0.003	0.090 ± 0.008*[,a]	0.086 ± 0.007[a]	0.074 ± 0.005[a]

For further details, see legend of Table 1. Values are the means ± SE ($n = 8$ fish per group).
* Significantly different ($P < 0.05$) from fish implanted with vegetable oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (SW, LSW, and HSW) within each treatment (control and AVT).

values but with a decline. It is interesting to note that 6 days post-injection AVT-treated fish did not show a linear relationship with respect to environmental salinity. Further studies are necessary in order to check this situation. In addition, results obtained in Experiments 1 and 2 showed changes in osmoregulatory and metabolic parameters assessed in AVT-treated groups, suggesting that the method for delivery and the dose of AVT used increased plasma levels of this hormone in both experiments.

In the first experiment, modifications in plasma osmolality and ion balance following transfer between SW and LSW or HSW indicate that the fish are in an adaptative period in agreement with previous studies (Mancera et al., 2002; Laiz-Carrión et al., 2005; Sangiao-Alvarellos et al., 2005). However, hormonal treatment did not produce any significant change in plasma osmolality or gill Na^+,K^+-ATPase activity before or after salinity transfer. Only in the treated group transferred to HSW, there was a positive correlation between dose of AVT used and plasma Na^+ concentration. Altogether, these results suggest that short-term AVT treatment has no influence on osmoregulatory mechanisms activated during the adaptative period after hypoosmotic and hyperosmotic transfer. However, it is also possible that the time used to assess these changes (24 h) was not long enough to observe modifications due to hormonal treatment.

In the second experiment, changes observed in osmoregulatory parameters of control fish due to salinity transfer agree with those previously reported for the same species under similar experimental condition (Laiz-Carrión et al., 2005; Sangiao-Alvarellos et al., 2005). In addition, a stimulatory effect of AVT on gill Na^+,K^+-ATPase activity was observed after 6 days of treatment in fish maintained in SW. Branchial tissue of different species express an AVT receptor, with higher numbers in SW- than in FW-adapted fish. However, there are no data regarding the specific localization of the receptor(s) in the different cell types present in the gill (Guibbolini et al., 1988; Warne et al., 2002). At present, we have no data about the existence of AVT receptors in gill mitochondrial-rich cells of gilthead sea bream. However, the enhancement observed in gill Na^+,K^+-ATPase activity after AVT treatment for 6 days suggests mitochondrial-rich cells as a target. The effect of AVT on gill Na^+,K^+-ATPase activity would agree with previous ion transport studies using gill epithelium in vivo, where this hormone increased Na^+ efflux in gill of SW-adapted flounder (Maetz and Lahlou, 1974), and is consistent with the necessity of SW-adapted fish to excrete ions (Na^+ and Cl^-)

Table 3
Kidney changes in metabolite levels and enzyme activities

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Glycogen (μmol glycosyl units/g wet wt.)	Control	1.37 ± 0.18	2.16 ± 0.32[b]	1.03 ± 0.15[a]	1.76 ± 0.22[a,b]
	AVT	1.71 ± 0.19	3.38 ± 0.36[*,b]	0.99 ± 0.18[a]	1.80 ± 0.21[a,b]
Glucose (μmol/g wet wt.)	Control	1.98 ± 0.22	2.05 ± 0.30[a,b]	2.88 ± 0.28[a]	1.32 ± 0.09[b]
	AVT	2.73 ± 0.22*	2.26 ± 0.19[a]	2.68 ± 0.33[a]	3.01 ± 0.26[*,a]
Lactate (μmol/g wet wt.)	Control	2.20 ± 0.27	2.10 ± 0.16[a]	2.27 ± 0.17[a]	2.36 ± 0.16[a]
	AVT	2.08 ± 0.22	2.94 ± 0.21[*,b]	2.07 ± 0.29[a]	3.31 ± 0.29[*,b]
GPase activity					
Total activity (U/mg protein)	Control	0.082 ± 0.009	0.078 ± 0.009[a]	0.055 ± 0.007[a]	0.064 ± 0.007[a]
	AVT	0.073 ± 0.005	0.074 ± 0.007[b]	0.042 ± 0.007[a]	0.082 ± 0.009[b]
% GPase *a*	Control	31.1 ± 4.24	49.2 ± 6.87[a]	49.9 ± 6.28[a]	29.9 ± 4.58[a]
	AVT	36.5 ± 3.29	47.1 ± 6.21[a]	46.7 ± 7.52[a]	30.8 ± 4.73[a]
PK activity					
Optimal activity (U/mg protein)	Control	5.55 ± 0.41	4.00 ± 0.42[a]	3.56 ± 0.30[a]	4.89 ± 0.52[a]
	AVT	6.15 ± 0.24	4.86 ± 0.54[a]	3.58 ± 0.33[a]	4.47 ± 0.57[a]
Activity ratio (%)	Control	26.7 ± 2.39	27.6 ± 2.52[b]	15.5 ± 1.19[a]	24.2 ± 2.44[b]
	AVT	15.5 ± 1.19*	35.5 ± 2.36[*,b]	18.2 ± 1.54[a]	34.4 ± 2.01[*,b]
Fructose 1,6-P_2 activation ratio (%)	Control	65.1 ± 7.10	41.9 ± 3.44[a,b]	35.6 ± 1.41[a]	56.9 ± 7.20[b]
	AVT	51.8 ± 3.25	83.4 ± 6.58[*,b]	51.7 ± 3.40[*,a]	73.8 ± 7.67[a,b]
HK activity (U/mg protein)	Control	0.29 ± 0.04	0.41 ± 0.04[b]	0.17 ± 0.01[a]	0.32 ± 0.04[a,b]
	AVT	0.10 ± 0.02*	0.22 ± 0.02[*,b]	0.42 ± 0.05[*,a]	0.19 ± 0.02[*,b]
G6Pase activity (U/mg protein)	Control	2.77 ± 0.61	1.36 ± 0.18[b]	3.30 ± 0.38[a]	2.79 ± 0.71[a]
	AVT	4.46 ± 0.65	2.50 ± 0.52[a]	2.57 ± 0.30[a]	3.05 ± 0.72[a]
LDH-O activity (U/mg protein)	Control	2.38 ± 0.20	2.54 ± 0.16[a]	2.75 ± 0.12[a]	2.39 ± 0.19[a]
	AVT	2.17 ± 0.11	2.10 ± 0.16[b]	2.62 ± 0.16[a]	2.11 ± 0.09[b]

For further details see legend of Table 1. Values are the means ± SE ($n = 8$ fish per group).
* Significantly different ($P < 0.05$) from fish implanted with vegetable oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (LSW, SW, and HSW) within each treatment (control and AVT).

through the gills (Marshall, 2002). In this way, AVT may also stimulate Cl^- secretion by an enhancement of Na^+,K^+-ATPase activity in gill mitochondrial-rich cells (as demonstrated in our experiment) via secondary active transport linked to Na^+,K^+-ATPase. Therefore, these results could suggest a role for AVT during the regulatory period of hyperosmotic acclimation in gilthead sea bream.

Kidney Na^+,K^+-ATPase activity of AVT-treated group decreased compared with control group in LSW-acclimated fish. AVT receptors have been described in teleost kidney (Perrot et al., 1993; Warne, 2001) and this could be also the case for gilthead sea bream kidney. Thus, the reduction in kidney Na^+,K^+-ATPase activity observed in AVT-treated group transferred to LSW could be related to decrease in ion transport during the adaptative period of hypoosmotic acclimation. However, no changes in plasma osmolality, Na^+ and Cl^- levels were observed between control and AVT-treated group kept in LSW.

In addition to a direct AVT action on osmoregulatory system, this hormone could also act indirectly via other endocrine pathways. In control fish, plasma cortisol levels increase after transfer to LSW and HSW but not to SW, in agreement with previous observations regarding the effects of salinity on plasma cortisol levels in this species that increased after transfer, remained elevated after 7 days, and returned to baseline leveled after 14 days of transfer (Sangiao-Alvarellos et al., 2005). Plasma cortisol levels were not affected by AVT treatment before or after transfer from SW to SW. However, a synergistic or additive effect of AVT and osmotic stimulation on plasma cortisol levels was observed after transfer to extreme salinities. Considering (i) the occurrence of specific binding sites for AVT in adrenocorticotropin (ACTH)-producing cells in the pituitary (Moons et al., 1989) and (ii) the fact that AVT can synergize with CRF to enhance ACTH release (Baker et al., 1996; Kulczykowska, 2001), our results suggest possible cooperation between exogenous AVT and endogenous CRF (stimulated by salinity transfer). It is very interesting, however, that this effect only occurred in fish transferred to extreme salinities, suggesting the necessity of another *stimuli* (i.e. salinity change) in addition to hormonal treatment. Accordingly, changes in osmoregulatory and metabolic parameters in fish treated with AVT and transferred to extreme salinities could be attributed to cortisol action. In this way, cortisol treatment of *S. auratus* (Mancera et al., 2002; Laiz-Carrión et al., 2003) induced similar osmoregulatory and metabolic changes to that observed in AVT-treated fish transferred to LSW and HSW (present results).

AVT also affects metabolic parameters in plasma and different osmoregulatory (gills and kidney) and non-osmoregulatory (liver and brain) tissues after long-term (second experiment) but not short-term (first experiment) treatment. Changes displayed by control fish 3 days after transfer to different salinities agree with those described

Table 4
Brain changes in metabolite levels and enzyme activities

Parameter	Treatment	Before transfer	After transfer		
			LSW	SW	HSW
Glycogen (μmol glycosyl units/g wet wt.)	Control	7.13 ± 0.76	1.64 ± 0.22^{b}	4.83 ± 0.87^{a}	1.41 ± 0.24^{b}
	AVT	$2.09 \pm 0.52^{*}$	$2.27 \pm 0.45^{a,b}$	$1.27 \pm 0.16^{*,a}$	$2.82 \pm 0.25^{*,b}$
Lactate (μmol/g wet wt.)	Control	8.52 ± 0.46	$6.92 \pm 0.41^{a,b}$	5.98 ± 0.51^{a}	8.20 ± 0.55^{b}
	AVT	$6.96 \pm 0.41^{*}$	8.37 ± 0.40^{b}	$7.31 \pm 0.24^{*,a}$	8.49 ± 0.51^{b}
Total α-amino acids (μmol/g wet wt.)	Control	8.07 ± 0.79	$7.23 \pm 0.61^{a,b}$	5.75 ± 0.49^{a}	9.20 ± 0.86^{b}
	AVT	7.11 ± 0.27	7.25 ± 0.67^{a}	$7.86 \pm 0.52^{*,a}$	7.46 ± 0.80^{a}
Triglyceride (μmol/g wet wt.)	Control	3.43 ± 0.45	1.78 ± 0.29^{a}	2.49 ± 0.36^{a}	3.02 ± 0.61^{a}
	AVT	$2.13 \pm 0.39^{*}$	1.50 ± 0.32^{a}	1.71 ± 0.26^{a}	2.35 ± 0.36^{a}
GPase activity					
Total activity (U/mg protein)	Control	0.27 ± 0.02	0.24 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.02^{a}
	AVT	0.26 ± 0.01	0.28 ± 0.02^{a}	0.23 ± 0.02^{a}	0.29 ± 0.01^{a}
% GPase *a*	Control	33.0 ± 2.67	35.5 ± 2.94^{a}	32.0 ± 2.55^{a}	35.3 ± 1.58^{a}
	AVT	30.7 ± 3.09	31.1 ± 1.52^{a}	31.2 ± 1.13^{a}	$25.5 \pm 1.76^{*,b}$
PFK activity					
Optimal activity (U/mg protein)	Control	8.31 ± 0.39	8.66 ± 0.46^{a}	8.61 ± 0.50^{a}	8.82 ± 0.49a
	AVT	9.14 ± 0.33	9.21 ± 0.74^{b}	$6.82 \pm 0.59^{*,a}$	$7.72 \pm 0.53^{a,b}$
Activity ratio (%)	Control	6.33 ± 0.57	4.98 ± 0.48^{a}	5.11 ± 0.47^{a}	6.36 ± 0.52^{a}
	AVT	5.30 ± 0.42	4.83 ± 0.47^{a}	4.40 ± 0.18^{a}	5.95 ± 0.58^{a}
Fructose 1,6-P_2 activation ratio (%)	Control	24.9 ± 3.47	14.7 ± 1.60^{b}	31.2 ± 1.37^{a}	29.5 ± 1.58^{a}
	AVT	25.3 ± 2.07	$24.7 \pm 2.11^{*,a}$	$22.0 \pm 1.43^{*,a}$	$43.1 \pm 2.09^{*,b}$
HK activity (U/mg protein)	Control	0.89 ± 0.03	1.11 ± 0.05^{b}	0.91 ± 0.03^{a}	1.11 ± 0.06^{b}
	AVT	0.92 ± 0.04	1.20 ± 0.02^{a}	$1.12 \pm 0.06^{*,a,b}$	1.04 ± 0.02^{b}
HOAD activity (U/mg protein)	Control	0.08 ± 0.01	0.10 ± 0.01^{b}	0.25 ± 0.01^{a}	0.09 ± 0.02^{b}
	AVT	$0.12 \pm 0.01^{*}$	$0.21 \pm 0.01^{*,b}$	$0.08 \pm 0.01^{*,a}$	0.11 ± 0.01^{a}

For further details see legend of Table 1. Values are the means ± SE ($n = 8$ fish per group).

* Significantly different ($P < 0.05$) from fish implanted with vegetable oil alone (control) under the same experimental conditions. Different letters indicate significant differences ($P < 0.05$) among groups (LSW, SW, and HSW) within each treatment (control and AVT).

previously during acclimation of this species to similar salinities (Sangiao-Alvarellos et al., 2003, 2005).

AVT treatment did not affect plasma glucose levels before transfer but clearly elevated these levels after transfer to extreme environmental salinities, but not in fish kept in SW. We have demonstrated previously that under the new environmental salinity conditions, osmoregulatory organs (like gills and kidney) demand more energy (Sangiao-Alvarellos et al., 2003, 2005). Therefore, the changes observed in the present experiment could suggest a mobilization of glucose to peripheral tissues to fuel the increased energetic demand observed in gills during LSW and HSW acclimation, as it has been suggested previously for this species (Sangiao-Alvarellos et al., 2003, 2005). The increase of glucose levels in treated fish could indicate that AVT exerts some metabolic role in acclimation to extreme environmental salinities. However, an indirect effect of AVT through cortisol, a clear hyperglycemic hormone (Mommsen et al., 1999), cannot be excluded (see below). The levels of the remaining metabolites assessed in plasma were not modified by AVT treatment. The lack of changes in lactate is in agreement to that reported in AVT-treated rainbow trout (Sangiao-Alvarellos et al., 2004). Altogether, it seems that the tissue mobilization of metabolites in *S. auratus* induced by AVT treatment is limited.

At hepatic level, the few studies performed to date in fish regarding metabolic effects of AVT described increased glycogenolytic potential in some cases (Janssens and Lowrey, 1987; Moon and Mommsen, 1990) but not in others (Mommsen et al., 1991). In the present study, several metabolic effects were noticed in liver after AVT treatment before transfer, such as increased G6Pase activity, and free glucose and amino acid levels. These results suggest a metabolic role for AVT in increasing glucose release from liver into plasma and could explain the hyperglycemia observed in AVT-treated fish. This increase is in contrast to that reported for carp hepatocytes treated with AVT (Janssens and Lowrey, 1987). However, these limited metabolic actions of AVT before transfer were more evident after transfer to different salinities. Thus, an enhancement of glycogenolysis was apparent in liver of AVT-treated fish but only after transfer to extreme salinities (i.e. LSW and HSW). This increased glycogenolysis was indicated by changes in both glycogen levels and GPase activity. In addition, an increased G6Pase activity was observed simultaneously, suggesting an enhanced capacity of liver to export glucose into plasma. These results suggest a role for AVT in producing a mobilization of liver glycogen and an enhanced glucose release but only during acclimation to extreme salinities and the necessity of another *stimuli* (i.e. salinity change) for inducing this metabolic role of AVT. Considering that treatment with cortisol enhanced liver glycogenolysis and hyperglycemia in this species (Laiz-Carrión et al., 2003), and the increase observed in plasma

cortisol levels in fish treated with AVT acclimated to extreme salinities, we can therefore largely attribute the metabolic effects of AVT in fish transferred to extreme salinities to cortisol action.

Besides increasing glycogenolytic and glucose release potential, no clear picture emerges regarding AVT actions in other pathways of carbohydrate (glycolysis, gluconeogenesis, or pentose phosphate shunt) or lipid metabolism for which no major changes were noticed. As for amino acid metabolism, the reduction of oxidative capacity in AVT-treated fish after transfer to SW, together with the increased amount of amino acids in AVT-treated fish, suggests a decreased catabolism of amino acids in AVT-treated fish kept in SW. A similar reduction in lactate levels was observed in AVT-treated fish after transfer to HSW, in contrast with lack of changes in lactate oxidation observed in hepatocytes of *Opsanus beta* treated with AVT (Mommsen et al., 1991).

In tissues other than liver known to possess receptors or binding sites for AVT like gills (Guibbolini et al., 1988), kidney (Warne, 2001), or brain (Moons et al., 1989) to our knowledge no studies have been carried out to assess the existence AVT effects on energy metabolism.

In gills, AVT treatment before transfer increased amino acid levels, capacity for oxidation of amino acids, and PK activity. These results suggest an enhancement in the use of amino acids as fuel due to this hormonal treatment. After transfer, the major effects of AVT were noticed in extreme salinities: free glucose levels increased in fish transferred to LSW or HSW, while LDH-O activity, GDH activity, and amino acid levels were enhanced in fish acclimated to HSW. All these data supported an increased use of fuels in addition to glucose (i.e. amino acids and lactate) in gills, as had been suggested previously in *S. auratus* during HSW acclimation (Sangiao-Alvarellos et al., 2003, 2005). Thus, it seems that the increased osmoregulatory work elicited by gills during acclimation to HSW is partially supported by amino acids and lactate and at least part of those changes are elicited by AVT treatment. In addition, and according to the metabolic role of cortisol during this kind of osmotic acclimation (Laiz-Carrión et al., 2003) and the increased levels measured in plasma after AVT treatment (present results), cortisol may also be involved in the metabolic changes induced by AVT treatment after salinity transfer.

In kidney, AVT treatment before transfer increased free glucose levels, while decreased HK activity and glycolytic potential. Since glycogen levels did not change, the origin for this glucose could be attributed to the reduced glycolytic capacity observed simultaneously. Lactate levels increase in AVT-treated fish acclimated to extreme salinities allowing us to hypothesize an increased uptake or a reduced use of lactate for fuelling purposes during acclimation to this low salinity. Since no changes were noticed for LDH-O activity under the same situation, an enhanced uptake seems possible. No major changes were observed in lipid and amino acid metabolism. During acclimation to HSW, major changes observed in AVT-treated fish included increased free glucose and lactate levels. Like in the case of LSW, an increase in glycolytic capacity was noticed in HSW also. Altogether, the most important change induced by AVT treatment was a reduced use of glucose and lactate as fuels. Considering that those metabolites are the most important for kidney (Mommsen et al., 1985), a reduced energy demand is apparent in kidney after AVT treatment.

Several studies carried out in recent years demonstrated that various hormones and/or neurotransmitters regulate brain energy metabolism in fish (see review by Soengas and Aldegunde, 2002). However, the possible role of neuropeptides in this issue has been scarcely assessed to date (Sangiao-Alvarellos et al., 2004), although in mammals at least part of the actions of neuropeptides are related to changes in brain energy metabolism (Magistretti, 1999). When addressing the energy metabolism parameters in brain after AVT treatment, we observed before transfer to extreme salinities a significant increase of the glycogenolytic potential together with increased oxidation of fatty acids and decreased lactate levels. These results suggest that AVT induces a mobilization of energy reserves in *S. auratus* acclimated to SW. The decreased glycogen levels observed in this experiment are in agreement with a similar decline observed in rainbow trout treated intracerebroventricularly with AVT (Sangiao-Alvarellos et al., 2004). In this regard, it is interesting to note that in *S. auratus* brain glycogen is also mobilized during acclimation to extreme environmental salinities (Sangiao-Alvarellos et al., 2003), suggesting a possible role for AVT as a mediator of these changes. The further decrease in glycogen levels in fish treated with AVT kept in SW should be related to a long-term effect of AVT at the same salinity, whereas a striking increase was noticed in fish transferred to HSW. Besides glycogenolysis, it seems that the energy requirements imposed by acclimation to extreme salinities are not imposing any extra change in AVT effects on brain energy metabolism, resulting in few changes in carbohydrate, lipid, or lactate metabolism.

In summary, treatment of *S. auratus* with AVT enhanced gill Na^+,K^+-ATPase activity, and induced several metabolic changes that may be related to the energy repartitioning process occurring in this species during osmotic acclimation to extreme environmental salinities. The most important metabolic changes were those displayed in liver by glycogenolytic potential and glucose release capacity, which can be attributed to the increased cortisol levels also elicited by AVT treatment in those extreme salinities. All these results suggest that AVT treatment is facilitating directly or indirectly osmoregulatory adaptive processes to extreme salinities as well as the energy costs associated with them. However, other hormone systems such as renin–angiotensin and natriuretic peptides cannot be excluded from involvement in changes following AVT treatment.

Acknowledgments

This study was partly supported by Grants BOS2001-4031-C02-02 and VEM2003-20062 (Ministerio de Ciencia y

Tecnología and FEDER, Spain), PGIDT04PXIC31208PN and PGIDIT05PXIC31202PN (Xunta de Galicia, Spain) to J.L.S., and grant BOS2001-4031-C02-01 (Ministerio de Ciencia y Tecnología and FEDER, Spain) to J.M.M. The authors thank Planta de Cultivos Marinos (CASEM, Universidad de Cádiz, Puerto Real, Cádiz, Spain) for providing experimental fish.

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