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Osmoregulatory action of PRL, GH, and cortisol in the gilthead seabream (*Sparus aurata* L.)

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Abstract

The osmoregulatory actions of ovine prolactin (oPRL), ovine growth hormone (oGH), and cortisol were tested in the euryhaline gilthead seabream *Sparus aurata*. Acclimated to sea water (SW, 40 ppt salinity, 1000 mOsm/kg H_2O) or brackish water (BW, 5 ppt, salinity, 130 mOsm/kg H_2O), injected every other day for one week (number of injections, 4) with saline (0.9% NaCl), oPRL (4 μg/g body weight), oGH (4 μg/g body weight) or cortisol (5 μg/g body weight), and transferred from SW to BW or from BW to SW 24 h after the last injection. Fish were sampled before and 24 h after transfer. Gill Na^+, K^+-ATPase activity, plasma osmolality, plasma ions (sodium and chloride), plasma glucose, and muscle water moisture were examined. SW-adapted fish showed higher gill Na^+, K^+-ATPase activity, plasma osmolality, and plasma ions levels than BW-adapted fish. Transfer from SW to BW decreased plasma osmolality and ions levels after 24 h, while transfer from BW to SW increased these parameters, whereas gill Na^+, K^+-ATPase activity was unaffected. oPRL treatment significantly decreased gill Na^+,K^+-ATPase activity and increased plasma osmolality and ions in SW- and BW-adapted fish. This treatment minimizes loss of osmolality and ions in plasma after transfer to BW and increased these values after transfer to SW. No significant changes were observed in gill Na^+,K^+-ATPase activity, plasma osmolality, and plasma ions in oGH-treated group with respect to saline group before or after transfer from SW to BW or from BW to SW. Treatment with cortisol induced, in SW-adapted fish, a significant increase of gill Na^+, K^+-ATPase activity and decrease of plasma osmolality and plasma ions. In BW-adapted fish this treatment induced a significant increases in gill Na^+, K^+-ATPase activity, plasma osmolality, and plasma ions. After transfer to SW cortisol-treated fish had higher plasma osmolality than the saline group. Our results support the osmoregulatory role of PRL in the adaptation to hypoosmotic environment in the gilthead seabream *S. aurata*. Further studies will be necessary to elucidate the osmoregulatory role of GH in this species. Cortisol results suggest a "dual osmoregulatory role" of this hormone in *S. aurata*.

Keywords: Prolactin; Growh hormone; Cortisol; Osmoregulation; Gill; Na^+, K^+-ATPase activity; Gilthead seabream

1. Introduction

Several hypophyseal and extrahypophyseal hormones control the activity of osmoregulatory organs. Prolactin (PRL), growth hormone (GH), and cortisol present an important osmoregulatory role in teleost species. However, the influence of these hormones on adaptation to hyperosmotic or hypoosmotic conditions depends on the environmental salinity and specie studied (Manzon, 2002; Mayer-Gostan et al., 1987; McCormick, 1995, 2001).

It is generally accepted that PRL is involved in freshwater (FW) adaptation (Hirano, 1986; Manzon, 2002; McCormick, 1995) and antagonizes GH and cortisol in seawater (SW) adaptation (Madsen and Bern, 1992; Seideln and Madsen, 1997). Several evidences support this hyperosmoregulatory role of PRL. Synthesis and release of hypophyseal PRL increased in teleost adapted to freshwater or brackish water (BW) (Olivereau et al., 1981; Ruijter and Wendelaar Bonga, 1988). In the gilthead seabream (*Sparus aurata*) adaptation to BW also activated the PRL cells (Mancera et al., 1993b). Accordingly expression of PRL mRNA (Martin et al., 1999) and plasma levels of PRL (Auperin et al., 1995; Morgan et al., 1997; Yamauchi et al., 1991)

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increased in hypoosmotic media. In salmonid and non-salmonid teleosts PRL treatment decrease gill Na^+, K^+-ATPase activity (see Manzon, 2002; McCormick, 1995). In this way, in the sparid silver seabream (*Sparus sarba*) oPRL reduced branchial Na^+,K^+-ATPase activity in SW- and BW-adapted fish (Kelly et al., 1999).

Conversely to PRL, the GH/IGF-I axis seems to play a clear role in seawater adaptation in salmonid teleosts (McCormick, 1995; Sakamoto et al., 1993). However, in non-salmonid teleosts the osmoregulatory role of this axis is less clear. Pituitary GH cells and plasma GH levels behave differently depending on the species studied and the environmental salinity (Mancera and McCormick, 1998b; Nishioka et al., 1988; Olivereau and Ball, 1970). In the gilthead seabream, immunocytochemical and morphological data suggested an activation of GH cells in hypoosmotic media (Mancera et al., 1995). The role of GH and/or IGF-I as osmoregulatory hormones has been studied in only a few non-salmonid species. GH treatment increased opercular chloride cell number and Na^+,K^+-ATPase density in tilapia (*Oreochromis mossambicus*) (Dang et al., 2000; Flik et al., 1993). In addition, GH stimulated salinity tolerance and gill Na^+,K^+-ATPase in this species (Borski et al., 1994; Sakamoto et al., 1997; Xu et al., 1998). In the anadromous striped bass (*Morone saxatilis*) this hormone increased gill Na^+,K^+-ATPase activity and ion regulatory capacity (Madsen et al., 1996). In the euryhaline mummichog, *Fundulus heteroclitus*, treatment with GH and/or IGF-I also improved salinity tolerance and gill Na^+,K^+-ATPase activity (Mancera and McCormick, 1998a). However, in *S. sarba*, no differences were observed between GH- and saline-treated fish either adapted to SW or BW (Kelly et al., 1999).

The hypoosmoregulatory role of cortisol is well established in many teleosts. Cortisol induced salinity tolerance, development, and proliferation of gill chloride cells, gill Na^+,K^+-ATPase activity, and expression of Na^+,K^+-ATPase α-subunit (McCormick, 1990, 1995, 1996; Madsen et al., 1995; Seidelin and Madsen, 1999). Moreover, there are also evidences indicating the involvement of cortisol in ion uptake in salmonid and non-salmonid teleosts (Gaitskell and Chester Jones, 1970; Mayer-Gostan et al., 1987; Perry et al., 1992; Perry, 1997; Yada and Ito, 1999). In *S. aurata* treatment with cortisol increased ion regulatory capacity after transfer to low salinity environments (Mancera et al., 1994). In this way, some evidences suggest a positive interaction of cortisol with PRL for maintenance of ion balance in freshwater fish (Chester Jones et al., 1969; Eckert et al., 2001; Gallis et al., 1979; Mayer-Gostan et al., 1987; Parwez and Goswami, 1985).

Gilthead seabream is an euryhaline teleost capable of adapting to extreme changes in environmental salinity (Chervinski, 1984; Mancera et al., 1993a). We have analysed the osmoregulatory system of this species, by studying aspect such as the response of adenohypophyseal cells to environmental salinity (Mancera et al., 1993b, 1995), plasmatic responses to abrupt changes in salinity (Mancera et al., 1993a) or the effect of treatment with cortisol on BW adaptation (Mancera et al., 1994). To our knowledge, there are no studies concerning the hormonal control of gill Na^+,K^+-ATPase activity in hypo- and hyperosmotic environments in this species. The aim of the present study was to analyse the osmoregulatory actions of oPRL, oGH, and cortisol in SW- and BW-adapted *S. aurata* before and after transfer from SW to BW and from BW to SW. The results will be discussed in relation to the osmoregulatory role of these hormones in other euryhaline teleost.

2. Materials and methods

2.1. Fish

Immature male gilthead seabream (*S. aurata* L., 40–60 g body weight) were provided by a commercial fish culturing center (CUPIMAR S.A., San Fernando, Cádiz, Spain) and transferred to the web laboratories at Faculty of Marine Science (Puerto Real, Cádiz). They were acclimated to SW in 300 liter aquaria for, at least, 2 weeks in an open system (40 ppt salinity, 1000 mOsm/kg H_2O) and to BW in a closed system (5 ppt salinity, 130 mOsm/kg H_2O). BW was obtained by mixing SW with dechlorined tap water. Water salinity was checked every day and corrected when necessary. During the experiments (April–June 1999), fish were maintained under natural photoperiod and constant temperature (18 °C). Fish were fed daily with 1% body weight commercial dry pellets (Dibaq-Diprotg SA, Segovia, Spain). They were fasted for 24 h before hormone injection and throughout the experiment.

2.2. Experimental protocol

Fish were anaesthetized with 2-phenoxyethanol (Sigma P-1126) (0.5 ml/liter water), weighed, injected intraperitoneally with vehicle or hormone plus vehicle, and placed back to SW or BW. Hormones were dissolved in saline (0.9% NaCl) and injected intraperitoneally (10 μl/g body weight). Fish were treated with: (a) ovine PRL (oPRL, NIADDK-oPRL-21, National Institutes of Health, Bethesda, MD, USA) (4 μg/g body weight), (b) ovine GH (oGH, NIADDK-oGH-15, National Institutes of Health, Bethesda, MD, USA) (4 μg/g body weight), or (c) cortisol (Sigma H-2270, hydrocortisone hemisuccinate sodium salt) (5 μg/g body weight). These doses have been previously used by other authors who showed osmoregulatory effects in other teleost species (Borski et al., 1994; Bœuf et al., 1994; Madsen and Bern, 1992; Sakamoto et al., 1997; Seidelin and Madsen, 1997,1999). The use of

mammalian hormones, such as oPRL and oGH, to study the osmoregulatory system of teleost is also well established (see Seidelin and Madsen, 1999).

Fish were injected every other day during one week (number of injections, 4). After 24 h of the last injection, half of the experimental fish ($n = 8$) were sampled and the other half ($n = 8$) were transferred from SW to BW (Experiment 1) or from BW to SW (Experiment 2). They remained for 24 h in the new salinity and then they were sampled. Injections were given between 9:00 and 10:00 AM. No mortality was observed during the experiments.

2.3. Sampling

Fish were anaesthetized with 2-phenoxyethanol (1 ml/liter water), weighed, and sampled. The blood was extracted from the caudal artery using ammonia-heparinized syringes. Plasma was separated from cells by centrifugation for 20 min at 3000 r.p.m. and stored at −80 °C. A biopsy of gill tissue was placed in 100 µl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80 °C. A piece of paraxial muscle was dissected and weighed for determination of total water content.

2.4. Analytical techniques

Na^+,K^+-ATPase activity was determined using the microassay method of McCormick (1993). Gill tissue was homogenized in 125 µl of SEI buffer with 0.1% deoxycholic acid, then centrifuged at 2000*g* for 30 s. Duplicate 10 µl homogenate samples were added to 200 µl assay mixture with and without 0.5 mM ouabain in 96-well microplates at 25 °C and read at 340 nm for 10 min with intermittent mixing. Ouabain-sensitive ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation and expressed as µmol ADP mg/protein/h. The Pierce BCA Protein kit (Pierce, Rockford, IL, USA) was used with bovine albumin as standard. Both assays were run on a microplate reader (EL340i, Bio-Tek Instruments, Winooski, VT, USA) using Delta Soft3 software for Macintosh.

Plasma osmolality was measured with a vapor pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA) and expressed as mosm/kg. Plasma Na^+ was measured by using an atomic absorption spectrophotometer and plasma Cl^- levels with the Chloride Sigma kit (no. 461). Plasma glucose was measured using a commercial kit from Sigma (Glucose HK 16–20). Muscle water content (MWC) was determined as percent weight loss after drying at 100 ° for 2 days.

2.5. Statistics

Significant differences among groups were tested by one-way ANOVA, followed by the Student–Newman–Keuls multiple comparison test (SNK). Significant differences between the same experimental condition, before and after transfer, were tested by *t* test. Results were considered significantly different when $p < 0.05$.

3. Results

3.1. Experiment 1 (transfer from SW to BW)

In SW-adapted fish, oPRL treatment significantly decreased gill Na^+,K^+-ATPase activity and induced a small increase in plasma osmolality. Treatment with cortisol induced significant increases in gill Na^+,K^+-ATPase activity and plasma osmolality. oGH treatment was without effect on gill Na^+,K^+-ATPase activity and plasma osmolality (Fig. 1). Plasma sodium and chloride levels varied concomitantly to osmolality (Table 1).

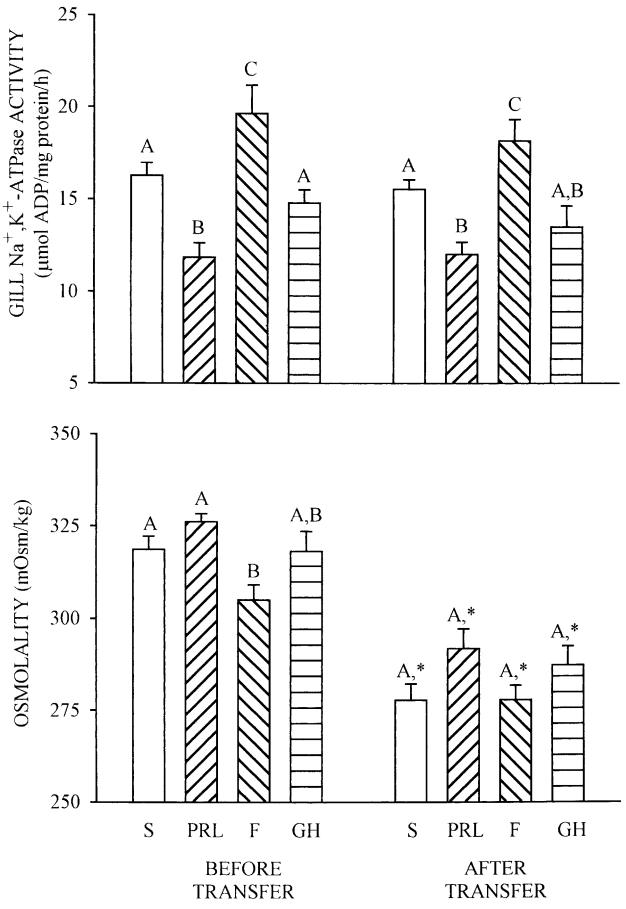

Fig. 1. Effect of oPRL, cortisol (F) and oGH treatment on gill Na^+,K^+-ATPase activity (top) and osmolality (bottom) before and after salinity transfer from SW to BW. Fish were injected four times every other day. One group ($n = 8$) were sampled 24 h after the last injection and other ($n = 8$) were transfer to BW for 24 h and sampled. Dates are expressed as means ± SEM ($n = 7$–8). Same letters indicate not differences among groups before or after transfer ($p < 0.05$). Asterisks indicate significant difference relative to same group before transfer ($p < 0.05$).

Table 1
Effect of oPRL, cortisol (F) and oGH treatment on plasma Na^+ and Cl^- levels before and after transfer from SW to BW

	Before transfer				After transfer			
	Saline	oPRL	F	oGH	Saline	oPRL	F	oGH
Na^+ (mM)	180 ± 5^{a}	191 ± 4^{b}	173 ± 6^{a}	177 ± 4^{a}	$168 \pm 5^{a,*}$	$179 \pm 4^{b,*}$	170 ± 4^{a}	$173 \pm 5^{a,b}$
Cl^- (mM)	150 ± 3^{a}	186 ± 4^{b}	144 ± 4^{a}	168 ± 3^{c}	$139 \pm 4^{a,*}$	$160 \pm 5^{b,*}$	138 ± 3^{a}	$146 \pm 3^{a,b,*}$

Values are the means ± SEM ($n = 7$–8). Same letters indicate not differences among groups before or after transfer ($P < 0.05$). Asterisks indicate significant difference relative to same group before transfer ($p < 0.05$).

Transfer from SW to BW for 24 h induced a small reduction in gill Na^+,K^+-ATPase activity. The relative values between groups followed the same pattern as those observed in SW (Fig. 1). This transfer also induced a significant plasma hypoosmolality in all groups and reduced ion levels (Fig. 1). In oPRL and oGH groups, this reduction was less evident than in saline and F group (Table 1). No statistically significant differences were observed in MWC in the hormone treated groups with respect to the saline group after the transfer (data not shown).

In SW-adapted fish, oGH treatment significantly increased plasma glucose levels. The transfer to BW did not induce significant changes in plasma glucose levels and, after 24 h in BW, values were similar to those of in fish kept in SW. No significant differences in plasma glucose were detected among experimental groups in BW (data not shown).

3.2. Experiment 2 (transfer from BW to SW)

In BW-adapted fish, oPRL treatment significantly decreased gill Na^+,K^+-ATPase activity and induced a small increase in plasma osmolality and ion levels. Cortisol increased significantly gill Na^+,K^+-ATPase activity, plasma osmolality, and ions, while oGH treatment was without effect on these parameters (Fig. 2; Table 2).

After transfer from BW to SW for 24 h, gill Na^+,K^+-ATPase activity increased in saline and oGH treated fish, but significant differences were not observed with respect to the same groups in SW-adapted fish. However, in oPRL and cortisol treated groups, a small reduction was observed (Fig. 2). The transfer induced hyperosmolality and increase in plasma ion levels in all groups, but only in saline and oPRL groups it was statistically significant when compared to BW-adapted fish (Fig. 2; Table 2). Twenty-four hours after the transfer, oPRL and cortisol groups had significantly higher levels than saline treated group. MWC only showed significant differences in the oPRL treated group (Fig. 3).

In BW-adapted fish, plasma glucose levels showed not significant differences with respect to saline group in any of the experimental groups. The transfer to SW did not significantly induce changes in plasma glucose levels with respect to the observed in BW-adapted fish (data not shown).

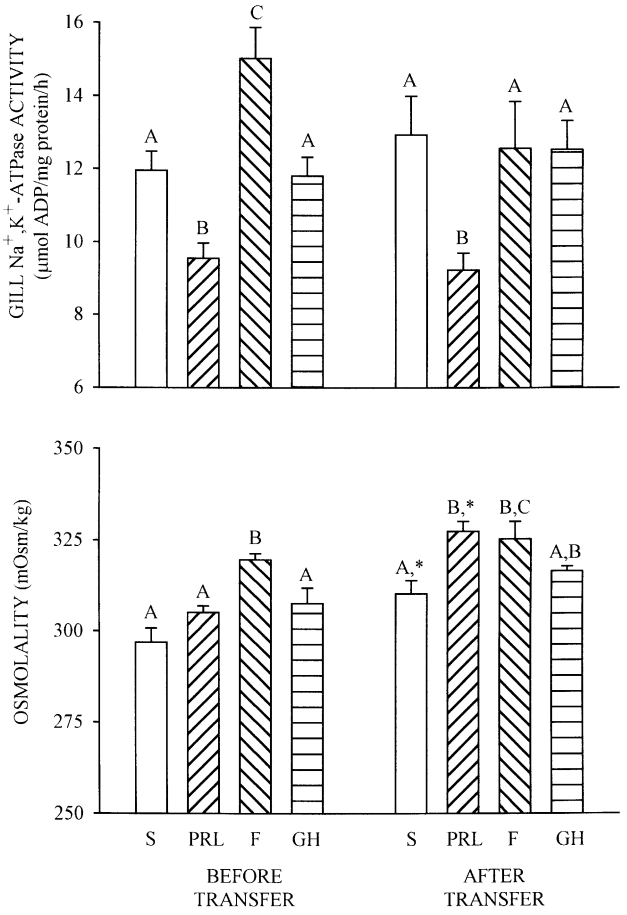

Fig. 2. Effect of oPRL, cortisol (F) and oGH treatment on gill Na^+, K^+-ATPase activity (top) and osmolality (bottom) before and after salinity transfer from BW to SW. Fish were injected four times every other day. One group ($n = 8$) were sampled 24 h after the last injection and other ($n = 8$) were transfer to SW for 24 h and sampled. Dates are expressed as means ± SEM ($n = 7$–8). Same letters indicate not differences among groups before or after transfer ($p < 0.05$). Asterisks indicate significant difference relative to same group before transfer ($p < 0.05$).

4. Discussion

In euryhaline fish, abrupt transfer from SW to BW or from BW to SW induces changes in osmotic plasma parameters and the consequent activation of osmoregulatory system to try to recover the original values. In this process, two periods were described: (i) adaptative

Table 2
Effect of oPRL, cortisol (F) and oGH treatment on plasma Na^+ and Cl^- levels before and after transfer from BW to SW

	Before transfer				After transfer			
	Saline	oPRL	F	oGH	Saline	oPRL	F	oGH
Na^+ (mM)	169 ± 2^a	174 ± 3^a	185 ± 3^b	171 ± 4^a	$179 \pm 2^{a,*}$	$190 \pm 4^{b,*}$	188 ± 5^b	177 ± 4^a
Cl^- (mM)	145 ± 3^a	164 ± 3^a	175 ± 2^c	160 ± 3^b	$165 \pm 4^{a,*}$	$174 \pm 3^{a,b,*}$	180 ± 3^b	168 ± 2^a

Values are the means ± SEM ($n = 7$–8). Same letters indicate not differences among groups before or after transfer ($P < 0.05$). Asterisks indicate significant difference relative to same group before transfer ($p < 0.05$).

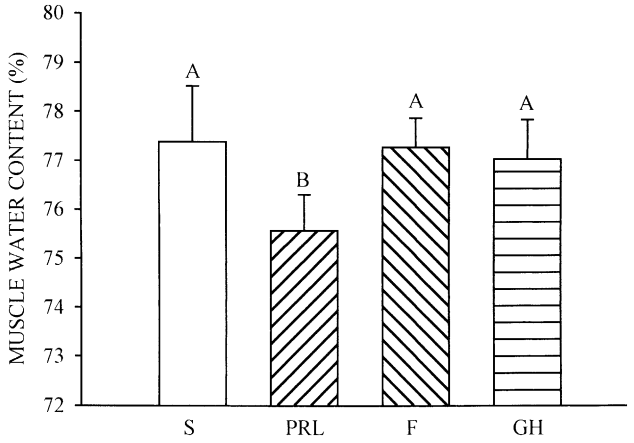

Fig. 3. Effect of oPRL, cortisol (F) and oGH treatment on MWC in fish transferred from BW to SW. Fish were injected four times every other day, transferred 24 h after last injection and sampled after 24 h in the new salinity. Dates are expressed as means ± SEM ($n = 7$–8). Same letters indicate not differences among groups ($P < 0.05$).

period, which changes in osmotic parameters, and (ii) chronic regulatory period, where these parameters reach again homeostasis (Holmes and Donaldson, 1969; Maetz, 1974). In a previous work, we studied the osmoregulatory response to abrupt salinity changes (from SW to BW and from BW to SW) in the gilthead seabream *S. aurata* and we showed the existence of a short adaptative period (24–48 h) (Mancera et al., 1993a). In agreement with this previous study, our results showed plasma hypoosmolality and a reduction in plasma ion levels after transfer from SW to BW, and hyperosmolality and increased ionic values after transfer from BW to SW.

The osmoregulatory role of PRL as a hormone that promotes adaptation to hypoosmotic environments is well established in teleosts (Hirano, 1986; McCormick, 1995; Manzon, 2002). Our results showed a clear osmoregulatory action of oPRL both in SW- and BW-adapted *S. aurata*. In both salinities oPRL induced a reduction in gill Na^+,K^+-ATPase activity and an increase in plasma osmolality and ion concentration. This agrees previous reports on euryhaline teleost adapted to SW or freshwater (*F. heteroclitus*: Pickford et al., 1970; *Chelon labrosus*: Gallis et al., 1979; *Heteropneustes fossiles*: Parwez and Goswami, 1985; *Oncorhynchus mykiss*: Madsen and Bern, 1992; and *O. mossambicus*: Sakamoto et al., 1997). In the sparid silver seabream *S. sarba*, a reduction of gill Na^+,K^+-ATPase activity and of Na^+,K^+-ATPase mRNA have been observed in SW- or BW-adapted fish treated with PRL (Kelly et al., 1999; Deane et al., 1999). In addition, it has been demonstrated that PRL antagonizes the hypoosmoregulatory action of cortisol and GH in salmonids (*Oncorhynchus mykiss*: Madsen and Bern, 1992; *Salmo salar*: Bœuf et al., 1994; *Salmo trutta*: Seidelin and Madsen, 1997).

Treatment with oPRL is less effective in decreasing gill Na^+,K^+-ATPase activity in BW-adapted fish (reduction of 19% respect to saline-treated group) as compared to SW-adapted fish (reduction of 28% respect to saline-treated group). In several teleost, morphological evidences indicated activation of PRL cells in hypoosmotic environments (Olivereau et al., 1981; Ruijter and Wendelaar Bonga, 1988), concomitantly with an increase in PRL plasma levels (Auperin et al., 1995; Hasegawa et al., 1987; Morgan et al., 1997). In *S. aurata*, we also detected activation of PRL cells in BW- with respect to SW-adapted fish (Mancera et al., 1993b). This could explain the lower effect of PRL treatment in BW-adapted fish, with potentially higher PRL plasmatic levels that in SW-adapted fish.

The use of mammalian hormones, such as oPRL and oGH, to study the osmoregulatory system of teleost is well established (see Manzon, 2002; McCormick, 1995; Seidelin and Madsen, 1999). These heterologous hormones present less affinity for teleost receptors than piscine hormones. Also, they have similar biological activity but with lower intensity (Prunet and Auperin, 1995). In our studies, we used oPRL. Due to the structural similarity between oPRL and oGH, some somatotropic effect could be expected after administration of oPRL. However, several studies have shown that teleost GH receptors do not virtually have affinity for oPRL (Gray et al., 1990; Sakamoto and Hirano, 1991). Accordingly, our results suggest that these two hormones have different osmoregulatory and metabolic functions: oPRL treatment play an hyperosmoregulatory role without influence on glycemia (data not shown) while oGH treatment increased plasma glucose levels (data not shown) and were without effect on osmoregulatory parameters. Thus supporting the absence of an heterosomatotropic effect of oPRL in *S. aurata*.

The present results suggest an important role of PRL during the adaptative period after transfer to different salinities. In addition, the previous data obtained at adenohypophyseal levels together with the present results suggested an important role of this hormone in *S. aurata* during the regulatory period in hyposmotic environment (Mancera et al., 1993b). Further studies on plasma levels of PRL in full SW- and BW-adapted fish and modifications of these levels during abrupt transfer to extreme salinity will be interesting to increase our knowledge on the osmoregulatory role of this hormone in *S. aurata*. The recently cloned PRL hormone and receptor in this species (Santos et al., 1999, 2001,) will provide news methodological tools for these studies.

GH presents an important role in SW adaptation in salmonid species. Treatment with this hormone increases salinity tolerance, gill chloride cells proliferation, gill Na^+,K^+-ATPase activity, and Na^+,K^+-ATPase α-subunit expression (Madsen et al., 1995; McCormick, 1995; Sakamoto et al., 1993; Seidelin and Madsen, 1999). In non-salmonid species, the osmoregulatory role of GH is less clear (McCormick, 1995; Mancera and McCormick, 1998b). In tilapia, *O. mossambicus* oGH treatment clearly improves adaptation to SW (Flik et al., 1993; Borski et al., 1994; Dang et al., 2000; Sakamoto et al., 1997). A similar feature has been reported for *F. heteroclitus* (Mancera and McCormick, 1998a, 1999). In a previous immunocytochemical, morphometrical, and ultrastructural study, we had evidences for an activation of GH cells in BW-adapted *S. aurata* with respect to SW-adapted (Mancera et al., 1995). The same observation has been reported in other euryhaline teleosts (*Anguilla anguilla*: Olivereau and Ball, 1970; *Mugil cephalus*: Abraham, 1974; *Pungitius pungitius*: Benjamin, 1978). Then, higher plasma GH levels could be expected in fish maintained in hypoosmotic environment.

The doses and protocol for administration GH used in our study are similar to those used in previous studies (Madsen and Bern, 1992; Bœuf et al., 1994; Seidelin and Madsen, 1997,1999). In our study, GH induced a clear hyperglycemia in SW-adapted fish, and with less intensity also in BW-adapted fish (data not showed). This agrees with the metabolic role of GH reported for other teleosts (Leung et al., 1991; O'Connor et al., 1993), suggesting that a similar biological activity of GH may occur in *S. aurata*. In the present study, the results obtained could suggested a certain hyperosmoregulatory role of GH during the regulatory and adaptative period post-transfer in *S. aurata* adapted to hypoosmotic environment. In the anadromous striped bass (*M. saxatilis*), treatment of hypophysectomized FW-adapted with recombinant striped bass GH also improved gill Na^+,K^+-ATPase activity and the capacity to maintain plasma osmolality (Madsen et al., 1996). In the air-breathing climbing perch *Anabas testudineus* administration of GH increased Na^+,K^+-ATPase activity (Leena and Oommen, 2000). In the silver seabream, *S. sarba* adapted to SW or BW, Kelly and Woo (1999) observed no changes in ions levels and a reduction in gill Na^+,K^+-ATPase activity in GH-treated respect to saline-treated fish. These authors also studied gill chloride cell morphological parameters and suggested an adaptative role of GH to hypoosmotic environments in this species. However, in the same species, GH treatment did not cause any significant changes in Na^+,K^+-ATPase α- and β-subunit mRNA expression (Deane et al., 1999).

From the present results it is not possible to conclude a clear osmoregulatory role of GH in *S. aurata*. Further studies on the influence of GH on osmoregulatory system will be necessary to clarify. The study of other osmoregulatory organs (such as kidney and intestine), use of homologous GH and other experimental approaches will be useful. Similarly to PRL, the knowledge of plasmatic values and mRNA expression of GH in SW- and BW-adapted fish and in fish under abrupt salinity changes also will be interesting.

Cortisol plays also an important metabolic role in relation to stress (Mommsen et al., 1999; Wendelaar Bonga, 1997). The experimental approach used here implicates the manipulation and injection of fish every other day which may have resulted in increased cortisol levels due to stress. We have no data on plasma cortisol values in our experimental groups, however, plasma glucose levels are also considered a good indicator of stress level in fish (Wendelaar Bonga, 1997). In our experiments, glucose values of saline groups were similar to that reported previously for *S. aurata* in non-stressed fish (data not showed) (Arends et al., 1999; Mancera et al., 1993a). Thus suggesting that stress should not be significantly present in our experiments.

In full SW- and BW-adapted *S. aurata*, which are in the chronic regulatory period, cortisol treatment increased gill Na^+,K^+-ATPase activity. However, plasma osmolality and ion levels decreased in SW-adapted fish and increased in BW-adapted fish. These results suggested a different osmoregulatory role of cortisol depending on environmental salinity. In teleosts, it is generally accepted that cortisol promotes SW adaptation. Cortisol treatment stimulates the size and number of gill chloride cells, gill Na^+,K^+-ATPase activity, α-subunit Na^+,K^+-ATPase expression, and salinity tolerance in a wide variety of teleost species (for references see Section 1). The effects of cortisol treatment on gill Na^+,K^+-ATPase activity, plasma osmolality, and ion levels in SW-adapted *S. aurata* agree with this general idea of cortisol as a hormone that promote adaptation to SW.

Several lines of evidence indicate a role of cortisol either in ion uptake in FW or BW-adapted fish. Indeed, cortisol treatment increased plasma osmolality, ion levels (Ca^{2+}, Na^+, and Cl^-), gill chloride cell abundance, surface area, and Na^+,K^+-ATPase density in gill chloride cells of several species maintained in hypoosmotic con-

ditions (rainbow trout, tilapia, eel, catfish, and medaka) (Dang et al., 2000; Eckert et al., 2001; Flik and Perry, 1989; Laurent and Perry, 1990; Perry, 1997; Yada and Ito, 1999). This could also be the case of BW-adapted *S. aurata*, with increased gill Na^+,K^+-ATPase activity, plasma osmolality, and ions levels after cortisol treatment. In this way, some marine euryhaline species, including *S. aurata*, that showed higher values of cortisol in BW respect to SW, suggesting that high cortisol levels are necessary for hypoosmotic adaptation (Johnson, 1973; Mancera et al., 1993b, 1994; Roche et al., 1989).

In a previous study on this species we demonstrated that a single injection of cortisol 3 h before transfer from SW to BW attenuates the ionic decrease after transfer. This suggests a protective role of cortisol impairing ionic loss or improving ionic intake (Mancera et al., 1993a). In the present study, four injections of cortisol every other days, prevent partly the decrease of plasma osmolality and ions levels during the adaptative period after the transfer from SW to BW. Thus suggesting data suggest a hyperosmotic role of cortisol in *S. aurata*. On the other hand, transfer from BW to SW induced an increase of plasma osmolality and ions levels in both saline and cortisol treated fish. This pattern agrees previously report in *S. aurata* (Mancera et al., 1993a). It is interesting to remark that the increase observed in plasma osmolality in cortisol-treated fish is higher than in saline-treated fish. If cortisol presents an ion uptake stimulating activity in BW-adapted fish and this activity is maintained during the first hours post-transfer this could explain the differences observed between groups. However, the results obtained in gill Na^+,K^+-ATPase activity after the transfer from BW to SW indicate a decrease in this value. This suggests that ion uptake stimulating activity of cortisol could be related to other osmoregulatory organs, such as kidney and intestine.

In several teleosts maintained in hypoosmotic media, a cooperation of PRL and cortisol to maintain normal osmolality and ions levels it has been suggested (Eckert et al., 2001; see McCormick, 2001). In *S. aurata*, it has been reported a activation of PRL and ACTH cells with higher plasma cortisol values in BW-adapted fish with respect to SW-adapted fish and it has been also suggested a cooperation of these hormones in low salinities (Mancera et al., 1993b). However, in BW-adapted fish we reported two different effects of hormone treatment on gill Na^+,K^+-ATPase activity: PRL treatment decreased this parameters, while cortisol treatment increased it. However, in both cases plasma osmolality increased. Future research about the influence of these hormonal treatments on other osmoregulatory aspects of gill, such as permeability to ions and water and on other osmoregulatory organs such as kidney and intestine will be necessary to answer this question.

In hypoosmotic media, it has been reported that a V-type H^+-ATPase is a principal pump involved in Na^+ uptake (Lin and Randall, 1995). However, a role of Na^+,K^+-ATPase pump in this ion uptake cannot be discarded. In some euryhaline species a ''U-shaped'' salinity dependence of gill Na^+,K^+-ATPase activity has been reported (see Jensen et al., 1998). Similarly, gilthead seabream also showed a ''U-shaped'' relation between gill Na^+,K^+-ATPase activity and environmental salinity in a range from 5 to 60 ppt (Mancera, unpublished data). We have no data about gill Na^+,K^+-ATPase activity in lower salinity, since in full FW *S. aurata* do not survive (Chervinski, 1984; personal observation). From our results, it is possible to suppose that in this species a V-type H^+-ATPase could be also involved in Na^+ uptake. However, our results showed a significant increases of gill Na^+,K^+-ATPase activity together with a plasma hyperosmolality in BW-adapted fish after cortisol treatment. These results suggested some role of Na^+,K^+-ATPase pump in hypoosmotic environment that is under cortisol regulation. Further studies on gill, kidney, and intestines are necessaries for the full biochemical characterization of hypoosmotic adaptation in *S. aurata.*

In conclusion, as for other teleosts, the present results suggest an important role of PRL in the adaptation to hypoosmotic environment and of cortisol in the adaptation to hyperosmotic environments in *S. aurata*. Additional studies will be necessary to elucidate the osmoregulatory role of GH in this species. In addition to its role in SW adaptation a role of cortisol in the hypoosmotic adaptation also is suggested. However, the biochemical pathways involved in this type of adaptation are not yet clear. The possibility of a dual osmoregulatory role of cortisol in *S. aurata*, as it has been suggested previously in other teleosts (Eckert et al., 2001; McCormick, 2001), deserves further research.

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