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Growth hormone and prolactin actions on osmoregulation and energy metabolism of gilthead sea bream (*Sparus auratus*)

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Abstract

The gilthead sea bream (*Sparus auratus*) is an euryhaline fish where prolactin (PRL) and growth hormone (GH) play a role in the adaptation to different environmental salinities. To find out the role of these pituitary hormones in osmoregulation and energy metabolism, fish were implanted with slow release implants of ovine GH (oGH, 5 μg g^{-1} body mass) or ovine prolactin (oPRL, 5 μg g^{-1} body mass), and sampled 7 days after the start of the treatment. GH increased branchial Na^+,K^+-ATPase activity and decreased sodium levels in line with its predicted hypoosmoregulatory action. GH had metabolic effects as indicated by lowered plasma protein and lactate levels, while glucose, triglycerides and plasma cortisol levels were not affected. Also, GH changed liver glucose and lipid metabolism, stimulated branchial and renal glucose metabolism and glycolytic activity, and enhanced glycogenolysis in brain. PRL induced hypernatremia. Furthermore, this hormone decreased liver lipid oxidation potential, and increased glucose availability in kidney and brain. Both hormones have opposite osmoregulatory effects and different metabolic effects. These metabolic changes may support a role for both hormones in the control of energy metabolism in fish that could be related to the metabolic changes occurring during osmotic acclimation.

Keywords: Gilthead sea bream; Growth hormone; Prolactin; Energy metabolism; Osmoregulation

1. Introduction

Growth hormone (GH) and prolactin (PRL) are pleiotropic and play a role in osmoregulation (McCormick, 1995; Björnsson, 1997) and energy metabolism in fish (Sheridan, 1986; Leung et al., 1991; Sangiao-Alvarellos et al., 2005b). Osmoregulation is an energetically costly process, as it requires adjustments of plasma metabolite levels and repartitioning of energy reserves to osmoregulatory organs, as well as new set point for plasma ion levels (Sangiao-Alvarellos et al., 2003b, 2005c).

With respect to osmoregulation in salmonids, GH facilitates seawater acclimation through chloride cell proliferation; by doing so it, enhances branchial Na^+,K^+-ATPase capacity, and stimulates expression of the Na^+,K^+-ATPase α-subunit (Sakamoto et al., 1993; Björnsson, 1997). In non-salmonid species, GH enhances hyper- or hypoosmoregulatory capacity depending on the species (Mancera and McCormick, 1998b; McCormick, 2001).

GH stimulates lipolysis in several fish species (Sheridan, 1986; O'Connor et al., 1993). Moreover, GH is anabolic as it increases protein synthesis and turnover (Foster et al., 1991; Seiddiki et al., 1995; Herbert et al., 2001). The effects of GH on

Abbreviations: Ala-AT, alanine aminotransferase (EC. 2.6.1.2.); Asp-AT, aspartate aminotransferase (EC. 2.6.1.1.); FBPase, fructose 1,6-bisphosphatase (EC. 3.1.3.11.); 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-hydroxyacyl-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.).

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carbohydrate metabolism in fish are less well studied. GH treatment reduces glycogen synthetase activity and decreased hepatic glycogen levels in tilapia (Leung et al., 1991) and, in line with this observation, this treatment also induces hyperglycemia in several fish species (Sweeting et al., 1985; Leung et al., 1991). In freshwater (FW) adapted rainbow trout, GH increases plasma glucose levels, decreases the glycolytic potential and the capacity to export glucose from liver; and also affects carbohydrate metabolism of gills, kidney and brain (Sangiao-Alvarellos et al., 2005b).

In fish, PRL controls water and electrolyte balance (especially in FW conditions), growth and development. Moreover, its pleiotropic character is illustrated by its effects on metabolism, behaviour, reproduction and immunoregulation (Hirano, 1986; Manzon, 2002). Indeed, PRL receptors were demonstrated in gills, kidney, gut, skin, and liver of several fish species (Prunet and Auperin, 1995; Manzon, 2002).

In waters of low salinity or FW environments, pituitary expression of PRL mRNA (Martin et al., 1999) and plasma levels of PRL (Auperin et al., 1995) increase to produce various osmoregulatory changes (Manzon, 2002). In addition, acclimation to hypoosmotic media induces changes in energy metabolism (Sangiao-Alvarellos et al., 2003b, 2005c). Surprisingly, very few investigations have examined the effects of PRL on the energy metabolism of osmoregulatory (Leena and Oommen, 2000) and non-osmoregulatory organs of teleosts. The few studies available regarding metabolic effects of PRL in fish only address effects on lipid metabolism (Sheridan, 1986; Leena and Oommen, 2000, 2001; Leena et al., 2001), and limited results on carbohydrate metabolism are available (Mancera et al., 2002).

During osmotic acclimation, the gilthead sea bream (*Sparus auratus*) shows osmoregulatory (Mancera et al., 1993a; 2002) and metabolic changes (Sangiao-Alvarellos et al., 2003b, 2005c). In this species, adaptation to brackish water or lower salinity activates PRL cells (Mancera et al., 1993b) whereas acclimation to hyperosmotic salinity enhances GH cells (Mancera et al., 1995). Previously, we assessed osmoregulatory actions of PRL and GH in sea bream (Mancera et al., 2002). However, effects of these hormones on energy metabolism of this species are unknown. Here we present effects of GH and PRL treatment on osmoregulation and energy metabolism in gilthead sea bream.

2. Materials and methods

2.1. Animals and experimental design

Sexually immature male gilthead sea bream (*S. auratus* L., 200–250 g body mass) were provided by Planta de Cultivos Marinos (C.A.S.E.M., Universidad de Cádiz, Puerto Real, Cádiz, Spain) and transferred to the laboratory at the Faculty of Marine Science (Puerto Real, Cádiz). Fish were acclimatised to seawater (36 p.p.t. salinity) in 400 L aquaria in an open system. During the experiments (May–June 2004), fish were kept under natural photoperiod and constant temperature (18 °C). Fish were fed a daily ration of 1% of the estimated body weight with commercial dry pellets (Dibaq-Diprotg SA, Segovia, Spain). Fish were fasted 24 h before sampling. The experiments described comply with the Guidelines of the European Union Council (86/609/EU) and those of the University of Cádiz (Spain) for the use of laboratory animals.

After 1 month of acclimatisation to laboratory conditions, fish were anaesthetized with 0.05% phenoxyethanol, weighed, and intraperitoneally implanted with slow-release vegetable oil implants following procedures previously described for administration of GH and PRL (Mancera and McCormick, 1999; Pelis and McCormick, 2001). Fish were injected with 10 μL g^{-1} body mass of vegetable oil (controls) or ovine growth hormone (oGH; National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, NIADDK-oGH-15, 5 μg g^{-1} body mass) or ovine prolactin (oPRL; NIADDK-oPRL-21, 5 μg g^{-1} body mass). Fish were sampled ($n=12$ per group) 7 days later. A group of 12 fish was manipulated and anesthetized identically, but not injected, and sampled after 7 days (untreated controls).

The use of mammalian hormones in fish is well established to determine hormonal control of osmoregulation (see Seidelin and Madsen, 1999), and both hormones were successfully used before in gilthead sea bream (Mancera et al., 2002). The doses, way of administration and exposure time are similar to those previously reported by others (Seidelin and Madsen, 1999; Zhou et al., 2004).

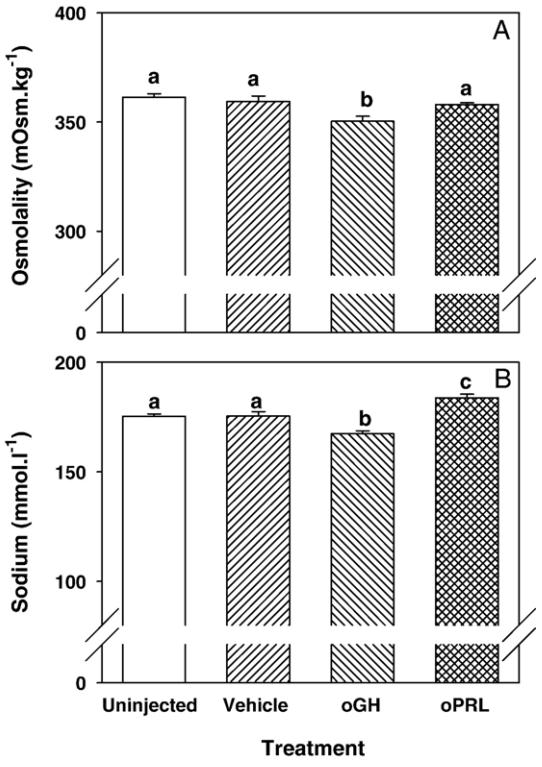

Fig. 1. Changes in plasma osmolality (A) and sodium (B) levels in gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 μg g^{-1} body mass) or oPRL (5 μg g^{-1} body mass). Basal levels of parameters were assessed from uninjected fish. Each value is the mean±S.E.M. of $n=12$ fish in each group (uninjected, vehicle, oGH and oPRL). Different letters indicate significant differences among groups ($P<0.05$, Student-Newman-Keuls test).

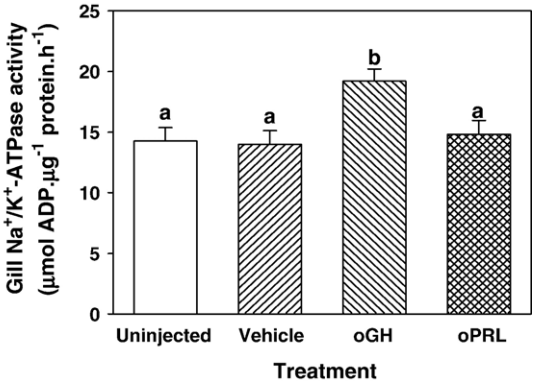

Fig. 2. Changes in gill Na^+,K^+-ATPase activity in gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 µg g^{-1} body mass) or oPRL (5 µg g^{-1} body mass). Basal levels of parameters were assessed from uninjected fish. Further details as in legend of Fig. 1.

After 7 days, fish were quickly netted from the holding tanks, anaesthetized with 2-phenoxyethanol (0.1%), weighed and sampled. Blood was obtained with ammonium-heparin treated syringes from the caudal vessels. Plasma was obtained by centrifugation (30 s at 13,000×*g*), and stored in two aliquots. One aliquot was immediately frozen in liquid nitrogen for the assessment of plasma osmolality, and levels of cortisol, sodium, and protein; the other aliquot was deproteinized with 6% perchloric acid, neutralized with 1 mol L^{-1} potassium bicarbonate, frozen in liquid nitrogen and stored at −80 °C. To assess gill Na^+,K^+-ATPase activity, 3–5 filaments from the second branchial arch (cut just above the septum with fine point scissors) were placed in 100 µL of ice-cold SEI buffer (150 mmol L^{-1} sucrose, 10 mmol L^{-1} EDTA, 50 mmol L^{-1} imidazole, pH 7.3) and frozen at −80 °C. To evaluate renal Na^+, K^+-ATPase activity, a small portion of posterior kidney was put in ice-cold SEI buffer and frozen at −80 °C. Brain, liver, the remainder of the kidney, and the other branchial arches were removed quickly from each fish, rinsed in saline, freeze-clamped in liquid nitrogen, and stored at −80 °C.

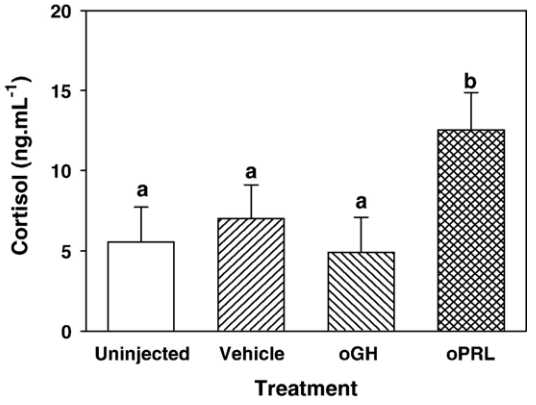

Fig. 3. Changes in plasma cortisol levels in gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 µg g^{-1} body mass) or oPRL (5 µg g^{-1} body mass). Basal levels of parameters were assessed from uninjected fish. Further details as in legend of Fig. 1.

2.2. Analytical procedures

Plasma glucose, lactate and triglyceride were measured using commercial microplate kits from Spinreact (Spain). Plasma protein was measured using the bicinchoninic acid method with a BCA protein kit (Pierce, Rockford, USA) for microplates; bovine serum albumin served as standard. Plasma osmolality was measured with a vapour pressure osmometer (Fiske One-Ten Osmometer, Fiske, VT, USA). Plasma Na^+ was measured using an atomic absorption spectrophotometer (Philips PU7000).

Gill and kidney Na^+,K^+-ATPase activity was determined by use of a microassay procedure based on ADP release (McCormick, 1993) adapted to *S. auratus* (Mancera et al., 2002).

Plasma cortisol levels were measured by indirect enzyme immunoassay (ELISA) validated for gilthead sea bream (Tintos et al., 2006). The ELISA satisfied the criteria of specificity (testing cross-reactivity with other steroids), reproducibility (interassay coefficient of variation <6%), precision (intra-assay coefficient of variation <4%), and accuracy (average recovery >98%).

Frozen liver, brain, kidney, and gill filaments were finely minced on an ice-cooled Petri dish, vigorously mixed and divided into two aliquots to assess enzyme activities and metabolite levels. The frozen tissue used for the assessment of metabolite levels was homogenized by ultrasonic disruption with 7.5 vol. of ice-cooled 6% perchloric acid, neutralized (using 1 mol L^{-1} potassium bicarbonate), centrifuged (2 min at 13,000×*g*); the supernatant was used to assay tissue metabolites. Lactate levels were determined spectrophotometrically by use

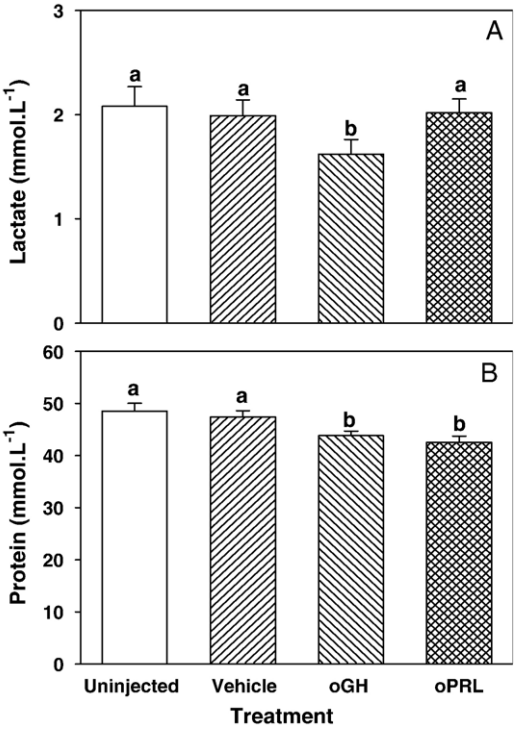

Fig. 4. Changes in plasma levels of lactate (A) and triglyceride (B) in gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 µg g^{-1} body mass) or oPRL (5 µg g^{-1} body mass). Basal levels of parameters were assessed from uninjected fish. Further details as in legend of Fig. 1.

of a commercial kit (Spinreact, Spain). Tissue glycogen levels were assessed using the method of Keppler and Decker (1974). Glucose obtained after glycogen breakdown in tissues (after subtracting free glucose levels), as well as tissue glucose levels, were determined with a commercial kit (Biomérieux, Spain).

The aliquots of tissues used for the assessment of enzyme activities were homogenized by ultrasonic disruption with 10 vol. of ice-cold stop-buffer containing: 50 mmol L^{-1} imidazole–HCl (pH 7.5), 15 mmol L^{-1} 2-mercaptoethanol, 100 mmol L^{-1} KF, 5 mmol L^{-1} EDTA, 5 mmol L^{-1} EGTA, and a protease inhibitor cocktail (Sigma, P-2714). The homogenate was centrifuged (2 min at 13,000×*g*) and the supernatant used in enzyme assays. In those cases where microsomal enzymes were assessed appropriate centrifugations were carried out to obtain 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 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. Therefore enzyme activities are expressed per mg protein. Protein was assayed in triplicate in homogenates according to Bradford (1976) with bovine serum albumin (Sigma, USA) as standard. Enzymatic analyses were carried out at conditions meeting requirements for optimal velocities. The specific conditions for enzyme assays were described previously (Laiz-Carrión et al., 2002, 2003; Sangiao-Alvarellos et al., 2003a,b, 2004, 2005a,b,c).

2.3. Statistics

Comparisons among groups were performed with one-way ANOVA using the SigmaStat statistical package. Post-hoc comparisons were made using Student-Newman-Keuls test; differences were considered statistically significant when $P<0.05$.

Table 1
Liver changes in the levels of metabolites and enzyme activities of gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 μg g^{-1} body mass) or oPRL (5 μg g^{-1} body mass)

Parameter	Uninjected	Vehicle	Treatment (5 μg g^{-1} body mass)	
			oGH	oPRL
Metabolites				
Glycogen levels (μmol glycosyl units g^{-1} wet wt.)	617±40.5 a	623±32.3 a	497±33.9 b	590±43.5 a
Glucose levels (μmol g^{-1} wet wt.)	22.4±2.89	19.8±2.26	18.3±1.21	21.5±2.63
Lactate levels (μmol g^{-1} wet wt.)	0.51±0.09	0.44±0.06	0.41±0.09	0.41±0.05
Enzyme activities				
Carbohydrate metabolism				
Glycogenolysis				
GPase activity				
Total activity (U mg^{-1} protein)	0.80±0.07	0.82±0.06	0.85±0.05	0.87±0.06
%GPase *a*	31.2±0.76 a	31.6±0.54 a	35.9±0.69 b	32.6±1.04 a
Glycolysis				
PFK activity				
Optimal activity (U mg^{-1} protein)	0.69±0.01 a	0.72±0.04 a	0.62±0.01 b	0.73±0.02 a
Activity ratio	0.45±0.01	0.42±0.03	0.45±0.03	0.44±0.03
F2-6P_2-activation ratio	0.41±0.03	0.36±0.02	0.37±0.02	0.40±0.03
Gluconeogenesis				
FBPase activity (U mg^{-1} protein)	0.88±0.04a	0.89±0.05 a	0.73±0.05 b	0.80±0.06 a
Glucose export capacity				
G6Pase activity (U mg^{-1} protein)	5.50±0.56 a	6.09±1.22 a	8.72±0.73 b	7.04±0.79 ab
Pentose phosphate pathway				
G6PDH activity (U mg^{-1} protein)	3.06±0.20	2.79±0.11	2.62±0.13	2.73±0.19
Amino acid metabolism				
Asp-AT activity (U mg^{-1} protein)	3.75±0.11 a	3.74±0.14 a	3.07±0.12 b	3.40±0.15 ab
Ala-AT activity (U mg^{-1} protein)	3.03±0.14 a	3.28±0.15 a	2.63±0.18 b	2.98±0.33 ab
GDH activity (U mg^{-1} protein)	13.9±0.32 a	13.7±0.45 a	12.3±0.39 b	12.9±0.44 ab
Lipid metabolism				
HOAD activity (U mg^{-1} protein)	0.21±0.02 a	0.23±0.03 a	0.17±0.01 b	0.18±0.01 b
Lactate metabolism				
LDH-O activity (U mg^{-1} protein)	0.33±0.03 a	0.31±0.03 a	0.22±0.01 b	0.30±0.05 a

Each value is the mean±S.E.M. of $n=12$ fish in each group (uninjected, vehicle, oGH, and oPRL). Different letters indicate significant differences among groups ($P<0.05$, Student-Newman-Keuls test). Basal levels of parameters were obtained from uninjected fish.

3. Results

All fish used recovered well from anesthesia and the implantation procedure. No mortality was observed throughout the experiment. No differences were noticed for any parameter assessed when comparing untreated and vehicle-treated fish.

3.1. GH effects

Plasma osmolality and sodium levels decreased in fish treated with oGH (Fig. 1). This treatment increased gill Na^+,K^+-ATPase activity significantly (Fig. 2) while kidney Na^+,K^+-ATPase activity did not change (data not shown). Plasma cortisol levels were not affected by treatment (Fig. 3) whereas plasma levels of lactate and protein decreased (Fig. 4). No effects were observed in plasma glucose and triglyceride levels (data not shown).

Changes in activity of metabolic enzymes and levels of metabolites in liver after hormone treatment are detailed in Table 1. The percentage of GPase in the active form and G6Pase activity had increased following oGH treatment. In contrast, the optimal activity of PFK, the activities of FBPase, Ala-AT, Asp-AT, GDH, HOAD, LDH-O, and glycogen levels had decreased.

In gills, oGH treatment increased the total activity of PK, and HK activity (Table 2). In kidney, this treatment enhanced total activity of GPase, optimal activity and activity ratio of PK, and activities of HK and G6PDH, but decreased glycogen levels (Table 3). Finally, in brain (Table 4), oGH treatment diminished the activity ratio and cofactor activation ratio of PK, as well as glycogen levels, and increased HK activity and glucose levels.

3.2. PRL effects

oPRL treatment increased plasma sodium levels, but not plasma osmolality (Fig. 1), whereas no changes were observed in gill (Fig. 2) and kidney (data not shown) Na^+,K^+-ATPase activitity. Basal plasma cortisol levels were significantly increased by oPRL treatment (Fig. 3). In plasma metabolic parameters assessed, protein levels decreased after treatment (Fig. 4) whereas no changes were noticed in lactate (Fig. 4), and glucose and protein levels (data not shown).

The significant effects induced by oPRL treatment in metabolic parameters were (i) decreased HOAD activity in liver (Table 1), (ii) increased glucose levels in gills (Table 2), (iii) a decrease in the cofactor activation ratio of PK and an increase in HK and G6Pase activities in kidney (Table 3), and (iv) an increase in glucose levels as well as in the activities of HK and G6PDH in brain (Table 4).

4. Discussion

4.1. GH effects

oGH treatment decreased plasma osmolality and sodium levels, and increased gill Na^+,K^+-ATPase activity in agreement with data observed previously in several teleosts (Mancera and McCormick, 1998a; Leena and Oommen, 2000; Pelis and McCormick, 2001; Sangiao-Alvarellos et al., 2005b). These data are reflecting an enhanced hypoosmoregulatory capacity and agree with the role of GH during seawater acclimation. The lack of effect of oGH treatment on kidney Na^+,K^+-ATPase activity is also in agreement with data reported for *S. trutta*

Table 2
Gill changes in the levels of metabolites and enzyme activities of gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 μg g^{-1} body mass) or oPRL (5 μg g^{-1} body mass)

Parameter	Uninjected	Vehicle	Treatment (5 μg g^{-1} body mass)	
			oGH	oPRL
Metabolites				
Glycogen levels (μmol glycosyl units g^{-1} wet wt.)	1.16±0.15	1.42±0.18	1.64±0.15	1.48±0.19
Glucose levels (μmol g^{-1} wet wt.)	0.30±0.09 a	0.29±0.06 a	0.27±0.03 a	0.50±0.07 b
Lactate levels (μmol g^{-1} wet wt.)	1.83±0.15	1.72±0.09	1.78±0.12	1.70±0.20
Enzyme activities				
Carbohydrate metabolism				
Glycogenolysis				
GPase activity				
Total activity (U mg^{-1} protein)	0.13±0.007	0.13±0.006	0.14±0.004 ab	0.15±0.006 b
%GPase *a*	23.5±2.58	22.9±2.27	26.5±1.65	22.6±2.34
Glycolysis				
PK activity				
Optimal activity (U mg^{-1} protein)	5.73±0.34 a	5.83±0.22 a	6.51±0.18 b	6.17±0.21 ab
Activity ratio	0.40±0.01	0.42±0.03	0.45±0.03	0.44±0.03
F1-6P_2-activation ratio	0.71±0.02	0.74±0.03	0.78±0.03	0.77±0.04
Glucose phosphorylating capacity				
HK activity (U mg^{-1} protein)	1.04±0.04 a	1.05±0.03 a	1.15±0.03 b	1.10±0.03 ab
Pentose phosphate pathway				
G6PDH activity (U mg^{-1} protein)	1.79±0.09	1.78±0.05	1.95±0.10	1.81±0.07

Each value is the mean±S.E.M. of $n=12$ fish in each group (uninjected, vehicle, oGH, and oPRL). Different letters indicate significant differences among groups ($P<0.05$, Student-Newman-Keuls test). Basal levels of parameters were obtained from uninjected fish.

Table 3
Kidney changes in the levels of metabolites and enzyme activities of gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 μg g^{-1} body mass) or oPRL (5 μg g^{-1} body mass)

Parameter	Uninjected	Vehicle	Treatment (5 μg g^{-1} body mass)	
			oGH	oPRL
Metabolites				
Glycogen levels (μmol glycosyl units g^{-1} wet wt.)	1.15±0.05 a	1.12±0.06 a	0.96±0.03 b	1.17±0.04 a
Glucose levels (μmol g^{-1} wet wt.)	2.21±0.23	2.20±0.29	2.54±0.30	2.42±0.18
Lactate levels (μmol g^{-1} wet wt.)	1.84±0.20 a	1.78±0.12 a	1.49±0.21 b	1.95±0.25 a
Enzyme activities				
Carbohydrate metabolism				
Glycogenolysis				
GPase activity				
Total activity (U mg^{-1} protein)	0.09±0.004 a	0.09±0.003 a	0.11±0.003 b	0.10±0.004 ab
%GPase *a*	29.0±2.26	30.4±1.13	27.5±2.46	31.3±1.52
Glycolysis				
PK activity				
Optimal activity (U mg^{-1} protein)	5.54±0.30 a	5.59±0.26 a	6.57±0.22 b	5.69±0.16 a
Activity ratio	0.15±0.01 a	0.14±0.01 a	0.19±0.01 b	0.15±0.01 a
F1-$6P_2$-activation ratio	0.51±0.04 a	0.50±0.06 a	0.52±0.05 a	0.41±0.04 b
Glucose phosphorylating capacity				
HK activity (U mg^{-1} protein)	0.23±0.03 a	0.25±0.02 a	0.35±0.03 b	0.32±0.02 b
Glucose export capacity				
G6Pase activity (U mg^{-1} protein)	1.26±0.23 a	1.29±0.21 a	1.19±0.18 a	1.72±0.20 b
Pentose phosphate pathway				
G6PDH activity (U mg^{-1} protein)	1.55±0.09 a	1.64±0.08 a	1.99±0.11 b	1.79±0.06 ab

Each value is the mean±S.E.M. of $n=12$ fish in each group (uninjected, vehicle, oGH, and oPRL). Different letters indicate significant differences among groups ($P<0.05$, Student-Newman-Keuls test). Basal levels of parameters were obtained from uninjected fish.

(Madsen et al., 1995) though decreased activity was observed in GH-treated *Sparus sarba* (Kelly et al., 1999).

The effect of GH treatment in plasma glucose levels depends on the species studied with both increases (Sweeting et al., 1985; Leung et al., 1991; Mancera and McCormick, 1998a; Vega-Rubín de Celis et al., 2003; Sangiao-Alvarellos et al., 2005b), or no changes being reported (Inui et al., 1985; McCormick, 1996; this study). Plasma triglyceride levels were not modified by oGH treatment in the present experiment in agreement with studies performed in other species (Farbridge and Leatherland, 1988;

Table 4
Brain changes in the levels of metabolites and enzyme activities of gilthead sea bream after treatment with vegetable oil (vehicle) alone or containing oGH (5 μg g^{-1} body mass) or oPRL (5 μg g^{-1} body mass)

Parameter	Uninjected	Vehicle	Treatment (5 μg g^{-1} body mass)	
			oGH	oPRL
Metabolites				
Glycogen levels (μmol glycosyl units g^{-1} wet wt.)	1.18±0.06 a	1.23±0.07 a	0.99±0.07 b	1.11±0.12 ab
Glucose levels (μmol g^{-1} wet wt.)	0.98±0.14 a	0.89±0.14 a	1.31±0.09 b	1.27±0.08 b
Lactate levels (μmol g^{-1} wet wt.)	5.34±0.41	4.99±0.45	5.85±0.60	6.34±0.67
Enzyme activities				
Carbohydrate metabolism				
Glycogenolysis				
GPase activity				
Total activity (U mg^{-1} protein)	0.48±0.02	0.45±0.02	0.46±0.02	0.45±0.02
%GPase *a*	48.1±2.34	47.9±2.01	50.6±2.02	50.9±1.35
Glycolysis				
PFK activity				
Optimal activity (U mg^{-1} protein)	8.06±0.30	7.65±0.23	8.08±0.40	7.96±0.41
Activity ratio	0.02±0.002 a	0.02±0.002 a	0.01±0.002 b	0.02±0.001 a
F2-$6P_2$-activation ratio	0.07±0.007 a	0.07±0.008 a	0.05±0.007 b	0.08±0.009 a
Glucose phosphorylating capacity				
HK activity (U mg^{-1} protein)	2.02±0.05 a	2.07±0.05 a	2.33±0.03 b	2.24±0.04 b
Pentose phosphate pathway				
G6PDH activity (U mg^{-1} protein)	0.70±0.02 a	0.71±0.02 a	0.79±0.02 ab	0.84±0.04 b

Each value is the mean±S.E.M. of $n=12$ fish in each group (uninjected, vehicle, oGH, and oPRL). Different letters indicate significant differences among groups ($P<0.05$, Student-Newman-Keuls test). Basal levels of parameters were obtained from uninjected fish.

Leung et al., 1991). However, lactate levels decreased in contrast with that observed in rainbow trout kept in FW (Seiddiki et al., 1995; Sangiao-Alvarellos et al., 2005b). Plasma protein levels also decreased in the present study whereas no changes have been reported after GH treatment in rainbow trout (Farbridge and Leatherland, 1988) and tilapia (Leung et al., 1991). Therefore, changes in plasma metabolites following GH treatment apparently are species-dependent.

In liver of oGH-treated gilthead sea bream, a significant decrease in glycogen levels as well as increased GPase activity were observed, suggesting an enhancement of glycogenolytic potential in agreement with results obtained in tilapia (Leung et al., 1991) but not with the lack of changes reported in rainbow trout (Inui et al., 1985; Farbridge and Leatherland, 1988; Sangiao-Alvarellos et al., 2005b). In mammals, GH is known to produce a decrease in liver GSase activity (Bak et al., 1991) in support of a glycogenolytic action for this hormone. The increased mobilization of glycosyl residues from glycogen stores could be predicted to produce increases in the capacity of liver to export glucose, in line with the increased G6Pase activity observed.

Liver gluconeogenic potential appears to be decreased by GH treatment in gilthead sea bream. The changes observed in the activity of liver Asp-AT, Ala-AT, and GDH also suggest a reduction in the catabolism of amino acids thus reinforcing the hypothesis of a reduced gluconeogenic flux, which is in agreement with the known role of GH as a stimulator of protein synthesis in fish (Foster et al., 1991). The remaining metabolic parameters assessed in liver of GH-treated fish pointed to a decrease in the glycolytic potential of this tissue. This decreased oxidative capacity would corroborate similar findings in rainbow trout (Sangiao-Alvarellos et al., 2005b) and European sea bass (Vega-Rubín de Celis et al., 2003).

oGH treatment also induced a marked decrease in the activity of the fatty acid oxidative enzyme HOAD in agreement with another study in the climbing perch (Leena et al., 2001) which is in contrast to the lipolytic action demonstrated in liver of several fish species (Sheridan, 1986; O'Connor et al., 1993). With respect to lactate metabolism, the decreased LDH activity in the oxidative direction is suggesting a reduction in the potential of liver to use lactate. The absence of changes in liver lactate levels seems to reinforce this hypothesis.

As a whole, data obtained from liver of gilthead sea bream substantiate that oGH treatment decreases the capacity of liver to produce ATP from glycolysis but increases the capacity to export glucose to plasma. Metabolites and enzyme activities involved in lipid, amino acid and lactate metabolism in liver are reduced after oGH treatment suggesting a metabolic reallocation of energy substrates. In fish treated with oGH plasma glucose levels do not increase simultaneously, and this could suggest an enhanced use of glucose in other tissues (i.e. osmoregulatory organs like gills and kidney, see below). All these metabolic changes clearly differ from those observed in rainbow trout treated with similar doses oGH (Sangiao-Alvarellos et al., 2005b). Apparently, different species-specific metabolic response to GH treatment occur, and the difference could relate to differences in osmoregulatory strategies i.e. truly euryhaline (gilthead sea bream) versus partially euryhaline (rainbow trout).

Considering that GH receptors are present in gill chloride cells of teleost (Tse et al., 2003; Fukada et al., 2004) including gilthead sea bream (Calduch-Giner et al., 2003), and the increase observed in gill Na^+,K^+-ATPase activity after oGH treatment (Mancera and McCormick, 1998a; Leena and Oommen, 2000; Pelis and McCormick, 2001; Sangiao-Alvarellos et al., 2005b), an action of GH on gill energy metabolism seems reasonable. Gilthead sea bream treated with oGH showed a small increase in the capacity of gill tissue to phosphorylate glucose and this is in agreement with a similar observation in rainbow trout (Sangiao-Alvarellos et al., 2005b). A higher increase of this capacity has also been reported in gilthead sea bream during acclimation to different salinities (Sangiao-Alvarellos et al., 2003b, 2005c). The increased capacity of phosphorylating exogenous glucose would support a higher use of glucose in this tissue after oGH treatment, which could be used to provide the ATP needed for sustaining Na^+,K^+-ATPase activity elicited by oGH treatment. The increase observed in gill glycolytic potential would also support an enhanced use of glucose in gills.

GH receptors have also been found in kidney of several teleosts, including gilthead sea bream (Tse et al., 2003; Calduch-Giner et al., 2003; Fukada et al., 2004), and their presence has been related to an osmoregulatory action of GH in this tissue (McCormick, 2001). The increased HK activity in kidney of oGH-treated fish points to an enhanced capacity for phosphorylating exogenous glucose within this tissue. An increased glycogenolytic potential was also observed in kidney of oGH-treated fish in agreement with that observed in GH-treated rainbow trout (Sangiao-Alvarellos et al., 2005b). The increased amounts of glucose in kidney resulting from both processes could be exported to plasma or being used by the renal cells. Since G6Pase activity did not show any change in oGH-treated gilthead se abream, the second possibility is more likely. In supporting of this hypothesis, oGH increases glycolytic potential through increased PK activity in fish kidney.

In brain, an enhanced glycogenolytic potential is apparent in oGH-treated gilthead sea bream in agreement with that previously suggested for rainbow trout (Sangiao-Alvarellos et al., 2005b). The mobilization of stored glycogen, the major energy reserve of fish brain (Soengas and Aldegunde, 2002), is accompanied by a marked increase in the capacity of brain to phosphorylate exogenous glucose.

The results of this study suggest that GH acts by inducing changes in energy metabolism of several tissues of gilthead sea bream. Since GH is known to induce increased production of IGF-I mRNA in liver, gills, kidney, and brain (Biga et al., 2004; Carnevali et al., 2005), at least part of the metabolic changes herein described could be also attributed to IGF-I action. In addition, the osmoregulatory and metabolic roles of GH could be mediated by cortisol (see McCormick, 2001). Cortisol levels were not affected by GH treatment suggesting that this possibility may be ruled out. However, GH treatment must have increased the number and affinity of gill cortisol receptors or cortisol turnover (Shrimpton et al., 1995) enhancing hypoosmoregulatory capacity and metabolic changes associated without any change in plasma cortisol levels.

4.2. Prolactin effects

The hyperosmoregulatory role of PRL is well established in teleosts since treatment with this hormone decreases gill Na^+, K^+-ATPase activity (Hirano, 1986) through changes in the expression of the subunits of the enzyme (Deane et al., 1999). However, depending on environment, in some species PRL treatment increased or had no effect on gill Na^+,K^+-ATPase activity (Flik et al., 1994; McCormick, 1995; Leena and Oommen, 2000; Manzon, 2002). In the present study, PRL treatment did not affect gill Na^+,K^+-ATPase activity in contrast with the decrease reported in specimens of the same species acclimated to seawater or brackish water (Mancera et al., 2002).

It is assumed that PRL acts on kidney producing an increase in sodium reabsorption and water excretion. However, effects of PRL treatment on kidney Na^+,K^+-ATPase activity are contradictory in FW-adapted fish (Braun and Dantzler, 1987). In the present study oPRL treatment did not affect kidney Na^+,K^+-ATPase activity in agreement with previous studies carried out in salmonids (McCormick et al., 1989; Madsen et al., 1995) and in silver sea bream (Kelly et al., 1999). Manzon (2002) suggested that the role of PRL on kidney function is related to water balance. If this hypothesis is correct, this could explain the failure of PRL treatment inducing any change in kidney Na^+,K^+-ATPase activity in the present experiment.

PRL receptors have been cloned from several teleosts including gilthead sea bream, characterised and its distribution in osmoregulatory (gill, kidney and intestinal tract) and non-osmoregulatory (liver) organs reported (Prunet and Auperin, 1995; Santos et al., 2001). PRL also affects energy metabolism (Sheridan, 1986; Leena and Oommen, 2000, 2001; Leena et al., 2001), and the modifications of metabolic parameters observed in gilthead sea bream are in line with such action of oPRL. However, other indirect pathways cannot be excluded. Cortisol exerts important effects on metabolism of osmoregulatory and non-osmoregulatory organs of gilthead sea bream (Laiz-Carrión et al., 2002, 2003) and oPRL treatment increased cortisol levels in tilapia (Flik et al., 1994) and also gilthead sea bream (present results). Accordingly, this hormone could induce some of the metabolic changes observed after oPRL-treatment.

All major pathways of carbohydrate, amino acid, and lactate metabolism do not appear to be significantly altered by oPRL treatment in liver. As for lipid metabolism, HOAD activity displayed a decrease in oPRL-treated fish. PRL treatment increased triglyceride-lipase and decreased lipid levels in several tissues of coho salmon (Sheridan, 1986), whereas in the climbing perch this treatment decreased liver G6PDH and ICDH activities without altering malic enzyme activities (Leena and Oommen, 2001; Leena et al., 2001). Therefore, the decreased fatty acid oxidative capacity observed in the present experiment does not apparently coincide with the antilipogenic activity reported in studies using the climbing perch. Different species-specific metabolic response to PRL could explain these discrepancies.

No references are available for any fish species on changes in energy metabolism in organs other than liver after PRL treatment. In gills, the only significant effect that oPRL treatment induced in the metabolic parameters assessed was an increase in free glucose levels, which can be considered a marginal effect since no other metabolic changes were apparent. In kidney and brain, oPRL treatment enhanced the potential for exogenous glucose use as indicated by elevation of HK activity. This higher use of glucose is also related to the increased levels of free glucose available in brain, but not in kidney, at the same time. Considering that no apparent changes were noticed for glycolytic potential and glycogen levels it seems that the increased availability of exogenous glucose is used in brain to provide reducing power through increased capacity of the pentose phosphate pathway, which is normally used for increased lipogenesis.

4.3. General considerations

In conclusion, treatment of seawater gilthead sea bream with oGH induced changes in osmoregulatory parameters similar to those observed naturally during hyperosmotic acclimation (McCormick, 2001). In addition, oGH also modified parameters of energy metabolism in liver, gills, kidney and brain, which in some cases are different from those previously reported in other fish species suggesting (i) a role for this hormone in regulation of energy metabolism in gilthead sea bream, and (ii) a different action of GH on energy metabolism depending on the fish species assessed. These results suggest that GH could be involved in the regulation of changes in energy metabolism associated with hyperosmotic acclimation, and the different metabolic responses observed compared with other teleost fish could be attributable to the different osmoregulatory ability of species assessed. In contrast, oPRL had not effect on osmoregulatory system and induced only a few changes in parameters of energy metabolism in liver, gills, kidney and brain supporting a minor (if any) role for this hormone in regulation of energy metabolism in seawater adapted gilthead sea bream. Considering that PRL is necessary for adaptation to low salinity environments (Hirano, 1986; Manzon, 2002), it will be interesting to check the metabolic effects of exogenous oPRL on specimens of gilthead sea bream adapted to this kind of environment where PRL system (expression, number and affinity of receptors, etc.) is activated.

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