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Energy metabolism in fish tissues related to osmoregulation and cortisol action

Raúl Laiz-Carrión[1], Susana Sangiao-Alvarellos[2], José M. Guzmán[1], María P. Martín del Río[1], Jesús M. Míguez[2], José L. Soengas[2,*] and Juan M. Mancera[1]
[1]*Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain*
[2]*Laboratorio de Fisioloxía Animal, Facultade de Ciencias, Universidade de Vigo, 36200 Vigo, Spain*
[*]*Author for correspondence (Phone: +34-986 812 564; Fax: +34-986 812 556; E-mail: jsoengas@uvigo.es)*

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Abstract

This is an overview of our recent studies of energy metabolism in fish brain and other organs regulated by exogenous (feeding, salinity) and endogenous (hormones) factors. To highlight our approach, we present latest results concerned osmoregulation in the gills of gilthead seabream, *Sparus auratus*. Our model, the seabream, is a euryhaline teleost capable of adaptation to extreme changes in environmental salinity. Treatment with cortisol allowed us to achieve circulating cortisol levels similar to those observed during osmotic adaptation and to assess how elevated hormonal levels affected simultaneously metabolic and osmoregulatory capacities of the gill tissue. Cortisol-implanted fish showed higher gill Na^+,K^+-ATPase activity than control fish but no changes were observed in plasma osmolality and ion levels. Plasma levels of glucose and lactate increased in cortisol-implanted fish while protein levels decreased. Cortisol treatment elicited metabolic changes in liver and brain reflecting an activation of the glycogenic and gluconeogenic potential in liver, and the glycogenic potential in brain, which are confirmatory of data obtained in previous experiments. In gills, we demonstrated that cortisol treatment elicited changes in their energy metabolism that can be summarized as a decreased capacity in the use of exogenous glucose (decreased HK activity), a decrease in the capacity of the pentose phosphate pathway (decreased G6PDH activity), and an increased glycolytic potential (increased PK activity). Observed metabolic changes in gills can be associated with those occurring in nature during osmotic adaptation in the same fish species.

Introduction

Our recent research has been focused on two different aspects of fish physiology. The first aspect was the characterization of energy metabolism in fish brain and its changes due to the action of endogenous and exogenous factors (see Soengas and Aldegunde 2002). Some of this research has been carried out in collaboration with the group of Dr. Manuel Aldegunde, who in recent years has been working on the physiology of neurotransmitter systems in fish brain. The vertebrate brain is isolated from the systemic circulation by a blood-brain barrier that allows the transport of glucose, monocarboxylates and amino acids. We have studied the transport of glucose (Aldegunde et al. 2000a) and neutral amino acids (Aldegunde et al. 1998; 2000b) suggesting transport kinetics similar to those previously described in other vertebrates. The limited information available (Soengas et al. 1996b, 1998a; Laiz-Carrión et al. 2003) supports an idea that once in the brain, oxidation of exogenous glucose and oxidative phosphorylation provide most of the ATP required for brain function in teleosts. Inputs regarding this issue derived from assessing utilization rates of different metabolites such as glucose, lactate and ketone bodies in rainbow trout brain (Soengas et al. 1998a), as well as by measuring enzyme activities

and metabolite levels within brain in several fish species (Soengas et al. 1996b, 1998a; Laiz-Carrión et al. 2003). Moreover, several metabolic parameters of fish are changing on a daily basis (Figueroa et al. 2000), whereas in some other instances post-prandial changes are observed (Soengas et al. 1996a; Figueroa et al. 2000). We have also demonstrated that starvation elicits profound changes in brain energy metabolism in fish that can be summarized as increased glycogenolysis and use of ketones (Soengas et al. 1996b, 1998a; Figueroa et al. 2000). Environmental factors induce changes in energy parameters in teleosts brain enhancing glycogenolysis elicited by pollutants (Soengas et al. 1997; Aldegunde et al. 1999), increasing capacity for anaerobic glycolysis under hypoxia/anoxia or changing substrate utilization during adaptation to cold temperatures. We have also addressed the regulatory role of several hormones such as melatonin (Soengas et al. 1998b), insulin (Ruibal et al. 2002), glucagon (Magnoni et al. 2001), cortisol (Laiz-Carrión et al. 2003) and norepinephrine (Sangiao-Alvarellos et al. 2003a) in the energy metabolism of fish brain, though the precise mechanisms of hormonal action are unknown and much research remains to be done in this field. Finally, the few studies performed on the different cell types from the nervous system of fish allowed us to hypothesize some functional relationships among these cells. Our ongoing research (Gradín et al. 2002) is directed at establishing techniques for the culture of astrocytes to be used in the characterization of brain energy metabolism at the cellular level.

The second focus of our research program is on the interrelationship between osmoregulation and energy metabolism using as a model the gilthead sea bream, *Sparus auratus*. We established collaboration with Dr. Juan M. Mancera whose group has analysed the osmoregulatory system of gilthead sea bream, by studying the response of adenohypophyseal cells to environmental salinity (Mancera et al. 1993b, 1995), changes in plasma content in response to abrupt changes in salinity (Mancera et al. 1993a), the effect of treatment with cortisol on adaptation to brackish water (Mancera et al. 1994), and the influence of prolactin, growth hormone (GH) and cortisol on the osmoregulatory capacity of gilthead sea bream (Mancera et al. 2002). In collaboration with Dr. S.M. McCormick, they have tested the osmoregulatory actions of the GH/insulin growth factor (IGF-I) axis in the killifish (Mancera and McCormick 1988a,b, 1999). On the other hand, and in collaboration with Drs. Gert Flik and S. Wendelaar Bonga they have studied the stress system of gilthead sea bream under chronic and acute stimulation (Arends et al. 1999, 2000). As a result of the present collaboration, we have demonstrated that osmotic acclimation in gilthead sea bream produces significant changes in the energy metabolism of osmoregulatory as well as non-osmoregulatory organs including brain, liver, kidney and gills (Sangiao-Alvarellos et al. 2003b).

Our ongoing experiments are dealing with metabolic changes in fish associated with osmotic acclimation under such physiological conditions as growth or density stress. We demonstrated recently that metabolic changes in brain and other tissues during osmotic acclimation are similar to those produced by the treatment with hormones regulating osmotic adaptation, such as cortisol (Laiz-Carrión et al. 2003), 17 β-estradiol (Guzmán et al., submitted; Sangiao-Alvarellos et al., submitted) and arginine-vasotocin (Sangiao-Alvarellos et al. 2004).

Cortisol is the main steroid produced and released by interrenal tissue in teleost fish, and is known to possess both glucocorticoid and mineralocorticoid activities. Accordingly, a role for cortisol in the control of several processes such as intermediary metabolism, ionic and osmotic regulation, growth, stress, and immune function was repeatedly demonstrated in teleost fish (McCormick 1995; Wendelaar Bonga 1997; Mommsen et al. 1999).

The influence of cortisol on fish energy metabolism has been investigated mainly in liver (Van der Boon et al. 1991; Mommsen et al. 1999), with less attention being paid to other organs possessing receptors for cortisol: gills, kidney and brain (Knoebl et al. 1996; Kloas et al. 1998; Pottinger et al. 2000). In a previous study (Laiz-Carrión et al. 2003) we demonstrated the existence of dose-dependent changes in metabolic parameters of plasma, liver, and brain in cortisol-treated gilthead sea bream.

The role of cortisol in the physiology of gills has been studied in several fish species. This hormone increased hypoosmoregulatory capacity. As a result, cortisol-treated fish showed an increased salinity tolerance with development and proliferation of gill chloride cells, high gill Na^+,K^+-ATPase activity, and expression of Na^+,K^+-ATPase α-subunit (McCormick 1990, 1995; Madsen et al. 1995; Seidelin and Madsen 1997). The activation of all these processes implies an increased energy requirement that supposedly could lead to changes in gill energy metabolism (Morgan and Iwama 1996; Morgan et al. 1997). However, this possibility has been scarcely assessed

in fish (Laiz-Carrión et al. 2003; Sangiao-Alvarellos et al. 2003b).

Thus, we can summarize that in teleost fish (1) cortisol is involved in the control not only of osmoregulatory capacity (McCormick 1995, 2001) but also of energy metabolism (Mommsen et al. 1999), (2) gills play an important role in the hypoosmoregulatory capacity but few studies are available regarding their metabolic performance (Mommsen 1984; Perry and Walsh 1989), and (3) there are no available studies concerning changes in the energy metabolism of gills after cortisol treatment.

The gilthead sea bream (*Sparus auratus*), an euryhaline teleost capable of adapting to extreme changes in environmental salinity (Chervinsky 1984; Mancera et al. 1993a,b), was used as a model to achieve cortisol levels similar to those observed in this fish during osmotic adaptation. We then assessed how the elevated levels of cortisol simultaneously affected osmoregulatory and metabolic capacities of gills. The study presented below (partially reported by Laiz-Carrión et al. 2003) exemplifies our experimental approach for addressing this problem.

Experiments on gilthead seabream

For detailed description of our experiments, we refer the readers to the recent paper of Laiz-Carrión et al. (2003). Here we described only modifications in the latest set of experiments, aimed to assess the influence of cortisol on gill metabolic and osmoregulatory capacities.

Immature male gilthead sea bream (*Sparus auratus* L., 150 g body weight) were provided by ACUINOVA S.L. (San Fernando, Cádiz, Spain) where experiments were carried out. Fish were acclimated to seawater in 1000 l tanks for at least 2 weeks in an open system (40 ppt salinity, 1200 mOsm kg^{-1} H_2O). During the experiments, fish were maintained under natural photoperiod (October 2002) and temperature (19–21 °C). Fish were fed daily at 1% body weight using commercial dry pellets (Trouvit Europa D-5, Trout España, Burgos, Spain), which constituted a maintenance diet for this species. They were fasted for 24 h before hormone injection and throughout the experiment. No differences were observed in general body parameters like body weight, length, condition factor or hepatosomatic index between control and treated fish. 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.

Two different groups (12 fish per group) of seawater-acclimated sea bream were used. Fish were anaesthetized with 2-phenoxyethanol (0.5 ml l^{-1} water), weighed, intraperitoneally implanted with slow-release coconut oil implants following procedures previously described (Soengas et al. 1992; Laiz-Carrión et al. 2003) and placed back into seawater. Thus, fish were implanted with 5 μl g^{-1} body weight of coconut oil alone (controls) or containing cortisol resulting in this later case in a whole dose of 50 μg g^{-1} body weight. Fish were sampled ($n = 12$) 7 days after implant. Before implant, one group of fish ($n = 7$) was sampled and served as a time 0 control group (untreated fish). No mortality was observed during the experiment.

Samples were taken and parameters were assessed as described previously (Laiz-Carrión et al. 2003). The additional metabolites assessed in the present study were plasma protein, and tissue ATP levels. Plasma protein was measured using the bicinchoninic acid method (Smith et al. 1985) with the BCA protein kit (Pierce, Rockford, USA), with bovine albumin as standard, whereas tissue ATP levels were determined with an enzymatic method using a commercial kit (Sigma diagnostics, USA). Moreover, the activities of pyruvate kinase (PK), glycogen phosphorylase (GPase), and glucose 6-phosphate dehydrogenase (G6PDH) were measured in gills as described before for other tissues (Laiz-Carrión et al. 2003), except that for gill PK the activation ratio was obtained using 0.01 mmol l^{-1} fructose 1,6-bisphosphate concentration.

All data were statistically analysed by a Student t test, considering the differences being statistically significant at $P < 0.05$.

Experimental results

No differences were observed between untreated fish (time 0 days) and those fish implanted with coconut oil alone (control) either for plasma cortisol levels or for the osmoregulatory and metabolic parameters assessed (data not shown).

Plasma cortisol levels elevated in cortisol-treated fish compared with controls (Table 1). Other changes in plasma parameters observed in cortisol-treated fish included increased glucose and lactate levels, as well as decreased protein levels (Table 1). In contrast,

Table 1. Changes in the levels of cortisol, glucose, lactate, triglycerides, protein, sodium, and chloride, and osmolality in plasma of gilthead seabream after 7 days of intraperitoneal implantation of 5 μl g^{-1} body weight of coconut oil alone (control) or containing cortisol (50 μg g^{-1} body weight). Each value is the mean $\pm$ S.E.M. of $n = 12$ fish. *, significantly different ($P < 0.05$) from fish implanted with coconut oil alone (control).

Parameter	Treatment	
	Control	Cortisol 50 μg g^{-1}
Cortisol levels (ng ml^{-1})	6.62 $\pm$ 1.36	27.6 $\pm$ 3.02*
Glucose levels (μmol ml^{-1})	3.89 $\pm$ 0.26	5.39 $\pm$ 0.34*
Lactate levels (μmol ml^{-1})	2.30 $\pm$ 0.11	3.12 $\pm$ 0.14*
Triglyceride levels (μmol ml^{-1})	2.56 $\pm$ 0.08	2.36 $\pm$ 0.11
Protein levels (μmol ml^{-1})	32.5 $\pm$ 0.57	29.41 $\pm$ 0.88*
Sodium levels (μmol ml^{-1})	179.3 $\pm$ 2.8	177.4 $\pm$ 2.5
Chloride levels (μmol ml^{-1})	162.9 $\pm$ 3.4	158.2 $\pm$ 2.6
Osmolality (mOsm kg^{-1})	366.4 $\pm$ 2.11	369.7 $\pm$ 3.53

no differences were observed for triglyceride levels, plasma osmolality, or plasma levels of sodium and chloride in cortisol-treated fish compared with controls.

The osmoregulatory and metabolic parameters assessed in gills are presented in Table 2. A significant increase in Na^+,K^+-ATPase activity was found in cortisol-treated fish in which ATP levels were also increased. An increase was also observed in cortisol-treated fish, when considering the activity ratio and the fructose 1,6-P_2 activation ratio of PK. In contrast, significant decreases in activity were observed for HK and G6PDH activities in cortisol-treated fish compared with controls. Finally, no significant changes were observed for glycogen and lactate levels, or for GPase activity when comparing control and cortisol-treated fish.

The different parameters measured in liver are shown in Table 3. Glycogen levels increased while GPase activity decreased significantly in cortisol-treated fish. An enhancement of PK activity was observed in cortisol-treated fish, at least with respect to the activity ratio and the fructose 1,6-P_2 activation ratio of the enzyme. The remaining parameters assessed in cortisol-treated-fish did not display significant differences with respect to control fish.

Finally, several parameters were evaluated in brain (Table 4). An increase in glycogen and ATP levels, and PFK activity (activity, activity ratio, and fructose 2,6-P_2 activation ratio) were observed in cortisol-treated fish compared with controls. No significant differences were observed for glycogen and lactate levels or for GPase, HK and G6PDH activities.

General discussion

The system used for cortisol delivery (coconut oil plus cortisol as a slow-release implant), as well as time (7 days) and dose (50 μg g^{-1}) of cortisol used in the present experiment were chosen based on the results obtained in other teleosts (see review by Gamperl et al. 1994) including gilthead sea bream (Laiz-Carrión et al. 2003). Thus, the four-fold elevation observed in plasma cortisol levels of cortisol-treated fish as compared with controls (Table 1) is quite similar to that reported in a previous study with the same dose and time of administration (Laiz-Carrión et al. 2003). It is also similar to those experienced by gilthead sea bream after changes in environmental salinity (Mancera et al. 1993a, 1994). Those elevated cortisol levels were effective in inducing metabolic changes in plasma, liver and brain, and changes in the only parameter, i.e. HK activity, assessed in gills in the preceding experiment (Laiz-Carrión et al. 2003). The results obtained in the most recent experiment agree in general with those reported previously, which lend support for the comparison between them.

Plasma glucose of cortisol-treated gilthead sea bream was clearly higher as compared with controls. Elevation of glycemic levels is similar to one previously reported in cortisol-treated fish (Van der Boon et al. 1991; Mommsen et al. 1999) including gilthead sea bream (Laiz-Carrión et al. 2003). A significant increase was also recorded in plasma lactate levels of cortisol-treated fish. Similar effect was reported previously in cortisol-treated rainbow trout (Dugan and Moon 1998) and gilthead sea bream (Laiz-Carrión et al. 2003) but not in the majority of other studies (Mommsen et al. 1999). Plasma protein levels declined in cortisol-treated fish, in contrast with an absence of any changes in rainbow trout (Andersen et al. 1991) and brook trout (Vijayan et al. 1991). Triglyceride (TG) levels did not change following cortisol treatment, while an increase in plasma TG levels has been reported in gilthead sea bream under similar experimental conditions (Laiz-Carrión et al. 2003).

Table 2. Changes in the levels of glycogen, lactate and ATP, and in the activities of Na^+,K^+ATPase, glycogen phosphorylase (GPase), pyruvate kinase (PK), hexokinase (HK), and glucose 6-phosphate dehydrogenase (G6PDH) in gills of gilthead seabream. The rest of the legend as in Table 1.

Parameter	Treatment	
	Control	Cortisol 50 μg g^{-1}
Glycogen levels (μmol glycosyl units g^{-1} wet weight)	0.42 ± 0.04	0.51 ± 0.04
Lactate levels (μmol. g^{-1} wet weight)	2.33 ± 0.14	2.59 ± 0.24
ATP levels (μmol g^{-1} wet weight)	0.76 ± 0.22	2.89 ± 0.83*
Na^+,K^+ATPase activity (μmol ADP mg^{-1} protein h^{-1})	10.8 ± 0.60	15.1 ± 1.21*
GPase activity		
Total activity (U mg^{-1} protein)	0.15 ± 0.01	0.13 ± 0.01
% GPase *a*	9.20 ± 2.26	6.19 ± 1.46
PK activity		
Activity (U mg^{-1} protein)	4.16 ± 0.21	4.78 ± 0.24
Activity ratio	0.53 ± 0.01	0.57 ± 0.01*
Fructose 1,6-P_2 activation ratio	0.89 ± 0.03	0.97 ± 0.01*
HK activity (U mg^{-1} protein)	0.51 ± 0.03	0.40 ± 0.02*
G6PDH activity (U mg^{-1} protein)	0.90 ± 0.07	0.63 ± 0.05*

We have no explanation for the different effects of cortisol on plasma protein and TG levels in two trials. A hydration effect or differences in either the size of fish or in the season (October vs. May) when the experiments were carried out could help to explain these discrepancies. Overall, the changes observed in plasma metabolite levels in the present study allow us to suggest that cortisol is either acting directly on the liver or indirectly through increased energy demand in other tissues, induced the mobilization of metabolic substrates, namely glucose and lactate, into plasma.

A stimulatory role of cortisol on hypoosmoregulatory capacity has been reported previously in other teleosts (see McCormick 1995, 2001). Our results agree with this general model and demonstrate that after 7 days of cortisol release from the implant, gill Na^+,K^+-ATPase activity becomes higher than in control fish. Stimulatory role of cortisol has been also reported for gilthead sea bream treated with 5 μg g^{-1} body weight of cortisol during one week every other day (Mancera et al. 2002). However, this is in contrast with the lack of changes observed in a previous study, using a similar experimental design, where increased gill Na^+,K^+-ATPase activity was observed at 5 days but not at 7 days post-implant (Laiz-Carrión et al. 2003). A similar reaction has been reported in seawater-acclimated specimens of the sparid silver seabream (*Sparus sarba*) treated daily during one week with 4 μg g^{-1} cortisol dissolved in saline solution, which showed increased cortisol levels but not gill Na^+,K^+-ATPase activity (Deane et al. 2000). We related our previous results to a loss in sensitivity of gill chloride cells in response to exogenous cortisol (see Laiz-Carrión et al. 2003). If this hypothesis is correct, differences in concentration and/or affinity of gill corticosteroids receptors between fish used in both experiments could explain the observed difference since higher levels of cortisol (45–50 ng ml^{-1}) were found in treated fish in the preceding experiment than in the present one (25–30 ng ml^{-1}). Another possibility could be the existence of seasonal differences (October vs. May) in cortisol receptors. Moreover, plasma ion levels (sodium and chloride) and plasma osmolality did not show any significant differences between

Table 3. Changes in the levels of glycogen, lactate and ATP, and in the activities of glycogen phosphorylase (GPase), pyruvate kinase (PK), fructose 1,6-bisphosphatase (FBPase), and glucose 6-phosphate dehydrogenase (G6PDH) assayed in liver of gilthead seabream. The rest of the legend as in Table 1.

	Treatment	
Parameter	Control	Cortisol 50 μg g^{-1}
Glycogen levels (μmol glycosyl units g^{-1} wet weight)	453 ± 31	564 ± 36*
Lactate levels (μmol g^{-1} wet weight)	1.40 ± 0.34	1.25 ± 0.21
ATP levels (μmol g^{-1} wet weight)	0.43 ± 0.05	0.40 ± 0.04
GPase activity		
Total activity (U mg^{-1} protein)	0.09 ± 0.008	0.07 ± 0.005*
% GPase *a*	59.2 ± 4.1	59.5 ± 3.2
PK activity		
Activity (U mg^{-1} protein)	0.53 ± 0.03	0.50 ± 0.02
Activity ratio	0.09 ± 0.03	0.18 ± 0.02*
Fructose 1,6-P_2 activation ratio	0.05 ± 0.01	0.12 ± 0.02*
FBPase activity (U mg^{-1} protein)	0.59 ± 0.05	0.48 ± 0.02
G6PDH activity (U mg^{-1} protein)	1.58 ± 0.10	1.22 ± 0.07

cortisol-treated fish and controls. These results coincide with already reported in the same fish species under similar cortisol treatment and time of exposure (Laiz-Carrion et al. 2003).

Changes in plasma concentration of cortisol have been associated with the process of ion regulation and, consequently with seawater acclimation in fish (McCormick 1995, 2001). In addition, cortisol played a role in the mobilization of energy substrates in fish (Mommsen et al. 1999). Therefore, some of the effects of cortisol on osmotic acclimation may be mediated indirectly by providing substrates for gill metabolism and maintaining gill Na^+,K^+-ATPase activity. Thus, not unexpectedly, metabolic changes occurring in tissues like liver during osmotic adaptation (Vijayan et al. 1996; Sangiao-Alvarellos et al. 2003b) are quite similar to those usually attributed to cortisol action (Mommsen et al. 1999; Laiz-Carrión et al. 2003). However, no studies have addressed the role of cortisol in the energy metabolism of gills, which should become metabolically altered by the enhanced hypoosmoregulatory capacity stimulated by cortisol.

In a previous study (Laiz-Carrión et al. 2003), only one parameter of gill energy metabolism was assessed in cortisol-treated gilthead sea bream. This parameter, HK activity, was also evaluated in the present experiment and again clearly displayed decreased activity in cortisol-treated fish. This decrease in enzyme activity coincides with elevated levels of plasma glucose suggesting that a decrease in the capacity of gills to use exogenous glucose occurs in cortisol-treated fish. This contrasts with the data obtained on rainbow trout adapting to seawater where a parallel increase in plasma glucose and gill HK activity has been observed (Soengas et al. 1995). HK activity is known to increase in gills when gilthead sea bream is exposed to increased environmental salinity (Sangiao-Alvarellos et al. 2003b). Thus, the decrease observed in gill HK activity in the present study addresses an interesting issue regarding the effects of a direct cortisol action vs. the action of elevated plasma cortisol produced by changes in environmental salinity (Sangiao-Alvarellos et al. 2003b). However, considering that levels of other hormones are known to change as well during osmotic acclimation (McCormick 2001), both situations are not fully comparable. Based on these results, we may hypothesize that increased energy demand in the gills, as suggested by increased Na^+,K^+-ATPase activity,

Table 4. Changes in the levels of glycogen, lactate and ATP, and in the activities of glycogen phosphorylase (GPase), 6-Phosphofructo 1-kinase (PFK), hexokinase (HK), and glucose 6-phosphate dehydrogenase (G6PDH) assayed in brain of gilthead seabream. The rest of the legend as in Table 1.

	Treatment	
Parameter	Control	Cortisol 50 μg g^{-1}
Glycogen levels (μmol glycosyl units.g^{-1} wet weight)	0.15 ± 0.03	0.23 ± 0.02*
Lactate levels (μmol g^{-1} wet weight)	7.43 ± 0.61	7.01 ± 1.32
ATP levels (μmol. g^{-1} wet weight)	0.08 ± 0.01	0.16 ± 0.01*
GPase activity		
Total activity (U mg^{-1} protein)	0.22 ± 0.01	0.22 ± 0.01
% GPase *a*	55.9 ± 1.68	51.2 ± 1.96
PFK activity		
Activity (U mg^{-1} protein)	4.19 ± 0.08	4.47 ± 0.07*
Activity ratio	0.04 ± 0.003	0.06 ± 0.004*
Fructose 2,6-P_2 activation ratio	0.14 ± 0.01	0.19 ± 0.01*
HK activity (U mg^{-1} protein)	0.73 ± 0.02	0.71 ± 0.01
G6PDH activity (U mg^{-1} protein)	0.22 ± 0.01	0.23 ± 0.01

should not be sufficiently covered by exogenous glucose allowing an increased use of other fuels such as exogenous lactate and triglycerides or endogenous glycogen.

The other parameters of gill energy metabolism were evaluated by us for the first time in any teleost fish after cortisol treatment. No significant changes in endogenous glycogen were observed when comparing the glycogenolytic potential of control and cortisol-treated fish and this applies to the level of metabolites as well as GPase activity. Considering the small amount of glycogen accumulated in gills, it could be expected not to see any change in this parameter because elevated metabolic demand of the gill tissue would not be sufficiently covered by the mobilization of glycogen. This is also in agreement with the lack of changes observed during seawater acclimation in gill glycogen content (Sangiao-Alvarellos et al. 2003b), physiological condition known to increase plasma cortisol levels (McCormick 2001).

An enhancement of the glycolytic capacity of gills in cortisol-treated fish is suggested by changes in the activity ratio and activation ratio of PK activity. It is also known that elevated plasma cortisol levels coincide with enhancement in the glycolytic potential of gills in the same fish species when acclimated to hyperosmotic environments (Sangiao-Alvarellos et al. 2003b). Together, these results suggested that cortisol treatment increased the energy demand of gills, which is reflected by higher ATPase activity and increased glycolytic potential. This demand should not be sufficiently fuelled by either endogenous resources or exogenous substrates like glucose or TG, therefore we can assume that some other substrate may be increasingly used.

Considering that (1) the energy requirement of the fish gill is thought to be maintained by the oxidation of glucose and lactate obtained from the circulation (Mommsen 1984; Perry and Walsh 1989; Soengas et al. 1995), and (2) the elevation of plasma lactate levels in cortisol-treated fish is taking place, we hypothesize that there is an enhancement of the use of exogenous lactate through LDH working in the oxidative direction in gills of cortisol-treated fish. The increase observed in ATP levels in the gills of cortisol-treated fish is striking considering the increase in ATPase activity, but it may imply that the endogenous production of ATP exceeds the energy consumption driven

by ATPase activity. On the other hand, G6PDH activity was significantly lower in cortisol-treated fish than in controls suggesting that a reduced capacity of the pentose phosphate pathway occurs in cortisol-treated fish reflecting a reduced demand for NADPH.

In the liver of cortisol-treated gilthead seabream, the results were comparable to those previously reported (Laiz-Carrión et al. 2003). These metabolic changes included an enhancement of the gluconeogenic potential, increased liver glycogen content, and decreased GPase activity. They also agree in general with those reported previously for other fish species (Mommsen et al. 1999). Some other metabolic parameters, such as lactate and ATP levels, were assessed by us for the first time. These metabolites did not show any significant changes in cortisol-treated fish.

The stress response induced by raised cortisol levels in plasma seems to be similar to that naturally occurring in several physiological situations such as starvation, hypoxia, toxic disruption, etc. (Wendelaar Bonga 1997). These physiological conditions generally require enhancement of the energy demands of the brain (Soengas and Aldegunde 2002). Accordingly, the results of brain energy metabolism in cortisol-treated gilthead sea bream obtained in the latest as well as in the previous experiments (Laiz-Carrión et al. 2003) are in agreement regarding an enhancement of glycolytic capacity (as judged by increased activity of PFK), increased glycogen levels, and the lack of changes in the use of exogenous glucose (HK activity), and pentose phosphate pathway (G6PDH activity). The results also agree with those obtained on brain of the same species when acclimated to different osmotic environments (Sangiao-Alvarellos et al. 2003b), and with changes in brain energy metabolism after treatment with another stress hormone, i.e. norepinephrine (Sangiao-Alvarellos et al. 2003a). These data lend further support for a role of cortisol in regulation pathways of energy metabolism in fish brain as suggested previously (Lynshiang and Gupta 2000; Laiz-Carrión et al. 2003) reflecting an adaptive role of cortisol during stress. In a previous study (Laiz-Carrión et al. 2003) we hypothesized that, as a whole, brain tissue in cortisol-treated fish appears to enhance the use of those pathways involved in ATP production, whereas other synthetic pathways seem to be reduced. This hypothesis is further supported by the measurement of brain ATP levels that increased 100% in cortisol-treated fish compared with controls, and by the lack of changes in brain lactate levels in cortisol-treated fish, which is further suggesting an enhancement of the aerobic production of ATP.

In summary, our recent investigations have provided evidence regarding the basics of brain energy metabolism in fish and its modifications by exogenous (nutrition, salinity, pollutants), and endogenous (neurotransmitters, hormones) factors. The future research in this field must be directed towards the mechanisms involved in hormone action as well as in the assessment of metabolic functions at cellular level (particularly in astrocytes). The second direction of our research has provided evidence regarding changes in the energy metabolism of osmoregulatory and non-osmoregulatory organs of gilthead seabream during osmotic acclimation. To highlight our recent research in this field we presented results regarding the effects of cortisol on gill metabolic and osmoregulatory capacities. Increased cortisol levels in plasma of gilthead sea bream elicited not only an enhanced hypoosmoregulatory capacity but also several metabolic changes in liver, brain and gills. Whereas the metabolic changes observed in liver and brain confirmed observations made in previous studies (Mommsen et al. 1999; Laiz-Carrión et al. 2003), the latest results demonstrated that cortisol produces important changes in gill energy metabolism as well. Enhancement of energy demand in gills induced increased glycolytic potential, decreased capacity for use of exogenous glucose, and decreased capacity of the pentose phosphate pathway. These metabolic changes suggested that the increased energy requirements of the gills (due to the intensified hypoosmoregulatory process triggered by cortisol) must be increasingly fuelled by metabolites other than exogenous glucose. Thus, the exogenous lactate becomes an ideal candidate for such fuel. The measurement of uptake and oxidation rates for metabolites in isolated gill cells will hopefully provide further information regarding our hypothesis.

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