Effect of cortisol on brackish water adaptation in the euryhaline gilthead sea bream (Sparus aurata L.)

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Gilthead sea breams (Sparus aurata L.) adapted to sea water (SW, 39‰ salinity) were injected with either saline or cortisol (10 μg/g) 3 hours before being abruptly transferred to brackish water (BW, 7‰ salinity). Plasma osmolality, Na⁺, Cl⁻, Ca²⁺ and cortisol were measured before and after the transference. The transference led to a transitory hypoosmolality and hypomineralization in both groups. The Na⁺ and Cl⁻ levels showed a lower decrease in the cortisol-injected group. Osmolality and Ca²⁺ were similar in both groups. In the control group plasma cortisol reached a peak soon after the transference. Long term BW-adapted specimens showed a stable 2.5-fold increase in cortisol levels with respect to SW-adapted. Cortisol injected group showed an increased cortisolemia, in relation to control, for 9 hr after the injection, after this time, cortisol levels were similar to the control group.

Key words: Osmoregulation; Cortisol; Brackish water adaptation; Sparus aurata L.

Introduction

Cortisol is the main corticosteroid synthesized by the interrenal tissue in many teleosts, for which an important role in metabolic and osmoregulatory processes has been ascribed (Henderson et al., 1970; Henderson and Garland, 1980; Van der Boon et al., 1991).

In euryhaline teleost this hormone acts on several osmoregulatory organs such as gills, gut, kidney and urinary bladder. However the exact osmoregulator role is not clear and appears to depend on the studied species. In some species cortisol has been reported to have a role in sea water (SW) adaptation by stimulating the differentiation of the chloride cells and by increasing branchial Na⁺/K⁺-ATPase activity. In contrast, in other species, it has been shown that cortisol controls adaptation to hypoosmotic environments by acting, synergically, with prolactin, in the digestive tract, kidney and gills (Henderson et al., 1970; Johnson, 1973; Bern, 1975; Henderson and Garland, 1980; Foskett et al., 1983).

Measures of plasma cortisol in euryhaline teleosts submitted to changes of the environmental salinity have shown contradictory results in different species. Higher levels in SW than in brackish water (BW) have been reported in Carassius auratus (Singley and Chavín, 1975) and Oncorhynchus kisutch (Redding et al., 1984; Avella et al., 1990). A transitory increase after the transference from SW to BW was seen in Anguilla anguilla (Leloup-Hatey, 1974), Chrysopterus major (Ishiota, 1980), Sarotherodon mossambicus (Assem and Hanke, 1981), Fundulus heteroclitus (Jacob and Taylor, 1983) and Cyprinus carpio (Abo Hegab and Hanke, 1984). On the other hand, higher levels of cortisol were measured in BW with respect to SW in Mugil cephalus and Platichthys stellatus (Johnson, 1973), Dicentrarchus labrax (Roche et al., 1989) and Sparus aurata (Mancera et al., 1993a).

Sea bream, Sparus aurata, is a euryhaline teleost that lives in SW or BW, and can face extreme changes in the environmental salinity (Arias, 1976; Chervinski, 1984). We have
previously shown that variations in plasma cortisol during the abrupt transferences from SW to BW and vice-versa suggested an osmoregulatory role of this hormone in long-term adaptation to hyposmotic environments in *Sparus aurata* (Mancera et al., 1993a).

The aim of the present investigation was to study the effect of one single injection of cortisol on plasmatic parameters of SW-adapted animals during the abrupt transference to BW. Osmolality, plasma level of Na\(^+\), Cl\(^-\), Ca\(^{2+}\) and cortisol have been measured. The result will be discussed in relation with the role of cortisol in hypoosmotic adaptation for *Sparus aurata*.

**Materials and Methods**

**Experimental fish**

Specimens of gilthead sea bream (*Sparus aurata* L.) (body weight 50 ± 5 g) were provided by an experimental fish culturing center (El Toruño, PEMARES, El Puerto de Santa Maria, Cádiz, Spain); where the animals stayed during the time of the experiment (September–October, 1990), under environmental conditions of photoperiod and water temperature (16–22°C). The animals were divided in two groups (N = 100 each) and stayed in separate 1000 l tanks with permanent water turnover and supplied with oxygen. They were fed twice a day (9 a.m. and 6 p.m.) with *Illex* sp. and, after the transference, the fish fasted for 4 days.

**Experimental protocol**

The fishes were injected 3 hr before the abrupt transference from sea water (SW, 39% salinity) to brackish (BW, 7% salinity). Cortisol-treated fishes received 10 μg cortisol/g body weight (Sigma H-2882, as sodium salt of hydrocortisone hemisuccinate) dissolved in saline solution (0.9% NaCl). The sham-injected controls received the same amount of saline solution.

Fish were anaesthetized with phenoxethanol (0.2%, Sigma P-1126) dissolved in the water, intramuscularly injected with a volume of solution that varied (≥100 μl) according to fish size. Cortisol and saline injected fishes were sampled (N = 10 for each time) 3 hr before the transference, and 0 hr, 3 hr, 6 hr, 9 hr, 1, 2, 3, 4 and 7 days after being transferred to BW. Each animal was sampled only once. The fishes fasted for 24 hr before each sampling. No mortality was observed in either groups during the experimental time.

**Sampling technique and measures**

Fish were anaesthetized with 0.2% phenoxyethanol and the blood was extracted from the caudal artery using insulin heparinized syringes. An amount of 0.5 ml of blood was obtained in each extraction and centrifuged for 20 min at 3000 rpm. The plasma was stored at −30°C until analysed.

Ten microlitres of each sample were used to measure the osmolality in a Fiske One-Ten Osmometer. The Na\(^+\), Cl\(^-\) and Ca\(^{2+}\) levels were determined, in 200 μl of plasma, by means of selective electrodes in an Automated Stat/Routine Analyser System (Beckman’s Synchron ASTRA). The serum concentration of cortisol was measured, in 50 μl of each sample, with a Coat-A-Count Cortisol \(^{125}\)I Kit (Diagnostic Products Corporation, Los Angeles, U.S.A.). In the statistical analysis a Kruskal–Wallis test was done (N = 10 for each time).

**Results**

Tables 1 and 2 show the measures obtained in the control group injected with saline and the experimental group injected with cortisol, respectively. A strong decrease in plasma osmolality was observed in control group during the first 2 days after the transference. Then the level was recovered during the following days, to reach a value similar to SW. Cortisol-injected animals maintained in SW showed a strong peak in osmolality 3 hr after the injection (time 0 hr). Moreover, during the transference, osmolality showed a value very similar to control group (Fig. 1).

Plasma levels of Na\(^+\), Cl\(^-\) and Ca\(^{2+}\) changed concomitantly with the osmolality in both groups, with a transitory hypomeralization and a posterior recovery of the values (Tables 1 and 2). Hypomeralization was lower and more transitory in the cortisol-treated group. This group showed, as for osmolality, a strong increase in the ionic levels at 0 hr (3 hr after being injected) (Fig. 2). In contrast, the decrease in Na\(^+\) and Cl\(^-\) levels occurred before in cortisol-treated group than in control group (Fig. 2).

In both groups, the Na\(^+\)/Cl\(^-\) ratio showed variations during the experimental time, indicating different responses for Na\(^+\) and Cl\(^-\) after the transference (Tables 1 and 2). During the first 24 hr the control group had higher values but, afterwards, the Na\(^+\)/Cl\(^-\) ratio was higher in the cortisol-treated group with respect to control group. In BW-adapted fish this ratio had similar values to SW-adapted fishes.

Regarding plasma cortisol, the control group showed a peak, 6 hr after the transference, and then the original value was recovered at 24 hr. Later, as the time of permanency in BW proceeded, cortisol values increased and reached a high and stable value in BW-adapted fishes (Table 1). In cortisol-treated group, plasma cortisol levels were maintained quite high.
Table 1. Osmolality, Na⁺, Cl⁻, Ca²⁺; cortisol levels and Na⁺/Cl⁻ ratio in saline-injected fishes. Values are the mean ± standard deviation (N = 10). Osmolality in mOsm/Kg, ions in mM/l and cortisol in ng/ml. *P < 0.001; †P < 0.01 with respect to the values observed in SW (Kruskal-Wallis test).

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>Time (hr/day)</th>
<th>-3 hr</th>
<th>0 hr</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>4 days</th>
<th>7 days</th>
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<tbody>
<tr>
<td>Osmolality</td>
<td>315.3 ± 6.9</td>
<td>316.2 ± 6.8</td>
<td>287.8 ± 6.3*</td>
<td>285.2 ± 7.8*</td>
<td>290.0 ± 7.0*</td>
<td>289.6 ± 7.2*</td>
<td>2890.6 ± 4.5*</td>
<td>2966.6 ± 3.0*</td>
<td>3006.6 ± 6.2†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>182.0 ± 4.5</td>
<td>183.0 ± 4.0</td>
<td>182.6 ± 4.6</td>
<td>170.4 ± 3.7†</td>
<td>172.2 ± 4.3</td>
<td>170.4 ± 5.4</td>
<td>171.7 ± 5.4</td>
<td>175.2 ± 4.8</td>
<td>176.7 ± 3.5</td>
<td>183.2 ± 4.8</td>
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<tr>
<td>Cl⁻</td>
<td>157.3 ± 5.1</td>
<td>158.3 ± 5.1</td>
<td>158.0 ± 5.2</td>
<td>144.6 ± 6.1*</td>
<td>148.0 ± 3.2*</td>
<td>152.2 ± 5.6</td>
<td>157.6 ± 4.5</td>
<td>157.2 ± 3.5</td>
<td>157.0 ± 4.4</td>
<td>158.0 ± 4.6</td>
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<tr>
<td>Na⁺/Cl⁻ ratio</td>
<td>0.11</td>
<td>0.11</td>
<td>1.16</td>
<td>1.16</td>
<td>1.16</td>
<td>1.12</td>
<td>1.12</td>
<td>1.09</td>
<td>1.11</td>
<td>1.13</td>
<td>1.16</td>
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<tr>
<td>Ca²⁺</td>
<td>3.07 ± 0.12</td>
<td>3.07 ± 0.14</td>
<td>3.11 ± 0.09</td>
<td>2.94 ± 0.16</td>
<td>2.88 ± 0.12</td>
<td>2.84 ± 0.08</td>
<td>2.69 ± 0.09*</td>
<td>2.58 ± 0.12*</td>
<td>2.93 ± 0.05</td>
<td>3.00 ± 0.15</td>
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<tr>
<td>Cortisol</td>
<td>42.7 ± 12.4</td>
<td>45.6 ± 10.0</td>
<td>100.0 ± 16.3*</td>
<td>146.5 ± 28.3*</td>
<td>109.2 ± 19.1*</td>
<td>49.4 ± 12.6</td>
<td>112.2 ± 22.8*</td>
<td>125.1 ± 24.2*</td>
<td>122.5 ± 21.5*</td>
<td>126.0 ± 23.5*</td>
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</table>

Table 2. Osmolality, Na⁺, Cl⁻, Ca²⁺, cortisol levels and Na⁺/Cl⁻ ratio in cortisol-injected fishes. Values are the mean ± standard deviation (N = 10). Osmolality in mOsm/Kg, ions in mM/l and cortisol in ng/ml. *P < 0.001; †P < 0.01 with respect to the values observed in SW (Kruskal-Wallis test).

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<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality</td>
<td>315.7 ± 5.9</td>
<td>334.4 ± 6.8†</td>
<td>286.6 ± 8.9*</td>
<td>283.7 ± 7.3*</td>
<td>285.7 ± 8.7*</td>
<td>287.7 ± 7.9*</td>
<td>292.3 ± 6.4*</td>
<td>297.0 ± 8.5†</td>
<td>302.0 ± 8.7</td>
<td>310.0 ± 8.6</td>
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<tr>
<td>Na⁺</td>
<td>183.5 ± 4.0</td>
<td>194.0 ± 3.8†</td>
<td>176.8 ± 5.7</td>
<td>176.5 ± 5.7</td>
<td>177.6 ± 5.2</td>
<td>181.5 ± 5.3</td>
<td>178.3 ± 2.6</td>
<td>184.7 ± 5.1</td>
<td>188.0 ± 5.4</td>
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<tr>
<td>Cl⁻</td>
<td>158.3 ± 5.0</td>
<td>172.8 ± 3.8*</td>
<td>151.4 ± 5.8</td>
<td>152.8 ± 3.9</td>
<td>155.0 ± 4.5</td>
<td>161.3 ± 6.1</td>
<td>158.0 ± 4.8</td>
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<td>158.6 ± 4.9</td>
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<tr>
<td>Na⁺/Cl⁻ ratio</td>
<td>0.11</td>
<td>0.12</td>
<td>1.17</td>
<td>1.17</td>
<td>1.15</td>
<td>1.13</td>
<td>1.13</td>
<td>1.18</td>
<td>1.20</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>3.10 ± 0.11</td>
<td>3.26 ± 0.11</td>
<td>2.82 ± 0.12</td>
<td>2.96 ± 0.12</td>
<td>2.84 ± 0.11</td>
<td>2.61 ± 0.11*</td>
<td>2.62 ± 0.13*</td>
<td>2.65 ± 0.16*</td>
<td>2.52 ± 0.08*</td>
<td>2.80 ± 0.15</td>
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<tr>
<td>Cortisol</td>
<td>42.5 ± 10.1</td>
<td>1401.1 ± 130.5*</td>
<td>853.7 ± 91.2*</td>
<td>223.1 ± 66.3*</td>
<td>116.8 ± 29.0*</td>
<td>58.7 ± 14.5</td>
<td>123.1 ± 29.0*</td>
<td>107.9 ± 18.5*</td>
<td>122.8 ± 19.8*</td>
<td>120.2 ± 26.4*</td>
<td></td>
</tr>
</tbody>
</table>
during the first 9 hr post-injection. However, as for the control group, after 24 hr their values were similar to SW (Fig. 1), after that they reached a high stable value (Table 2).

**Discussion**

Abrupt transference from SW to BW (SW → BW) led a transitory decrease in osmolality and Na⁺, Cl⁻, and Ca²⁺ plasma levels in both control and cortisol-treated groups. The animals showed a transitory adaptative period until original values are recovered and then a permanent regulatory phase to keep these values stable. This pattern was similar to those reported for other teleosts submitted to decreases in the environmental salinity (Holmes and Donaldson, 1969; Holliday, 1971; Maetz, 1974; Evans, 1980) and was shown before for *Sparus aurata* (Mancera et al., 1993a).

Fishes long-term adapted to BW showed a slightly decreased osmolality with respect to SW-adapted although Na⁺, Cl⁻ and Ca²⁺ concentrations were similar (Mancera et al., 1993a; present results). This discrepancy may be due to the presence of unknown plasma osmolytes, as was suggested by Woo and Wu (1982) that would not completely recover their original levels in BW. On the other hand, differences on Na⁺ and Cl⁻ concentration between control and cortisol-treated groups contrast with a similar plasma osmolality. Maybe the loss of these putative osmolytes vary in a different manner in
both groups, with a better recovery in the controls than in the injected animals.

We have previously reported variations in cortisol levels after abrupt SW and BW transfersences in Sparus aurata (Mancera et al., 1993a). In this species cortisol is high in BW-adapted fishes and low in SW-adapted fishes. This was also reported for Mugil cephalus and Platichthys stellatus (Johnson, 1973), and Dicentrarchus labrax (Roche et al., 1989). However after the transference SW → BW a transitory increase in cortisol occurs in Sparus aurata (Mancera et al., 1993a; present results); this must be related with stress better than with osmoregulation as has been suggested for other teleosts: Anguilla anguilla (Leloup-Hatey, 1974), Chrysopsys major (Ishioka, 1980), Sarotherodon mossambicus (Assem and Hanke, 1981), Fundulus heteroclitus (Jacob and Taylor, 1983), and Oncorhynchs kisutch (Redding et al., 1984).

The effects of cortisol or ACTH varies with the species studied. In Anguilla rouilla (Epstein et al., 1971), Cyprinus carpio and Sarotherodon mossambicus (Abo Hegab and Hanke, 1984), Oncorhynchs kisutch (Richman III and Zaugg, 1987), Salmo gairdneri (Madsen, 1990) and Salmo salar (Bisbal and Specker, 1991) a hypo-osmotic response, by increasing the number of chloride cells and their Na⁺/K⁺-ATPase activity and thus ionic excretion, has been reported. In contrast in Gambusia (Chambolle, 1967), Amia calva (Hanson et al., 1976), Ictalurus melas (Fortner and Pickford, 1982), and Heteropneustes fossilis (Parwex and Goswami, 1985) injection of cortisol or ACTH permitted survival in fresh water of hypophysectomized fishes, hence indicating a hyperosmotic role for cortisol. In agreement, corticosteroid or ACTH injected Fundulus kensae showed increased Na⁺ plasma levels (Stanley and Fleming 1964, 1967).

In our results, cortisol-injected animals showed an increased osmolality and ionic levels in SW; moreover the ionic decrease, during the adaptive period after SW → BW transference, was lower than in saline-injected fishes. This speaks in favour of a protective role of cortisol impairing ionic loss or improving ionic intake. Thus for Sparus aurata cortisol has a clear hyperosmotic role and can be an important hormone in adaptation to hypoosmotic environments. This can justify the stable increase in cortisol levels after the transference SW → BW (Mancera et al., 1993a; present results) and the decrease after the transference BW → SW (Mancera et al., 1993a). Studies on Na⁺/K⁺-ATPase activity in gut, gills and kidney during SW and BW adaptation will be of a great interest to corroborate the present assumptions in this species (cf. Gallis et al., 1979a and b).

Cortisol injected animals showed high plasma levels of the hormone at 3 hr thus indicating a good incorporation of the hormone, however, 9 hr after the transference, cortisol concentration was similar in control and experimental groups; similar results were obtained in Cyprinus carpio (Abo Hegab and Hanke, 1984). Higher cortisolemia seen in the experimental group from 0 hr to 9 hr can account for the higher Na⁺ and Cl⁻ levels in this group. However from 9 hr post transference, cortisol levels were similar in control and cortisol-treated group whereas Na⁺ and Cl⁻ levels were higher in the last. It may be that cortisol action is slow and its effects remain for a while after it has decreased or that other hormones such as prolactin may be synergistically facilitated by cortisol. In this sense we have previously reported activation of prolactin and corticotropin adeno-hypophysial cells in long-term BW-adapted Sparus aurata (Mancera et al., 1993b). This indicates a possible cooperation of cortisol and prolactin in hypoosmotic environments, as has been suggested for other euryhaline teleosts (Johnson, 1973; Bern, 1975; Fossett et al., 1983; Hirano, 1986).

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References


