Cortisol integrates osmotic competence in the organs of North African catfish (Clarias gariepinus Burchell): Evidence from *in vivo* and *in situ* approach
Abstract

We examined the *in vivo* and *in situ* effects of cortisol in North African catfish (*Clarias gariepinus*) to identify how this major corticosteroid integrates the osmotic competence in the fish organ systems. The indices of hydromineral and metabolic regulations were analyzed in the fish after repeated cortisol injections (40 and 200 ng g\(^{-1}\) body mass) as three alternate intraperitoneal injections for five days, and compared its hydromineral effects with 20 min cortisol perfusion (75-300 ng ml\(^{-1}\)). As expected, the plasma cortisol increased (*P*<0.01 & *P*<0.001) in the fish with increasing doses of cortisol injections. A reduction (*P*<0.001) in the kidney Na\(^+\), K\(^+\)-ATPase activity was observed in the cortisol-injected fish, whereas cortisol perfusion increased (*P*<0.05 & *P*<0.001) its activity. In contrast, the liver Na\(^+\), K\(^+\)-ATPase activity showed an increase (*P*<0.01) after cortisol injection, but decreased (*P*<0.01) after cortisol perfusion. The gill Na\(^+\), K\(^+\)-ATPase activity, however, increased after both the cortisol injections and the perfusion. The cortisol injections increased (*P*<0.05) the intestinal Na\(^+\), K\(^+\)-ATPase activity, but the cortisol perfusion failed to elicit such a response. The plasma glucose (*P*<0.05) and plasma lactate (*P*<0.01) increased, but the plasma urea (*P*<0.05) declined in the cortisol-injected fish. Cortisol injections reduced the plasma [Na] (*P*<0.01) and [K] (*P*<0.001) concentrations, whereas the plasma [Mg] increased (*P*<0.05) substantially. Our *in vivo* and *in situ* data indicate that cortisol, at its physiologically-realistic concentrations, promotes and integrates the osmotic competence in the organs particularly kidney and liver by differentially regulating its rapid and prolonged effects on Na/K pump activity, thus support the hypothesis that cortisol integrates the specific and differential osmotic functions of the organ systems in fish.
2.1 Introduction

Fishes have developed mechanisms to maintain their hydromineral and metabolic homeostasis irrespective of the dynamic nature of environmental conditions. Gills, kidney and intestine of fish, thus coordinate hydromineral homeostasis (McCormick, 2001; Marshall et al., 2002; Evans, 2002, 2008), whereas liver directs metabolic homeostasis (Mommsen et al., 1999; Peter and Peter, 2007). With the multiple hormonal controls, the osmoregulatory (Hazon and Balment, 1998; McCormick, 2001) and nonosmoregulatory organs including liver regulate ion transport particularly Na and K across the plasma membrane, which is essential to support cell metabolism. The chloride cell in branchial epithelium, for instance, is an important site for ion handling in freshwater fish and is under hormonal control including cortisol (Evans et al., 2005; Bradshaw and McCormick, 2006). On the other hand, with the aid of hormones liver regulates pathways of intermediary metabolism to ensure energy homeostasis under varied physiologic conditions (Leatherland, 1994; Iwama et al., 2006; Peter and Peter, 2007; Peter et al., 2007).

Plasma cortisol, an indicator to assess the magnitude of stress response in fish (Barton and Iwama, 1991; Wendelaar Bonga, 1997), plays significant roles in hydromineral balance, growth and reproduction (Mommsen et al., 1999; Laiz-Carrion et al., 2003; Small, 2004a; McCormick et al., 2008). A dual role of cortisol in carbohydrate metabolism and hydromineral regulation has been demonstrated in a number of fishes (Gallo and Civinini, 2003; Sangio-Alvarellos et al., 2005; Takahashi et al., 2006). Under acute stress, cortisol level in fish can easily shoot up many folds to enhance the mobilization of energy reserves and metabolic rate (Wendelaar Bonga, 1997; Gregory and Wood, 1999; Flik et al., 2006). Cortisol regulates glucose mobilization (Vijayan et al., 1996) by inducing liver glycogenolysis and gluconeogenesis to handle the high energy demand especially during stress conditions (Vijayan et al., 1997). Hyperglycemia is, thus considered as the most
common indicator of stress which is mainly due to the elevated cortisol and adrenaline levels (Wedemeyer, 1997; Barton et al., 2002).

Cortisol contributes to hydromineral regulation in freshwater fish, but is often referred to as a seawater hormone (McCormick, 2001). Emerging molecular evidences, especially on the divergent osmotic functions of glucocorticoid and mineralocorticoid receptors in fish (Bury et al., 2003; Aluru and Vijayan, 2006; Bradshaw and McCormick, 2006; McCormick et al., 2008), further support this view. As a measure of hydromineral capacity at the organismal and cellular level, Na^+, K^+-ATPase activity is involved in ion transport and is widely used as an index of the functional state of the chloride cells (Dang et al., 2000a). Induction of chloride cell proliferation associated with an increase in its number, size and Na^+, K^+-ATPase activity (Hazon and Balment, 1998; Dang et al., 2000a; Nishimura and Fan, 2003) has been demonstrated in freshwater-acclimated fish after cortisol treatment. Besides stimulating branchial Na^+, K^+-ATPase activity (Seidelin et al., 1999; McCormick, 2001), cortisol also regulates the whole body Ca^+, Na^+ and Cl^- ion influxes (Flik and Perry, 1989). Cortisol, thus achieves its ionoregulatory recovery by regulating the activity (Wendelaar Bonga, 1997; Marshall and Grosell, 2006), the expression of Na^+, K^+-ATPase (McCormick et al., 2008), and by the expression of Na-K-2Cl co-transporter (Pelas and McCormick, 2001).

On the other hand, less attention has been given to examine the hydromineral effects of cortisol on kidney function compared to gills and intestine (Seidelin et al., 1999). Likewise, the osmotic function of liver has not been addressed adequately, although critical roles of liver in intermediary metabolism (Mommsen et al., 1999; Peter et al., 2007), ureogenesis (Walsh and Mommsen, 2001; Wright, 2007) and cortisol-directed metabolic regulation (Mommsen et al., 1999; Sangio-Alvarellos et al., 2005) have been established in fish. We thus hypothesized that cortisol may be involved in the integration of osmotic competence in organ systems of fish. To
this end, we analyzed the indices of hydromineral regulation in the African catfish *Clarias gariepinus* after administering *in vivo* cortisol injections and compared its hydromineral effects on both osmoregulatory and nonosmoregulatory organs with *in situ* cortisol perfusion. We also measured the metabolic and thyroidal response in catfish to cortisol injections.

### 2.2 Materials and methods

**Experimental animals**

Freshwater North African catfish *Clarias gariepinus* (Burchell) of order Siluriformes comprising both sexes in their prespawning phase (41 ± 4 g body mass) were collected and acclimated in 50 L glass tanks with aerated tap water (pH 6.8) under natural photoperiod (12L/12D) for three weeks at 28 ± 1°C. They were fed daily with commercial fish feed at a ration of 1.5% of body mass. The animal care and the experimentation were strictly according to the regulation of Animal Ethical Committee of the University and there was no mortality during the experimentation.

**In vivo effects of repeated cortisol injections**

This experiment (N=2) tested the *in vivo* effects of cortisol (Sigma St. Louis, MO) injections on metabolic, hydromineral and thyroid response in catfish after repeated cortisol injections for five days. Twenty-four acclimated fish were held as three groups of eight each. The first group, which considered as the control fish, was given injections of 0.85% saline on every alternate day for 5 days. The second and third fish groups were given cortisol injections in a total dose of 40 and 200 ng g⁻¹ body mass respectively as three injections on day 1, 3 and 5. All injections were made intra-peritoneally below the pelvic fin between 9.00 and 9.30 a.m. and the volume of hormone vehicle (saline) was kept as 0.1 ml. The hormone was first dissolved in propylene glycol (0.01%) and subsequently diluted with saline (0.85%
NaCl) as has been reported earlier (Jacob and Oommen, 1992). Feeding was stopped for 24 h prior to sampling to ensure optimum experimental conditions. Both control and hormone-treated groups were handled in the same manner. Twenty-four hour after the last injection, fish in each tank were quickly dip-netted and anaesthetized (9.00 a.m.) in 0.1% 2-phenoxyethanol solution (SRL, Mumbai). Blood was drawn from the caudal artery using a heparinized 23 gauge syringe. Plasma was separated immediately by centrifugation (10,000 g) for 5 min at 4°C and stored at -20°C until analyzed. Fish were then sacrificed by spinal transsection and pieces of second gill arch, 1 cm of anterior intestine, posterior kidney and a posterior lobe of liver were excised immediately and kept in 0.25MSEI buffer (pH 7.1) and stored at -20°C.

Effects of in situ cortisol-perfusion

The effect of 20 min in situ cortisol perfusion on Na+, K+-ATPase activity in fish organs was examined in this experiment (N=2). Fish were anesthetized in 2-phenoxyethanol solution and were placed in a wet towel. Blood was drawn from the caudal artery with the help of a heparinized syringe. A ventral incision was made to each fish from the anus to the pectoral girdle and a cannula (PE-50 tubing) was inserted into the ventricle through the bulbus arteriosus. Perfusion was done with the help of a peristaltic pump (ENPD-100 EnterTech, Mumbai) using a perfusion medium (Cortland saline; 119 mM NaCl, 5 mM NaHCO₃, 5.4 mM KCl, 0.35 mM Na₂HPO₄, 0.44 mM KH₂PO₄, 0.81 mM MgSO₄, 1.25 mM CaCl₂ and 5 mM D-glucose of pH 7.4) at a rate of 0.3 ml min⁻¹ for 20 min as was demonstrated earlier in Clarias batrachus (Saha et al., 2000) and in Anabas testudineus (Peter, M. C. S., unpublished). One group of fish (n=6) were perfused with perfusion medium alone and served as the control fish. Physiologically-realistic doses (75, 150 and 300 ng ml⁻¹) of cortisol were first dissolved in 0.01% propylene glycol and then diluted in saline (0.85% NaCl). These nominal concentrations of cortisol were then supplemented to the perfusion medium after serial dilutions and perfused into three
groups of catfish (n=6). After 20 min of cortisol or saline perfusion, gills, kidney, intestine and liver tissues were excised and stored at -20°C as described earlier.

**Analytical procedures**

**Na⁺, K⁺-ATPase specific activity**

The ouabain-sensitive Na⁺, K⁺ dependent adenosine triphosphatase (Na⁺, K⁺-ATPase, E.C. 3.6.3.9) specific activity was measured in tissue homogenates as described by Peter et al., (2000). Briefly, about 100 mg each of gill filaments was scraped off from the gill arch or 50 mg of anterior portion of intestine or a part of posterior kidney or a lower lobe of liver tissue was homogenized in of 0.25 M SEI buffer (pH 7.1) and centrifuged at 700 g for 10 min. The supernatant obtained was used to measure the specific activity of Na⁺, K⁺-ATPase and samples were incubated for 15 min with or without 1 mmol L⁻¹ ouabain (Sigma, St. Louis). Saponin (0.2 mg.mg⁻¹ protein) was routinely added to optimize substrate accessibility. Samples were incubated for 15 min at 37°C in a medium containing 100 mmol L⁻¹ NaCl, 30 mmol L⁻¹ imidazole, pH 7.4, 0.1 mmol L⁻¹ EDTA, 5 mmol L⁻¹ MgCl₂ and either 15 mmol L⁻¹ KCl (Medium A) or 1 mmol L⁻¹ ouabain (medium E). Na₂ATP was added to a final concentration of 3 mmol L⁻¹. The reaction was stopped by adding ice-cold 8.6% TCA solution and the liberated inorganic phosphate, Pᵢ, was quantified spectrophotometrically (Sytronics 2202, New Delhi). The specific activity of Na⁺, K⁺-ATPase was defined as the difference between the release of Pᵢ in medium A and in medium E, and was expressed as µmol Pᵢ h mg protein⁻². The protein contents in the tissues were measured using modified Biuret assay (Alexander and Ingram, 1980) with bovine serum albumin as standard.
Plasma metabolites and minerals

Plasma glucose (GOD/POD test kit; Span Diagnostics Ltd., New Delhi), urea (DAM kit; Span Diagnostics Ltd., New Delhi) and lactate (PAP Fluid test; Radiant Diagnostics, New Delhi) concentrations were measured colorimetrically in a Systronic Spectrophotometer 2202 (Systronics, New Delhi) using commercial test kits.

Plasma \([\text{Na}]\) and \([\text{K}]\) were measured with a flame photometric analyser (Systronics 129, New Delhi) using standards of Na and K (Remedix diagnostics, Palakkad) and values were expressed as mmol L\(^{-1}\). Plasma \([\text{Mg}]\) and \([\text{Ca}]\) were estimated with an Atomic Absorption Spectrometer (Solar S2, Thermo Elemental, UK) using appropriate standards (SRL, Mumbai) and values were expressed as mmol L\(^{-1}\).

Plasma cortisol, \(T_3\) and \(T_4\)

Cortisol concentrations in plasma samples were measured by competitive immunoenzymatic assay (DiaMetra, Foligno, Italy) and values were expressed as ng ml\(^{-1}\). The sensitivity and reliability of this method was examined and the values were comparable to RIA method reported earlier (Peter, 2007). In brief, plasma was deproteinised with ethanol phosphate buffer (1:9). Plate wells coated with mouse-anti-rabbit IgG were treated with standards and diluted samples (20 \(\mu\)l) and incubated with 200 \(\mu\)l cortisol-HP conjugate at 37°C for 1 hour. After washing, 100 \(\mu\)l TMB-H\(_2\)O\(_2\) was added and incubated at 20°C for 15 min in the dark. Absorbance was recorded on a plate reader (Span Autoreader 4011, New Delhi) at 450 nm after adding 0.15 moles sulphuric acid. The intra-assay coefficient of variation was 3%, and the inter-assay coefficient of variation was 9.32%.

Plasma \(T_3\) and \(T_4\) concentrations were measured by microwell enzyme immunoassay (EIA: magnetic solid phase) with kits (Syntron Bioresearch Inc,
Carlsbad, California, Catalog # 3810-96 for T₃ and Catalog # 2210-96 for T₄). The sensitivity of this method was checked by comparison of results from RIA based on competitive binding of $^{125}$I-labelled T₃ or T₄ with the EIA results (Peter et al., 2007). Briefly, the anti-T₄ (goat anti-mouse IgG) coated wells were treated with 50 µl standards, control and samples. After adding 100 µl T₄-HRP conjugate the wells were incubated at 37°C for 1 hour. After washing, 50 µl of 0.05 M acetate buffer and TMB were added and incubated at 20°C for 15 min. Absorbance was read at 450nm after stopping the reaction with 1N HCl. The intra-assay coefficient of variation was 7.2 and inter-assay coefficient of variation was 9.0.

Similarly, plasma T₃ was quantified as described for T₄ but used anti-T₃-antibody (goat anti-mouse IgG) and the T₃-conjugate HRP. The intra-assay coefficient of variation was 4.4 and inter-assay coefficient of variation was 8.5.

Statistics

The data were collected from both in vivo (n=8, N=2) and in situ (n=6, N=2) experiments and are presented as mean ± S. E. M. All data sets were analysed using one-way analysis of variance (ANOVA) followed by a post-hoc multiple (all-pairwise) SNK comparison test. Significant differences between groups were accepted if $P<0.05$ and all the statistical tests were performed using a software package (Graphpad Instat-3, San Diego, USA).

2.3. Results

In vivo effects of repeated cortisol injections and in situ cortisol perfusion on Na⁺, K⁺-ATPase activity

The kidney Na⁺, K⁺-ATPase activity declined ($P<0.001$) after five days of repeated cortisol injections, but showed increases ($P<0.05$ and $P<0.001$) after 20 min of cortisol perfusion (Fig.2.1A). In contrast, liver Na⁺, K⁺-ATPase activity increased ($P<0.01$) after cortisol injections, but declined ($P<0.01$) after cortisol perfusion
The Na⁺, K⁺-ATPase activity in the gills increased after both cortisol injections ($P<0.01$) and perfusion ($P<0.001$; Fig. 2.2A). The intestinal Na⁺, K⁺-ATPase activity increased ($P<0.05$) after cortisol injection, but remained unaltered in the cortisol-perfused fish (Fig. 2.2B).

Fig. 2.1 Kidney (A) and liver (B) Na⁺, K⁺-ATPase activities in African catfish after in vivo injections of cortisol and cortisol perfusion. The fish were injected with cortisol (40 and 200 ng g⁻¹ body mass) as three i. p. injections over five days and varied doses of cortisol (75, 150 and 300 ng ml⁻¹) were loaded in the perfusion medium and perfused for 20 min. ** $P<0.01$ and *** $P<0.001$ compared with in vivo control. a: $P<0.05$, b: $P<0.01$ and c: $P<0.001$ compared with perfused control.
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Gill Na⁺, K⁺ -ATPase activity

Intestinal Na⁺, K⁺ -ATPase activity

Fig. 2.2 Gill (A) and intestinal (B) Na⁺, K⁺-ATPase activities in African catfish after in vivo injections of cortisol and cortisol perfusion. The fish were injected with cortisol (40 and 200 ng g⁻¹ body mass) as three i. p. injections for five days and the branchial and intestinal Na⁺, K⁺-ATPase activity were measured. The varied doses of cortisol (75, 150 and 300 ng ml⁻¹) in the perfusion medium was loaded and perfused in the fish for 20 min. * P<0.05 and ** P<0.001 compared with in vivo control. c: P<0.001 compared with perfused control.

Plasma metabolites and minerals after in vivo cortisol injections

Significant increases in the plasma glucose (P<0.05), plasma lactate (P<0.01) and a decrease in the urea (P< 0.05) were recorded in the cortisol-injected fish (Fig. 2.3A, B & C). Cortisol injections showed decreases in the plasma [Na] (P<0.01) and
[K] \(P<0.001\) concentrations; increased \(P<0.05\) plasma [Mg], but had little effect on plasma [Ca] (Table 2.1).

**Plasma cortisol, T₃ and T₄ after in vivo cortisol treatment**

As expected, the plasma cortisol levels \(P<0.001\) rose significantly in the fish after five days of repeated cortisol injections compared to sham-control (Fig. 2.4B). The basal cortisol levels in the untreated control were \(11.2 \pm 1.05\) ng ml\(^{-1}\). The plasma T₃ decreased significantly \(P<0.01\), whereas plasma T₄ remained unaltered in the cortisol-injected fish (Fig. 2.4A).

<table>
<thead>
<tr>
<th>Status</th>
<th>Control</th>
<th>Cortisol 40 ng g(^{-1})</th>
<th>Cortisol 200 ng g(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>150.2 ± 4.5</td>
<td>127.8 ± 1.3***</td>
<td>144.6 ± 4.7</td>
</tr>
<tr>
<td>K</td>
<td>5.16 ± 0.11</td>
<td>2.18 ± 0.19***</td>
<td>2.05 ± 0.14***</td>
</tr>
<tr>
<td>Ca</td>
<td>2.80 ± 0.25</td>
<td>2.53 ± 0.20</td>
<td>2.10 ± 0.36</td>
</tr>
<tr>
<td>Mg</td>
<td>1.28 ± 0.18</td>
<td>1.74 ± 0.09*</td>
<td>1.71 ± 0.11*</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m, \(n=6\)

Plasma was analyzed after three intraperitoneal injections of cortisol (40 and 200 ng/g body mass) for five days.

\* \(P < 0.01\) ** \(P < 0.01\) *** \(P < 0.001\) compared to control plasma.
Plasma glucose

![Graph of Plasma glucose](image)

Plasma lactate

![Graph of Plasma lactate](image)

Plasma urea

![Graph of Plasma urea](image)

Fig- 2.3 Plasma glucose (A), plasma lactate (B) and plasma urea (C) in African catfish after *in vivo* injections of cortisol. The fish were injected with cortisol (40 and 200 ng g⁻¹ body mass) as three i. p. injections over five days. *P<0.05 and **P< 0.01 compared with control plasma.
Fig. 2.4 Plasma T₃ and T₄ (A) and plasma cortisol (B) in African catfish after repeated \textit{in vivo} injections of cortisol. The hormone was measured in plasma after three i. p. injections of cortisol (40 and 200 ng g⁻¹ body mass) for five days. Cortisol concentrations (75, 150 and 300 ng ml⁻¹) in the perfusion medium were also represented. ** $P<0.01$ and *** $P<0.001$ compared with control plasma.

2.4 Discussion

The major conclusion drawn from this study is that cortisol promotes osmotic competence by integrating specific and differential osmotic regulations in the osmoregulatory and nonosmoregulatory organs of North African catfish. To our knowledge, this is the first to explore the integrated and specific action of cortisol.
both in vivo and in situ on fish organ systems, as the integrative mechanism of cortisol effects in fish is less clear at the organ level (Evans, 2008).

Fish kidney, a vital osmoregulatory organ, plays an essential role in hydromineral homeostasis and in freshwater fish it filters at high rates and reabsorbs nearly all filtered solutes (Bone et al., 1995; Karnaky, 1998). The downregulated kidney Na⁺, K⁺-ATPase activity in the cortisol-injected fish and its upregulation in the cortisol-perfused fish implies that cortisol has a differential action on kidney Na⁺, K⁺-ATPase under in vivo and in situ conditions. An indication of poor Na reabsorption due to downregulated Na pump activity prevails in the kidney of cortisol-injected fish. On the contrary, a direct and rapid stimulatory action of cortisol on Na pump activity occurs in the kidney of perfused fish, implying that an enhanced Na reabsorption is essential in these fish. The varied time course of cortisol action and the disparity in the physiologic status of fish might be the basis of this differential action of cortisol. Our results thus provide evidence that this systemic organ is a direct target for cortisol action, although Seidelin et al., (1999) recorded no significant changes in kidney ATPase activity in freshwater-acclimated brown trout (Salmo trutta) treated with cortisol.

As systemic organ, liver harbours major metabolic machineries and plays a critical role in energy homeostasis. Simultaneously, liver possess its own osmoregulatory competence to handle the metabolic function as these two processes are coupled. It is therefore not surprising to find liver as a major target controlling energy metabolism mainly through the action of hormones particularly by thyroid hormones and cortisol (Leatherland, 1994; Mommsen et al., 1999; Peter et al., 2007). Unlike kidney, the differential response of liver to cortisol in vivo and in situ underscores the vital role of this organ in exerting both rapid and prolonged effects of cortisol on metabolic machinery and its coupling with osmotic competence. This coupling duly coordinated by cortisol, is thus critical and ensures its active
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metabolic participation during stress response. In addition, the opposing responses of kidney and liver to both in vivo and in situ cortisol treatments provide clues on the fine-tuning of osmotic and metabolic mechanisms in these organ systems.

Gills, the known target for cortisol action, are the prime organ involved in the hydromineral homeostasis of fish. Branchial chloride cells rich in Na⁺, K⁺-ATPase expel excess salts in hyperosmotic environment (Marshall, 2002; McCormick, 2001; Evans et al., 2005), but engages its absorption in hypoosmotic environment (Deane and Woo, 2004). Cortisol shows both rapid and prolonged stimulatory action on the gill Na pump activity in our catfish, supporting the cortisol-driven freshwater ion transport and chloride cell function. This gill response to cortisol, thus contributes to its role in the maintenance or restoration of hydromineral balance in freshwater fish. Enhanced branchial Na⁺, K⁺-ATPase activity and expression by cortisol have been documented in a number of fish species (Dang et al., 2000a; Laiz-Carrion et al., 2003; McCormick et al., 2008).

The intestine of agglomerular teleost has a profound role in hydromineral regulation and cortisol enhances the intestinal fluid absorption and ion permeability (Hazon and Balment, 1998; Marshall et al., 2002). Catfish intestine that produced substantial increase in the Na⁺, K⁺-ATPase activity after repeated cortisol injections, reveals that intestine is an important site for cortisol action. Rich basolateral Na⁺, K⁺-ATPase in the intestinal epithelium (Sardella et al., 2008) offers ion exchange and cortisol in catfish thus produce increased ion absorption. Similar response of sockeye salmon intestine to cortisol as evidenced by the maintenance of Na⁺, K⁺-ATPase activity has been demonstrated (Vieillette and Young, 2005). In freshwater, the ion-transporting capacities of teleost intestine actively take up essential ions primarily from the food, including Ca²⁺ and Na⁺, although these increases in ion transport ATPase activities also reflect ionoregulatory disturbance (Nolan et al., 1999).
stimulated osmotic function in conjunction with other osmoregulatory organs could thus be seen in catfish after prolonged cortisol administration.

A pronounced fall in the plasma Na after cortisol injections indicates a leaky gill epithelium as has been demonstrated earlier (McDonald and Milligan, 1997). This is in line with the observations of decreased plasma Na in the cortisol-injected rainbow trout (Madsen, 1990a) and the reduced ion movement across the trout gill epithelium (Kelly and Wood, 2001). In contrast, cortisol treatment showed no significant change in plasma Na⁺, Cl⁻ of cutthroat trout parr in freshwater (Morgan and Iwama, 1996) and elevated plasma Na in the cortisol-fed tilapia *Oreochromis mossambicus* (Dang et al., 2000a). The rise in Mg and fall in K in the plasma of cortisol-injected catfish even in the absence of a change in plasma Ca point to the cortisol effects on both monovalent and divalent ion transport as have been well documented in other fish species (Laiz-Carrion et al., 2003). The elevated plasma Mg indicates its increased reabsorption by the kidney. A similar relationship between Mg and cortisol has been demonstrated in freshwater smolts of Atlantic salmon exposed to atrazine (Nieves-Puigdoller et al., 2007).

High cortisol level, representing the magnitude of stress response in fish (Davis, 2006), mobilizes energy substrates (Vijayan et al., 1997; Mommsen et al., 1999) and compensate the osmoregulatory disturbances (Wendelaar Bonga, 1997; Nolan et al., 1999; Dang et al., 2000a; Peter, 2007). Coordination of physiologic responses during stress thus becomes a major strategy of integrated stress response (Peter et al., 2007). Cortisol combats the disturbed physiological functions by redistributing the energy from growth and reproduction to restore its homeostasis (Wendelaar Bonga, 1997). Hyperglycemia in our catfish, which might be primarily due to the increased hepatic gluconeogenesis and peripheral proteolysis, thus becomes a metabolic strategy to cope with the energy demand. Similar hyperglycemic effect of cortisol has been demonstrated in a number of teleosts
species (Mommsen et al., 1999; Laiz-Carrion et al., 2003). For example, in cutthroat trout (Oncorhynchus clarki clarki) parr kept in fresh water showed higher plasma glucose (Morgan and Iwama, 1996). Nevertheless, the effects of cortisol on gluconeogenesis and gluconeogenic enzymes have been demonstrated in liver of cortisol-implanted fish (Vijayan et al., 1994b; Mommsen et al., 1999).

Teleost fishes possess ureogenic enzymes and are capable of synthesizing urea (Wood, 2001; Walsh and Mommsen, 2001; Wright, 2007). Our catfish responded positively to cortisol treatment by decreasing plasma urea and that implies a direct effect of cortisol on nitrogen excretion probably favouring the branchial excretion of NH₄ as has been suggested earlier (Walsh et al., 1994). A three-fold rise in the plasma urea concentrations was, however, reported in rainbow trout (Oncorhynchus mykiss) treated with cortisol (McDonald and Wood, 2004). As most teleosts are ammoniotelic and excrete nitrogenous wastes through the gill chloride cells as ammonium (Wood, 2001), the increased gill excretion of NH₄ would eventually downregulate the ureogenic potential of catfish resulting in lowered plasma urea concentration. Plasma lactate, an index of aerobic/anaerobic glycolysis, increased substantially in the cortisol-treated catfish indicating an enhanced gluconeogenic potential in these fish as cortisol has been shown to promote gluconeogenesis in liver (Mommsen et al., 1999, Laiz-Carrion et al., 2002). Similar rise in plasma lactate has been demonstrated in net confined channel catfish (Small, 2004b) and rainbow trout (Trenzado et al., 2003), but net confined carp showed a reduction in lactate (Ruane et al., 2001). A significant increase in plasma lactate was also reported in cortisol-treated gilthead sea bream, Sparus auratus (Laiz-Carrion et al., 2003).

The increased plasma cortisol in catfish with increasing doses of repeated cortisol injections is consistent with the reports in freshwater trout (Morgan and Iwama, 1996) and in Atlantic salmon (McCormick et al., 2008). In our catfish, prolonged cortisol availability did not affect the plasma T₄ level, although fish
thyroid axis is sensitive to stressor-exposure (Redding et al., 1986; Leatherland, 1994; Peter, 2007). The plasma T₃ in catfish, however, declined after repeated cortisol injections and that implies a negative interaction of cortisol with T₃. Suppressed plasma T₃ level has also been reported in European eel (*Anguilla anguilla*) and coho salmon (*Oncorhynchus kisutch*) after cortisol treatments (Redding et al., 1986). On the other hand, hyperthyroidism evoked a marked reduction in basal plasma cortisol level in *Cyprinus carpio* (Geven et al., 2006) and in tilapia (Peter, 2007). Plasma thyroid hormone levels are sensitive to toxic and non-toxic stressors including handling (Leatherland, 1994; Peter et al., 2007; Leji et al., 2007). An inverse relationship between plasma cortisol and thyroid hormone has been observed in *Solea senegalensis* (Arjona et al., 2008) and in *Anguilla anguilla* (Redding et al., 1986). Studies on Mozambique tilapia revealed that changes in the status of thyroid affects the cortisol-directed stress response (Peter, 2007).

In conclusion, our results provide evidence that cortisol promotes and integrates the osmotic competence in both osmoregulatory and nonosmoregulatory organs of catfish by coordinating its specific and differential actions on Na⁺, K⁺-ATPase activity particularly in kidney and liver. Cortisol thus regulates plasticity in its ion transport by exerting both rapid and prolonged actions at the organ level which becomes fundamental to the regulation of hydromineral homeostasis in fish. Our data also establish that kidney and liver are the key targets for cortisol action as it play major roles in hydromineral and metabolic homeostasis of fish.