Bioenergetics of Adaptation to a Salinity Transition in Euryhaline Teleost (*Oreochromis mossambicus*) Brain

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Freshwater (FW) teleosts are capable of acclimating to seawater (SW) following such a transfer from FW. However, their osmoregulating mechanisms are still unclear, particularly those in the brain. The present study was conducted to examine acute changes that occur in brain Na^+-K^+-ATPase activity, creatine kinase (CK) activity, creatine, creatinine contents, and ATP levels of tilapia (*Oreochromis mossambicus*) in response to this transition. After transfer to SW (25 ppt), the Na^+-K^+-ATPase activity was maintained for 8 hr at higher levels than that in FW. In contrast, in 35 ppt SW, Na^+-K^+-ATPase was maintained at a even higher level than in FW for the first 2 hr. Brain Na^+-K^+-ATPase contents in both the 25 and 35 ppt SW groups were significantly elevated within 1 and 0.5 hr after transfer from FW, respectively. Interestingly, brain CK activities and content (homodimer of the B subunit [BB] form) in both the 25 and 35 ppt SW groups were significantly elevated within 1 hr after transfer from FW. The ATP contents in 35 ppt SW increased abruptly within 0.5 hr, and then gradually decreased during the next 2 hr. Unlike the 35 ppt group that declined in ATP contents, the 25 ppt group leveled off within 24 hr. The elevations in CK activity and creatine levels after transfer from FW to SW imply that abrupt salinity changes alter phosphocreatine/CK ratio. Such changes are needed to satisfy the increases in the energetic requirement of the cotransport mechanisms mediating osmoregulation. [Exp Biol Med Vol. 227(1):45–50, 2002]

Key words: brain Na^+-K^+-ATPase; creatine kinase; ATP; tilapia (*Oreochromis mossambicus*)

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Creatine kinase (CK; EC 2.7.3.2) is used to transfer energy between phosphagens in the reversible reaction: ADP + phosphocreatine → ATP + creatine. CK exists as different isoforms comprised of either an alkaline homodimer of the M subunit (MM), an acidic homodimer of the B subunit (BB), or a heterodimer of the M and B subunits (MB). These isoforms can be identified by differences in their electrophoretic mobility, tissue, subcellular distribution, and primary sequence (1, 2). Three different types of CK isoforms (brain, muscle, and mitochondria) exist in teleosts (reviewed in Refs. 3 and 4). Interestingly, trout spermatozoa CK activity is high (s-CK isozyme) (5), but a previous study showed that it was not present in gills (6).

The activity of plasma CK increases as a physiological stress response in big game fish after capture (7). Total CK activity significantly decreased by 20% in fish (*Oreochromis mossambicus*) brain after exposure to hypergravity for 7 days (8). On the other hand, CK levels are elevated in Atlantic salmon (*Salmo salar*) with severe degenerative myopathy (pancreas disease) (9, 10). Recently, an increase in CK activity was associated with developing skeletal muscle degeneration in sea bass, *Dicentrarchus labrax* (11). In addition, there is some evidence of seasonal fluctuations of CK in rainbow trout, *Oncorhynchus mykiss* (12); tissue variability in CK isozyme expression in rainbow trout, *Oncorhynchus mykiss*, and genetic variability of tissue CK between fish species including rainbow trout (4, 13). The presence of MM-type CK, BB-type CK, and mitochondrial CK was demonstrated with MM-type CK being predominant in *Gillichthys mirabilis* gills (14). Muscle-type CK is functionally coupled to Na^+-K^+-ATPase activity, in that ATP consumption is offset by CK-mediated dephosphorylation of creatine phosphate (15, 16). The CK of gills acts as an energy source to satisfy increases unenergetic requirements of gill Na^+-K^+-ATPase for osmoregulation after transfer

from freshwater (FW) to seawater (SW; Weng CF, *et al.*, unpublished data).

When a euryhaline teleost goes from hyperosmotic (SW) to hypo-osmotic (FW) media, it tends to gain water followed by loss of osmolytes and water in a regulatory volume response referred to as regulatory volume decrease. Alternatively, upon exposure to an increase in salinity, the initial effect is a loss in water followed by accumulation of osmolytes until the intracellular and extracellular osmolalities match one another. Therefore, regulatory volume responses are responsible for maintenance of a stable internal milieu, ionic regulation, and water balance. When tilapia were transferred directly to SW (35 ppt), they died within 4 hr. However, they survived after transfer to 25 ppt SW (Weng CF, *et al.*, unpublished data) (17). The organs involved in osmoregulation in teleosts include the opercular membrane, gill, gut, kidney, and urinary bladder. The gill is the primary organ that responds to the critical problem of salinity changes in teleostean fish; however, it is not known whether other vital organs such as the brain also play a functional role (ion transport and water balance) in adapting to transfer from FW to SW. Previously, it was determined that CK is needed during critical phases of brain development in the cichlid fish *Oreochromis mossambicus* and the clawed toad *Xenopus laevis*. It is very sensitive during this period to changes in environmental conditions (e.g., gravity) (8).

In the current study, we determined which type of CK is regulated by salinity. The results showed that gill CK activity in tilapia (*O. mossambicus*) was characterized and altered by salinity, directly or indirectly, especially muscle-type CK (Weng CF, *et al.*, unpublished data). Furthermore, we characterized changes in brain Na^+-K^+-ATPase activity, ATP levels, creatine and creatinine contents, and CK after short-term transfer from FW to 25 and 35 ppt SW in the present study.

Materials and Methods

Animals. Tilapia (*O. mossambicus*) were originally obtained from the Tainan Fish Culture Station of the Taiwan Fisheries Research Institute and were maintained in a freshwater recirculating tank at 25°–28°C at the Institute of Zoology, Academia Sinica (Taipei, Taiwan). They varied from 5 to 7 cm in total length and weighed from 2.5 to 4.0 g. Tilapia were transferred from FW to 25 or 35 ppt SW for various times (0.5, 1, 1.5, 2, 4, 8, 16, and 24 hr, n = 6–8, at each time point). Fish were anesthetized on ice and were sacrificed immediately. The brain was removed and weighed followed by homogenization with a motorized Teflon pestle at 1000 rpm for 2 min on ice in a solution containing 100 mM imidazole-HCl buffer, pH 7.0, 5 mM Na_2EDTA, 200 mM sucrose, and 0.1% sodium deoxycholate. After centrifugation (12,000 rpm for 30 min at 4°C), the supernatant was kept at −70°C until assay. Total protein was determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) and bovine serum albumin was used as a standard (Fraction V., Sigma, St. Louis, MO).

CK Activity. Brain CK activity was measured using a commercial kit (Sigma) as per enclosed instructions, with minor modifications (small volume). The reactions are ADP + phosphocreatine → ATP + creatine, and creatine + α-naphthol + diacetyl → colored complex. Diluted samples (0.2 ml) were mixed with 0.5 ml of phosphocreatine solution (0.2 ml). ADP-glutathione solution (start reaction) was added to each tube and they were mixed vigorously and incubated for 30 min at 37°C. Afterwards, 20 μl of *p*-hydroxymercuribenzoate was added to stop the reaction, and then 100 μl of α-naphthol solution, 100 μl of diacetyl solution, 700 μl of mH_2O or double distilled water (D.D.W.) were added and mixed well. After incubation at 37°C for 20 min, the tubes were centrifuged at 3500 rpm for 5 min. The absorbance of the supernatant was measured at 520 nm. The CK activity of unknown samples was calculated by comparing them with creatine standards. The data are expressed in micromoles of creatine released per milligram of protein.

Na^+-K^+-ATPase Activity. The measurement of brain Na^+-K^+-ATPase activity was determined by the rate of Pi formation. Briefly, a sample (80–100 μg of total protein) was added to a reaction buffer that contained (in millimoles): 100 imidazole-HCl buffer, pH 7.6, 125 NaCl, 75 KCl, 7.5 $MgCl_2$, and 5 Na_2 ATP. After incubation at 37°C for 30 min, the samples were placed on ice and 30% tricholoroacetic acid (TCA) was added to stop the reaction. The vials were centrifuged at 4000 rpm at 4°C for 10 min; the supernatant and the phosphate standards were added with 2.5% acid molybdate solution, 10% SDS, and 0.025% $NaHSO_3$ (Na_2SO_3, 1-amino-2-naphthol-4-sulfomic acid). After incubation at 20°C for 30 min. Pi production was measured at 700 nm following the method of Peterson (18). The Na^+-K^+-ATPase activity was defined as the difference in the inorganic phosphate liberated in the presence and absence of 0.5 mM ouabain. Data are expressed in micromoles of inorganic phosphate released per milligram of protein per hour.

ATP. The ATP level was measured as per instructions provided with a commercial kit (Sigma). The reactions are: ATP + 3-phosphoglycerate^{PGK} → ADP + 1,3-diphosphoglycerate, and 1,3-diphosphoglycerate + NADH^{GAPD} → glyceradehyde-3-P + NAD + P. The amount of ATP in a 10-μl sample was determined by the decrease in absorbance at 340 nm (Spectophotometer, Hitachi, Japan) that occurs when NADH is oxidized to NAD. The data are expressed as micromoles of ATP released per milligram of protein.

Creatine. The creatine content was measured spectrophotometrically using a protocol described in a commercial kit (Sigma). The reaction is: creatine + α-naphthol + diacetyl →→ colored complex. The absorbance of the supernatant was measured at 520 nm. The creatine content of unknown samples was calculated by comparison with the creatine standard. The data are expressed in micromoles of creatine released per milligram of protein.

Creatinine. Creatinine was measured as per instructions provided with a commercial kit (Sigma). The absorbances of the standard and test samples were read at 500 nm as an initial value. Thereafter, 0.1 ml of acid reagent (a mixture of sulfuric acid and acetic acid) was added and mixed. The mixture was again incubated at room temperature for 5 min. The final value of absorbance was read. The absorbance of the test sample is equal to the initial value minus the final value. The creatinine content of test samples was calculated by comparison with the creatinine standard.

Western Blotting. Brain homogenate (total protein 100 μg) was mixed with an equal volume of 2× electrophoresis sample buffer containing 1,4-dithiothreithol (DTT). The proteins were separated by electrophoresis on a 4%–12% gradient polyacrylamide slab gel (NuPAGE, Novex, CA) and were electrophoretically transferred to a PVDF membrane (Amersham Life Science, Buckinghamshire, UK). The blots were incubated overnight in 3% NET buffer (0.25% gelatin, 50 mM NaCl, 50 mM Tris-HCl, 5 mM EDTA, pH 7.5, and 0.05% Tween-20) and were washed three times in PBST buffer (0.01 M phosphate, 0.09% NaCl, pH 7.5, and 0.05% Tween-20). Membranes were incubated for 2 hr with mouse anti-chicken Na^+-K^+-ATPase α subunit monoclonal antibody in a 1:2000 dilution. The membranes were stripped and reprobed with (1:2000) for mouse anti-human muscle form (MM) CK monoclonal antibody or (1:1000) for rabbit anti-human brain form (BB) CK polyclonal antibody. After washing three times with PBST, immunoreactive proteins were visualized using an enhanced chemiluminescent (ECL) system (Pierce, Rockford, IL) according to the instructions supplied by the manufacturer. The differences in the band intensity of FW and SW were compared using densitometry (Personal Densitometer SI, Molecular Dynamics, Sunnyvale, CA).

Statistics. All data are expressed as the mean ± SEM. Differences among the groups were determined by one-way analysis of variance (ANOVA), and means among them were compared with FW values by the student *t* test. *P* values <0.05 were chosen to be significant. The relationships between CK and Na^+-K^+-ATPase activity were tested by correlation analysis.

Results

Tilapia died 4 hr after transfer from FW to 35 ppt SW, which is in agreement with a previous report on tilapia (17). In the present study, we measured the short-term effects of this transfer on brain Na^+-K^+-ATPase activity, ATP levels, CK activity, and creatine and creatinine contents.

Na^+-K^+-ATPase. Brain Na^+-K^+-ATPase activity in fish transferred to SW (35 ppt) increased within 0.5 hr and then dropped shortly thereafter to a level higher than that in FW. After transfer from FW to 25 ppt SW, brain Na^+-K^+-ATPase activity of fish increased within 1 hr and then remained elevated for the next 24 hr (Fig. 1). Western blotting data showed that brain Na^+-K^+-ATPase in FW was lower than that in SW. On the other hand, transfer to SW (35 ppt)

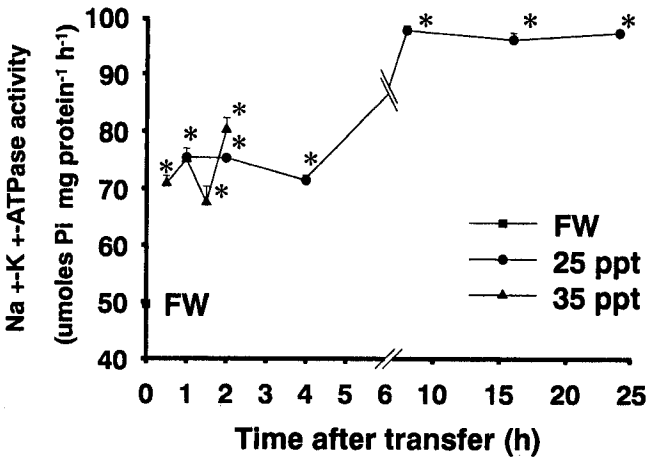

Figure 1. Acute responses of Na^+-K^+-ATPase activity in tilapia brain at various times after transfer from FW to 25 or 35 ppt SW. The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference (P < 0.05) between the FW control and the 25 or 35 ppt SW groups.

caused Na^+-K^+-ATPase to increase within 0.5 hr to a higher level, and then dropped shortly to a level lower than that in FW. In 25 ppt SW, the Na^+-K^+-ATPase expression increased within 1 hr after transfer. Interestingly, brain Na^+-K^+-ATPase reached a higher level within 2 hr after transfer and was then maintained at higher levels for the next 24 hr (Fig. 2, A and B).

CK. Brain CK activity in fish transferred to SW (35 ppt) increased within 0.5 hr and then elevated to a level

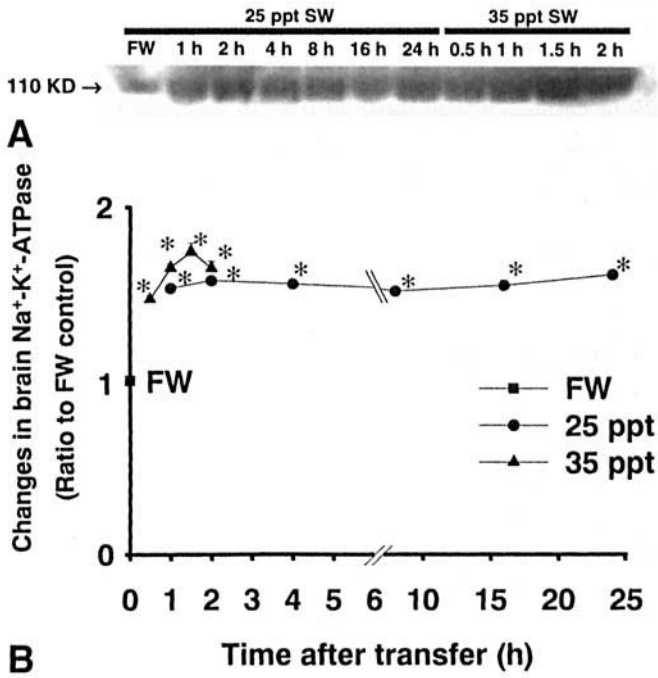

Figure 2. Acute responses of tilapia brain. (A) Western blot of Na^+-K^+-ATPase alpha subunit and (B) changes of Na^+- K^+-ATPase protein (Western blot) in tilapia brain at various times after transfer from FW to 25 or 35 ppt SW. The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference (P < 0.05) between the FW control (the value as 1, intensity is 38.2 ± 8.7) and the 25 or 35 ppt SW groups.

higher than that in FW. Similarly, after transfer from FW to 25 ppt SW, brain CK activity increased 5-fold within 1 hr and remained stable for 8 hr. Its activity declined after 8 hr as it did in FW (Fig. 3). In addition, transfer to SW (35 ppt) caused CK (BB form) content to increase gradually within 1.5 hr to a higher level, and then it dropped to a level lower (at 2 hr) than that in FW. In 25 ppt SW, the CK (BB form) content increased within 1 hr after transfer and was then maintained at higher levels for the next 8 hr. Afterward, it dropped to the same level as in FW at 16 hr and then increased to a higher level at 24 hr (Fig. 4, A and B). No CK (MM form) was detected in brain (data not shown). Correlation analysis indicated that there is no significant relationship between CK activity and Na^+-K^+-ATPase activity (r^2 = 0.28) following transfer from FW to SW.

ATP. The brain ATP level dramatically decreased after transfer to 25 ppt for 2 hr or 35 ppt for 1 hr, respectively. Compared with the FW level, the ATP levels in both the 25 and 35 ppt SW groups were lower than that in the FW group (Fig. 5).

Creatine and Creatinine. Alterations in brain creatine content paralleled changes in CK activity after transfer from FW to SW (Fig. 6). After transfer to 25 ppt SW, the creatine levels were elevated within 1 hr when compared with that of the FW group. Thereafter, the activity declined after 8 hr in both the 25 and 35 ppt SW groups. In contrast, the creatinine content was lower than creatine and declined after the transition compared with the FW group (Fig. 7).

Discussion

Na^+-K^+-ATPase activity in tilapia brain after transfer from FW to SW (25 ppt) was maintained at higher levels for 24 hr compared with those in FW. In addition, brain Na^+-K^+-ATPase activity of the 35 ppt SW group reached a higher level within 1 hr than did that of fish in 25 ppt SW.

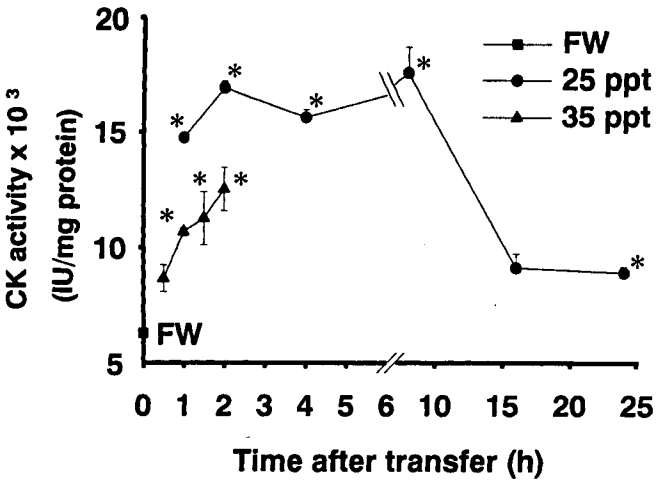

Figure 3. Acute responses of creatine kinase activity in tilapia brain at various times after transfer from FW to 25 or 35 ppt SW. The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference (P < 0.05) between the FW control and the 25 or 35 ppt SW groups.

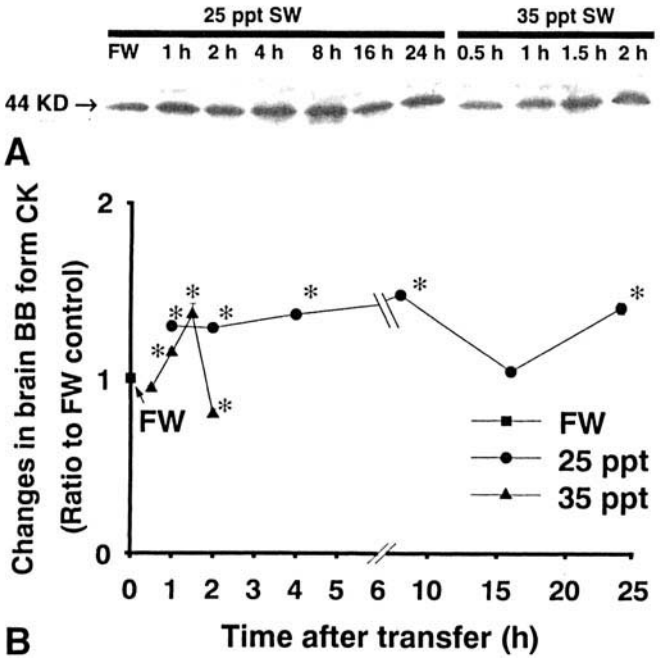

Figure 4. Acute responses of tilapia brain. (A) Western blot of brain-type CK (BB form) and (B) alterations of brain-type CK (BB form, Western blot) in tilapia brain at various times after transfer from FW to 25 or 35 ppt SW. The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference (P < 0.05) between the FW control (the value as 1, intensity is 13.6 ± 2.7) and the 25 or 35 ppt SW groups.

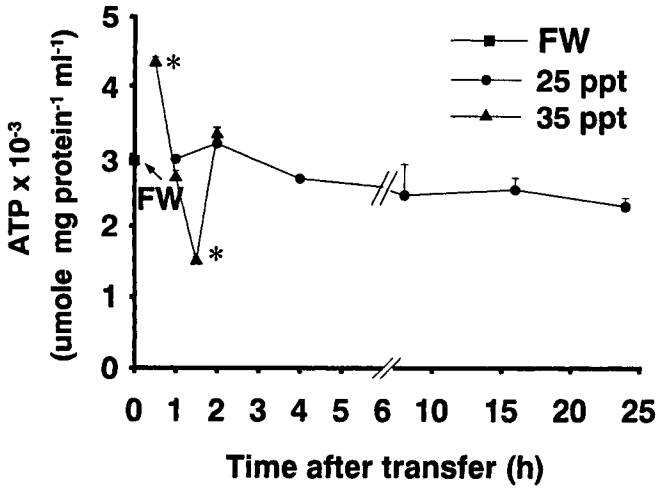

Figure 5. Acute response of tilapia brain ATP at various times after transfer from FW to SW (25 or 35 ppt). The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference (P < 0.05) between the FW control and the 25 or 35 ppt SW groups.

Brain Na^+-K^+-ATPase contents in both the 25 and 35 ppt SW groups were significantly elevated within 1 and 0.5 hr after transfer from FW, respectively. Interestingly, brain CK activities and content (BB form) in both the 25 and 35 ppt SW groups were significantly elevated within 1 hr after transfer from FW. ATP levels in 35 ppt SW increased abruptly within 0.5 hr, but then gradually decreased within 2 hr after transfer. In contrast, the ATP content remained at a constant level for 24 hr after transfer from FW to 25 ppt

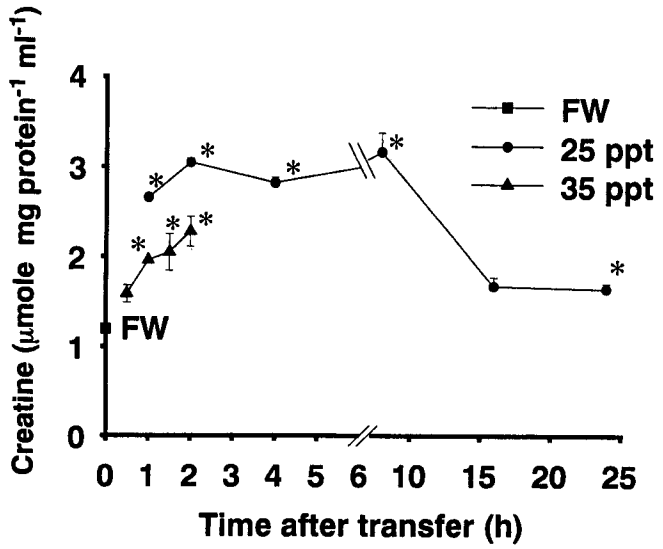

Figure 6. Acute response of tilapia brain creatine at various times after transfer from FW to SW (25 or 35 ppt). The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference ($P < 0.05$) between the FW control and the 25 or 35 ppt SW groups.

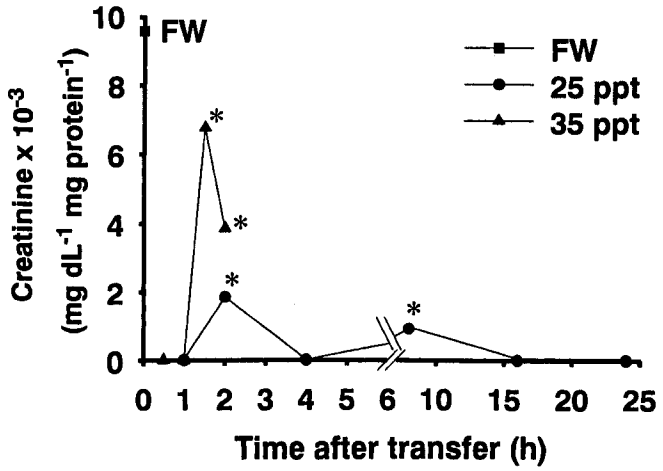

Figure 7. Acute response of tilapia brain creatinine at various times after transfer from FW to SW (25 or 35 ppt). The data are expressed as the mean ± SEM (n = 6, two independent experiments). An asterisk indicates a significant difference ($P < 0.05$) between the FW control and the 25 or 35 ppt SW groups.

SW, but this level was lower compared with the FW group. This lack of correlation suggests that ATP levels did not fall enough to affect Na^+-K^+-ATPase activity. This is tenable because the apparent K_m for ATP of the Na:K pump is far lower than cellular Km ATP content. Therefore, the energy buffering function of CK was not critical to the maintenance of pump activity during exposure to 25 ppt.

In teleosts, alterations of the environment (e.g., water pH, salinity, or heavy metal pollution) cause physiological responses such as changes in hormone concentrations (growth hormone, prolactin, cortisol, etc.); changes in plasma ion levels (e.g., Na^+, and Cl^-, K^+) and osmolarity; glucose and oxygen consumption rates (19); and water drinking activity (20). Fish expend large amounts of energy, particularly in the gill, intestine, kidney, and brain, to compensate for these salinity changes. The energetics of these additive responses to changes in salinity is not yet fully understood. In general, the function of phosphocreatine in excitable cells (particularly muscle and heart) is to provide an acute energy source in both aerobic and anaerobic metabolism. Phosphocreatine/CK is present in some excitable tissues (*Narcine brasiliensis* electric organ) (21) and non-excitable tissues (*Gillichthys mirabilis* gills; *Oreochromis mossambicus* gills; and *Squalus acanthias* rectal gland) (14, 22; Weng CF, *et al.*, unpublished data) with large fluctuating energy demands. Moreover, the normal demands placed upon Na^+-K^+-ATPase during the process of generation of electrical currents require large and rapid changes in activity of CK in the electric organ (22). Recently, it was found that CK provides an energy source for ion transport (Na^+-K^+-ATPase) in *G. mirabilis* gill based on the effects of iodoacetamide (14). This implies that changes in CK activity may be a good indicator of increases in energy conversion after transfer from FW to SW.

Brain CK activity in the 35 ppt SW group was lower than in the 25 ppt SW group. Similarly, the Na^+-K^+-ATPase activity of gills in 35 ppt SW fish was lower than that in the 25 ppt SW group. Therefore, the change in brain CK activity was insufficient to meet the energy demand increase during the critical phase after transfer to 35 ppt SW. This appears to be the case since the ATP levels fell following transfer from FW to either 25 or 35 ppt. Nevertheless, fish can adapt to 25 ppt. This finding is in agreement with previous reports (17, 23). In a previous study (Weng CF, *et al.*, unpublished data), CK activity of tilapia gill changed in concert with alterations in salinity (25 ppt SW), suggesting that initially, adaptation is dependent on increases in CK activity. Another indication of this association is that there was a positive correlation between increases in CK and Na^+-K^+-ATPase activity in tilapia gill. In the current study, increases in brain Na^+-K^+-ATPase reflect the need to increase ion transport rates after salinity increments even though the increases in Na^+-K^+-ATPase and CK activity are not related to one another; increases in CK activity contribute to the energetic requirement for survival in 25 ppt.

Changes in CK activity are needed during the critical phases of brain development, and changes in CK activity occurs during exposure to environmental stress in channel catfish (24). The marine fish, Red seabream (*Pagrus major*) and Pacific mackerel (*Scomber japonicus*) possess less thermostable muscle CK than do carp (*Cyprinus carpio*) (25). We found that increases in tilapia brain CK content (BB form) also occurred following transfer to 25 ppt, which further shows that this transition is a common feature needed for adaptation to an environmental challenge. Recently, three muscle CK isoforms of carp (*C. carpio*) were cloned and characterized (26). They proposed that increases in a specific isoform expression might be linked to acclimation during an increase in environmental temperature. In a preliminary study, two different isoforms of MM-CK in

gills one expressed during SW transfer, which have been cloned and sequenced (Kong, *et al.,*). Our current interest is to determine in tilapia brain whether the BB-CK isoform is specifically upregulated in response to such a transition.

Creatine is converted into creatinine in muscle and kidney. The urine creatinine level is a functional indicator of kidney clearance. The brain creatinine level of fish in FW was higher than that of fish in SW (25 or 35 ppt) in this study. The brain creatine level increased after SW, which is consistent with a higher ATP level at 0.5 hr after transfer to 35 ppt because creatine phosphate concentrations decreased. This association implies a tight coupling of the ATP/ADP reaction to the creatine phosphate/Creatine reaction (27). There is more creatine available in SW adapted fish than the FW counterpart for metabolism to creatinine since more creatine phosphate is dephosphorylated to creatine due to increases in CK activity. Taken together, creatine and creatinine levels in brain as in serum might contribute to meeting the increased energetic cost of increases in ion transport activity that are needed for cellular homeostasis in response to an increase in salinity.

1. Villarreal LG, Ma TS, Kerner SA, Roberts R, Perryman MB. Human creatine kinase: isolation and sequence analysis of cDNA clones for the B subunit, development of subunit specific probes and determination of gene copy number. Biochem Biophys Res Commun **144:**1116–1127, 1987.
2. Perryman MB, Kerner SA, Bohlmeyer TJ, Roberts R. Isolation and sequence analysis of a full-length cDNA for human M creatine kinase. Biochem Biophys Res Commun **140:**981–989, 1986.
3. Coppes ZL, Vecchi SD, Ferreira E, Hirschhorn M. Multilocus isozyme systems in fish. Comp Biochem Physiol **96B:**11–13, 1990.
4. Rottiers DV, Redell LA, Booke HE, Amaral S. Differences in stocks of American shad from the Columbia and Delaware rivers. Trans Am Fish Soc **121:**132–136, 1992.
5. Saudrais C, Garber AT, McKay DJ, Dixon GH, Loir M. Creatine kinase in trout male germ cells: purification, gene expression, and localization in the testis. Mol Reprod Dev **44:**433–442, 1996.
6. Garber AT, Winkfein RJ, Dixon GH. A novel creatine kinase cDNA whose transcript shows enhanced testicular expression. Biochem Biophys Acta **1087:**256–258, 1990.
7. Wells RM, McIntyre RH, Morgan AK, Davie PS. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comp Biochem Physiol **84A:**565–571, 1986.
8. Slenzka K, Appel R, Rahmann H. Brain creatine kinase activity during ontogeny of the cichlid fish *Oreochromis mossambicus* and the clawed toad *Xenopus laevis*, influence of gravity? Neurochem Int **22:**405–411, 1993.
9. Ferguson HW, Rice DA, Lynas JK. Clinical pathology of myodegeneration (pancreas disease) in Atlantic salmon (*Salmo salar*). Vet Rec **119:**297–299, 1986.
10. Rodger HD, Murphy TM, Drinan EM, Rice DA. Acute skeletal myopathy in farmed Atlantic salmon, *Salmo salar.*. Dis Aquatic Organisms **12:**17–23, 1991.
11. Meassager JL, Stephan G, Quentel C, Baudin-Laurencin F. Effects of dietary oxidized fish oil and antioxidant deficiency on histopathology, haematology, tissue and plasma biochemistry, *Dicentrarchus labrax.* Aquatic Living Resources **5:**205–214, 1992.
12. Bucher F. Organ patterns and natural fluctuations of blood enzymes of rainbow trout, *Salmo gairdneri* Rich. Comp Biochem Physiol **96B:**795–800, 1990.
13. Paaver TK. Electrophoretic variability of proteins and genetic features of breed groups and stocks of rainbow trout, *Salmo gairdneri,* reared in the USSR. Voprosy Ikhtiologii **28:**595–603, 1988.
14. Kultz D, Somero GN. Ion transport in gill of the euryhaline fish *Gillichthys mirabilis* is facilitated by a phosphocreatine circuit. Am J Physiol **268:**R1003–R1012, 1995.
15. Saks VA, Lipina NV, Sharov VG, Sminov VN, Chazov EI, Grosse R. The localization of the MM isoenzyme of creatine phosphokinase on the surface membrane of myocardial cells and its functional coupling to ouabain-inhibited Na^+-K^+-ATPase. Biochem Biophys Acta **465:**550–558, 1977
16. Saks VA, Belikova YO, Kuznetsov AV. In vivo regulation of mitochondrial respiration in cardiomyocytes: specfic restriction for intracellular diffusion of ATP. Biochem Biophys Acta **1074:**302–311, 1991.
17. Hwang PP, Sun CM, Wu SM. Changes of plasma osmolality, chloride concentration and gill Na^+-K^+-ATPase activity in tilapia (*Oreochromis mossambicus*) during seawater acclimation. Mar Biol **100:**295–299, 1989.
18. Peterson GL. A simplified method for analysis of inorganic phosphate in the presence of interfering substances. Anal Biochem **84:**164–172, 1978.
19. McCormick SD. In Hoar WS, Randall DJ, Farrell AP, Eds. Fish Physiology: Cellular and Molecular Approaches to Fish Ionic Regulation.. San Francisco: Academic Press, Vol. **14:**285–315, 1995.
20. Lin LY, Weng CF, Hwang PP. Effects of cortisol on water balance in SW transferred tilapia (*Oreochromis mossambicus*) larvae. Physiol Biochem Zool **73:**283–289, 2000
21. Blum H, Balschi JA, Johnson RG Jr. Coupled in vivo activity of creatine phosphokinase and the membrane-bound Na^+-K^+-ATPase in the resting and stimulated electric organ of the electric fish *Narcine brasiliensis.* J Biol Chem **266:**10254–10259, 1991.
22. Friedman DL, Robert R. Purification and localization of brain-type creatine kinase in sodium chloride transporting epithelia of the spiny dogfish, *Squalus acanthias.* J Biol Chem **267:**4270–4276, 1992.
23. Hwang PP. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in the euryhaline teleost *Oreochromis mossambicus.* Mar Biol **94:**643–649, 1987.
24. Ellsaesser CF, Clem LW. Blood serum chemistry measurements of normal and acutely stressed channel catfish. Comp Biochem Physiol **88A:**589–594, 1987.
25. Nakagawa T, Nagayama F. Enzymatic properties of fish muscle creatine kinase. Comp Biochem Physiol **98B:**349–354, 1991.
26. Sun HW, Hui CH, Wu JL. Cloning, characterization, and expression in *Escherichia coli* of three creatine kinase muscle isoenzyme cDNAs from carp striated muscle. J Biol Chem **273:**33774–33780, 1998.
27. Moon TW, Altringham JD, Johnston IA. Energetics and power output of isolated fish fast muscle fibres performing oscillatory work. J Exp Biol **158:**261–273, 1991.