Cell volume regulation in the perfused liver of a freshwater air-breathing catfish *Clarias batrachus* under aniso-osmotic conditions*:* Roles of inorganic ions and taurine

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The roles of various inorganic ions and taurine, an organic osmolyte, in cell volume regulation were investigated in the perfused liver of a freshwater air-breathing catfish *Clarias batrachus* under aniso-osmotic conditions. There was a transient increase and decrease of liver cell volume following hypotonic (–80 mOsmol/l) and hypertonic (+80 mOsmol/l) exposures, respectively, which gradually decreased/increased near to the control level due to release/uptake of water within a period of 25–30 min. Liver volume decrease was accompanied by enhanced efflux of K^+ (9.45 ± 0.54 μmol/g liver) due to activation of Ba^{2+}- and quinidine-sensitive K^+ channel, and to a lesser extent due to enhanced efflux of Cl^- (4.35 ± 0.25 μmol/g liver) and Na^+ (3.68 ± 0.37 μmol/g liver). Conversely, upon hypertonic exposure, there was amiloride- and ouabain-sensitive uptake of K^+ (9.78 ± 0.65 μmol/g liver), and also Cl^- (3.72 ± 0.25 μmol/g liver). The alkalization/acidification of the liver effluents under hypo-/hypertonicity was mainly due to movement of various ions during volume regulatory processes. Taurine, an important organic osmolyte, appears also to play a very important role in hepatocyte cell volume regulation in the walking catfish as evidenced by the fact that hypo- and hyper-osmolarity caused transient efflux (5.68 ± 0.38 μmol/g liver) and uptake (6.38 ± 0.45 μmol/g liver) of taurine, respectively. The taurine efflux was sensitive to 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid (DIDS, an anion channel blocker), but the uptake was insensitive to DIDS, thus indicating that the release and uptake of taurine during volume regulatory processes are unidirectional. Although the liver of walking catfish possesses the RVD and RVI mechanisms, it is to be noted that liver cells remain partly swollen and shrunken during anisotonic exposures, thereby possibly causing various volume-sensitive metabolic changes in the liver as reported earlier.

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1. Introduction

Cell volume regulation, both in invertebrates and vertebrates, is accomplished by activation of transmembrane fluxes of ions and/or organic solutes in the direction necessary to reinstate proper volume. A relatively limited number of solutes seem to serve the function of osmolytes. Such solutes may be inorganic ions like K^+, Na^+, Cl^- and possibly also some organic osmolytes (for reviews, see Goldstein and Perlman 1995; Häussinger 1996). Osmolytes need to be non-perturbing solutes that do not interfere with protein function, even when occurring in high intracellular concentrations (Chamberlin and Strange 1989; Kwon and Handler 1995; Yancey 2005). Such a prerequisite may explain why only a few classes of organic compounds, viz. polyols like inositol and sorbitol, methylamines like betain and α-glycerophos

Keywords. Hypertonicity; hypotonicity; liver mass; regulatory volume decrease; regulatory volume increase; taurine

Abbreviations used: DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulphuric acid; RVD, regulatory volume decrease; RVI, regulatory volume increase.

phorylcholine, and certain amino acids like taurine, have evolved as osmolytes in living cells.

Most cells respond to acute increase in volume by releasing osmotically active cytoplasmic solutes via specific volume-dependent pathways, allowing the cells to undergo a regulatory volume decrease (RVD). The functional properties of these pathways as well as molecular mechanisms by which they are regulated are not yet fully understood. When cells are exposed to solutions of decreased osmolarity, they rapidly swell; this response is followed by a RVD, which occurs despite the continued presence of the hypotonic medium (Kimelberg and Frangakis 1986). The time taken to restore their initial volume varies from some minutes to several hours depending on the cell types (for review, see Häussinger 1996). Conversely, upon sudden exposure to hypertonic medium, the cells behave like more or less perfect osmometer and shrink, but display within minutes a regulatory volume increase (RVI), which brings back cell volume largely but not completely to the initial level. Looking at the enormous importance of osmoregulation in fishes, some reports on this line are already available concerning the cell volume regulatory mechanisms in red blood cells (Fugelli and Thoroed 1986; Motais *et al* 1991; Haynes and Goldstein 1993; Cossins *et al* 1994), renal cells (Kanli and Terros 1997; Kanli and Norderhus 1998), intestinal cells (Lionetto *et al* 2001, 2002, 2005; Trischitta *et al* 2004), and few reports in fish liver cells (Bianchini *et al* 1988; Ballatori and Boyer 1992; Fossat *et al* 1997; Espelt *et al* 2003).

Several varieties of freshwater air-breathing teleosts, such as singhi catfish (*Heteropneustes fossilis*), walking catfish (*Clarias batrachus*), cuchia (*Amphipnous cuchia*), climbing perch (*Anabas* spp.), snake heads (*Channa* spp.) are known to occur in Indian subcontinent, which live in slow flowing, stagnant and polluted water bodies of ponds, lakes and swamps, and are reported to be more resistant against various environmental changes such as high environmental ammonia, hypoxic and desiccation stresses (for review, see Saha and Ratha 1998). Moreover, they frequently encounter with the problems of osmolarity changes during different seasons of the year, especially in the summer when the ponds and lakes dry up, and in the monsoon when the water in the same habitat gets diluted. Furthermore, these fishes are known to bury themselves in the mud to avoid total dehydration arising out of such extreme environmental situations. Thus, looking at their extreme habitat, it would be interesting to elucidate the possible occurrence of volume regulatory mechanisms under anisotonic conditions in some of these Indian air-breathing fishes. The present work examines the role of various ions and taurine in the volume regulatory responses of hepatocytes of one of the mentioned air-breathing catfishes (*Clarias batrachus*) under anisotonic conditions.

2. Materials and methods

2.1 *Animals*

Clarias batrachus, weighing 100 ± 15 g body mass, were purchased from commercial sources, and acclimatized in the laboratory approximately for 1 month at a room temperature of 28 ± 2ºC in plastic aquaria with 12 h:12 h light and dark photoperiod. Minced pork liver and rice bran (5% of body weight) was given as food, and the water (collected from a nearby natural stream) was changed on alternate days. No sex differentiation of fish was carried out during performing the experiments. Food was withdrawn 24 h prior to experiments.

2.2 *Liver perfusion technique*

The fish were anaesthetized in neutralized 3-aminobenzoic acid ethyl ester (MS222, 0.2 g/l) for 5 min before the operation to perform the perfusion of the liver. The liver was perfused via the portal vein in a noncirculating manner with a haemoglobin-free medium following Saha *et al* (1995). The isotonic medium (265 mOsmol/l, determined by the freezing point depression method) contained 119 mM NaCl, 5 mM $NaHCO_3$, 5.4 mM KCl, 0.35 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4$ and 1.25 mM $CaCl_2$ as a basic solution. The medium was gassed with O_2/CO_2 (99:1, v/v) to yield a pH of 7.5 and the liver was perfused at a flow rate of 4 to 5 ml/g liver/min. The temperature of the medium was 30ºC. The hypotonic medium (–80 mOsmol/l) was prepared by removing an equivalent amount of NaCl, and hypertonic medium (+80 mOsmol/l) was prepared by adding NaCl to the standard perfusion medium as mentioned above. While measuring the efflux and uptake of Na^+ and $Cl^–$ under anisotonic conditions, initially a hypotonic medium (–80 mOsmol/l) was prepared by omitting equivalent amount of NaCl from the above mentioned isotonic medium, followed by addition of equivalent amount of mannitol to adjust osmolarity to iso- and hypertonicity so that the Na^+ and $Cl^–$ concentrations remain constant both under iso- and anisotonic conditions.

To study the rate of K^+, Na^+ and $Cl^–$ fluxes in the effluent, livers were initially perfused for 20 min with isotonic medium, followed by infusion of hypo- (–80 mOsmol/l) or hypertonic (+80 mOsmol/l) medium. The K^+ efflux/uptake during anisotonic conditions from the perfused liver was also studied in the presence of various ion transporter blockers such as Ba^{2+} (1 mM), quinidine (1 mM), amiloride (1 mM) and ouabain (1 mM) individually. In experiments with Ba^{2+}, sulphate was omitted from the perfusion fluid.

To study the rate of taurine efflux/uptake under anisotonic conditions, the liver was initially perfused with isotonic

medium for 20 min, followed by infusion of hypo- (–80 mOsmol/l) or hypertonic (+80 mOsmol/l) medium. During hypertonic perfusion condition, extra 0.2 mM taurine was added in the perfusion medium. In another set of experiments, 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid (DIDS, 0.2 mM), an anion channel blocker, was infused in the perfusion medium through a precision pump. Effluents were collected at 1 min interval for the determination of the pattern of taurine efflux/uptake by the perfused liver. For the determination of anisotonicity induced total efflux/uptake of taurine, the total effluents were collected for the period of anisotonic exposures and calculated from the control rate of efflux under isotonic condition.

2.3 *Analysis*

The fluxes of K^+, Na^+ and Cl^- from the perfused liver were continuously monitored with K^+, Na^+ and Cl^--sensitive electrodes connected to an ion analyzer (Orion, USA). The net release or uptakes of these ions from the perfused liver under anisotonic conditions were determined by planimetry of areas under curves and calibrations were done by infusion of known amounts of KCl and NaCl with a precision pump (Lang *et al* 1989). For the measurement of changes in the effluents of pH profile, effluents were collected at 1 min interval and the pH was measured with a pH-sensitive microelectrode (Orion, USA).

The taurine content in the tissue and in the effluent was analysed with a Shimadzu HPLC (Model LC 4A) with a post-column derivatization method using opthaldehyde (OPA) reagent as a fluorescent dye, following the method of Fujiwara *et al* (1987) with certain modifications (Saha *et al* 2000). The separation column used was a strong cation exchanger (Shim-Pack ISC-07 Li). The detector (Shimadzu RF-535 fluorescent detector) was set at an emission of 455 nm and an excitation of 365 nm, and was coupled to a data integrator (Shimadzu CR6A) for quantification of the eluted peak area. The eluting mobile phase was a buffer of 0.16 N Li-citrate containing 7% methyl cellusolve (pH 2.5); the flow rate was 0.4 ml per min, and the column temperature was 40ºC. After the taurine elution was complete, which normally takes place within 3–4 min, the column was re-equilibrated for 20 min with 0.2 N Li-hydroxide before subsequent injections. Hypochlorite reagent for on-line oxidation was prepared by adding 0.4 ml of the commercially available hypochlorite solution to 1000 ml of the buffer solution (pH 10.0) containing sodium carbonate (0.384 M), boric acid (0.216 M) and potassium sulphate (0.108 M). The fluorescent reagent was prepared by adding 2.0 g opthaldehyde (dissolved in 14 ml of ethanol, 4 ml 10% Brij 35 and 2 ml 2-mercaptoethanol in 980 ml of the alkaline buffer).

Liver mass was also monitored in a separate set of experiment by placing the perfused fish liver on a specially constructed balance pan (Sartorius, Germany) (Lang *et al* 1989), which allowed continuous drain of bile and extra-hepatic fluid.

The water content in the perfused liver was determined by oven drying method in a separate set of experiment (Goswami and Saha 1998).

2.4 *Chemicals*

Quinidine, amiloride, ouabain, taurine, DIDS and opthaldehyde were obtained from Sigma Chemical Co., St. Louis, USA. All other chemicals used were of analytical grade and obtained from indigenous sources. Deionized double glass-distilled water was used for all preparations.

2.5 *Statistical analysis*

The data collected from different replicates were statistically analysed and presented as mean ± SEM (n), where n equals the number of animals in the sample. Comparisons of the unpaired mean values between the experimental and respective controls were made using unpaired Student's t-test and differences with $P<0.05$ were regarded as statistically significant.

3. Results

3.1 *Changes of cell volume and water content of the perfused liver following anisotonic exposures*

In a first series of experiments, the effects of anisotonic exposures on liver cell volume were investigated. Exposure of walking catfish liver to hypotonic medium (–80 mOsmol/l) evoked a rapid swelling of liver cells as evidenced from the increase of liver mass by 60 ± 5.5% within 5 min, followed by a gradual decrease of liver mass reaching a plateau almost after 25 min, but maintained a significant ($P<0.05$) increase of liver mass by 12.6 ± 2.2% compared to isotonic control value as long as the liver was maintained at hypotonic perfusion medium. A representative tracing is depicted in figure 1A.

Hypertonic perfusion condition (+80 mOsmol/l) elicited a rapid decrease in liver cell volume as evidenced from the decrease of liver mass by 52 ± 5% compared to the control mass within 8 min, with a subsequent increase of liver mass, which reached a plateau almost after 30 min. Again a significant ($P<0.05$) decrease in liver mass by 15.5 ± 2.5% compared to isotonic control value was maintained at hypertonic perfusion medium. Figure 1B reports the tracing of a typical experiment.

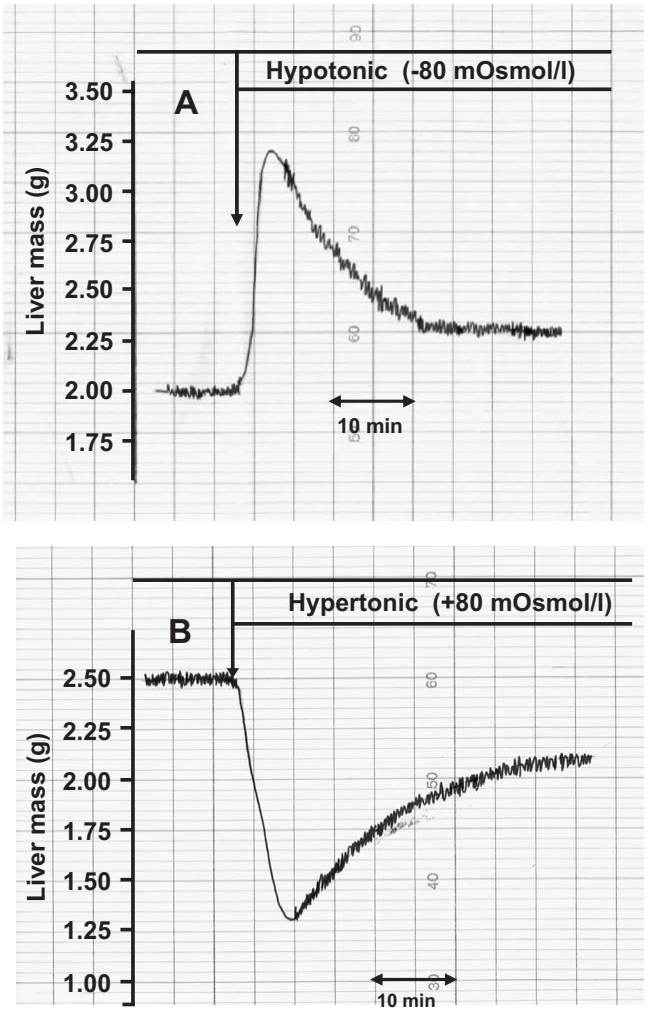

Figure 1. Effects of hypotonicity **(A)** and hypertonicity **(B)** on liver mass of *C. batrachus*. Representative tracings from a series of 3 similar experiments are shown.

Furthermore, exposure of walking catfish liver to hypo- and hypertonic media resulted in significant (P<0.05) increase and decrease of water content by 12.1 ± 1.5% and 14.2 ± 1.7% , respectively.

3.2 *Efflux/uptake of K^+ from the perfused liver following anisotonic exposures*

As depicted in figure 2A, the perfusion of walking catfish liver with hypotonic medium (–80 mOsmol/l), after initial perfusion with isotonic medium, led to a transient increase of K^+ efflux, which lasted for a period of 12–15 min, thereafter returning back to the basal level. The extra efflux of K^+ during this volume regulatory process was calculated to be 9.45 ± 0.54 μmol/g liver (table 1). The K^+ efflux due to hypotonically-induced cell swelling was, however, partly blocked by the presence of Ba^{2+} (1 mM; figure 2B) and maximally by quinidine (1 mM; figure 2C); these inhibitors resulting to a K^+ efflux of only 3.32 ± 0.25 and 1.28 ± 0.09 μmol/g liver, respectively. In comparison, the K^+ efflux due to hypotonically-induced cell swelling was not affected by the presence of amiloride (1 mM) and ouabain (1 mM) (table 1, graphs not shown).

In contrast, perfusion of liver with hypertonic medium (+80 mOsmol/l), after initial perfusion with isotonic medium, resulted in a transient uptake of K^+, which lasted for a period of 15–20 min, thereafter returning back to the basal level (figure 3A). The hypertonically-induced K^+ uptake by the perfused liver was calculated to be 9.78 ± 0.65 μmol/g liver, which remained largely unaffected by the presence of Ba^{2+} (1 mM) and quinidine (1 mM) (table 1). However, amiloride (1 mM) and ouabain (1 mM) drastically inhibited the hypertonically-induced K^+ uptake to a level of only 1.21 ± 0.08 and 1.54 ± 0.10 μmol/g liver, respectively (figure 3B, C; table 1).

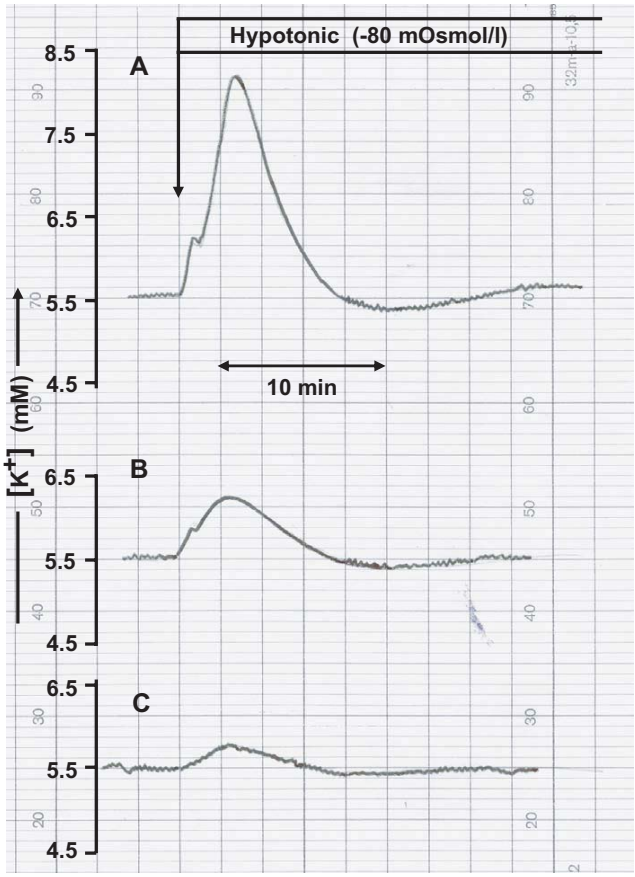

Figure 2. Effect of hypotonicity on K^+ efflux by the perfused liver of *C. batrachus* in the absence **(A)**, and presence of Ba^{2+} (1 mM) **(B)** and quinidine (1 mM) **(C)**. Representative tracings from a series of 3 similar experiments are shown.

Table 1. Extra efflux/uptake of K^+, Na^+, Cl^- and taurine (μmol/g liver) in the presence/absence of different inhibitors from the perfused liver of *C. batrachus* following anisotonic exposures.

Conditions	K^+	Na^+	Cl^-	Taurine
Hypotonic (–80 mOsmol/l)	+ 9.45 ± 0.54	+ 3.68 ± 0.37	+ 4.35 ± 0.25	+ 5.68 ± 0.38
Hypotonic + Ba^{2+} (1 mM)	+ 3.32 ± 0.25	–	–	–
Hypotonic + Quinidine (1 mM)	+ 1.28 ± 0.09	–	–	–
Hypotonic + Amiloride (1 mM)	+ 8.94 ± 0.38	–	–	–
Hypotonic + Ouabain (1 mM)	+ 8.19 ± 0.42	–	–	–
Hypotonic + DIDS (0.2 mM)	–	–	–	+ 0.41 ± 0.05
Hypertonic (+80 mOsmol/l)	– 9.78 ± 0.65	–3.72 ± 0.25	–4.17 ± 0.47	–6.38 ± 0.45
Hypertonic + Ba^{2+} (1 mM)	– 9.02 ± 0.32	–	–	–
Hypertonic + Quinidine (1 mM)	– 8.98 ± 0.28	–	–	–
Hypertonic + Amiloride (1 mM)	– 1.21 ± 0.08	–	–	–
Hypertonic + Ouabain (1 mM)	– 1.54 ± 0.10	–	–	–
Hypertonic + DIDS (0.2 mM)	–	–	–	– 5.74 ± 0.35

–, Not determined
Values are expressed as mean ± SEM (n=3).

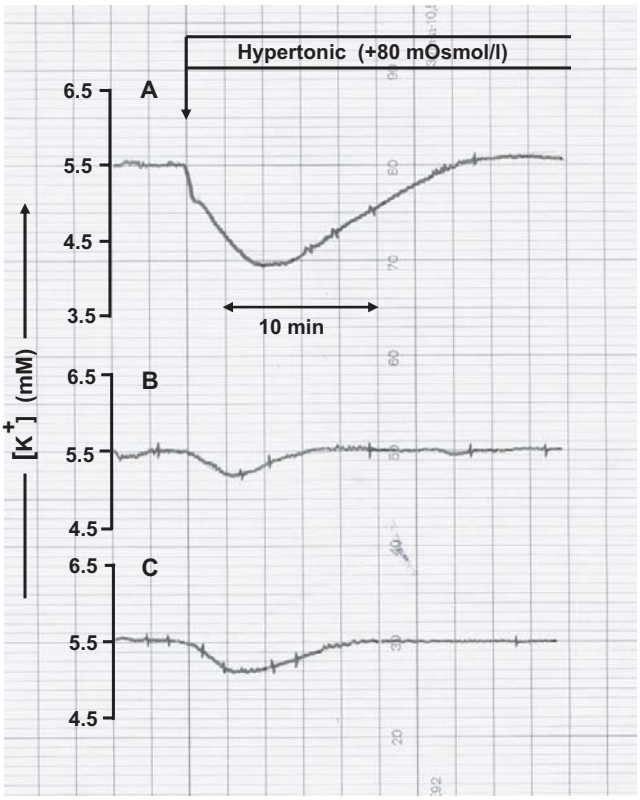

Figure 3. Effect of hypertonicity on K^+ uptake by the perfused liver of *C. batrachus* in the absence **(A)**, and in the presence of amiloride (1 mM) **(B)** and ouabain (1 mM) **(C)**. Representative tracings from a series of 3 similar experiments are shown.

3.3 *Efflux/uptake of Na^+ and Cl^- from the perfused liver following anisotonic exposures*

The perfusion of walking catfish liver with hypotonic medium (–80 mOsmol/l), after initial perfusion with isotonic medium, led to a transient increase of Na^+ and Cl^- efflux, which lasted for a period of 2–3 min, thereafter returning back to the basal levels (figures 4A and 5A). The hypotonically-induced Na^+ and Cl^- effluxes were calculated to be 3.68 ± 0.37 and 4.35 ± 0.25 μmol/g liver, respectively (table 1). Conversely, upon exposure to hypertonic perfusion condition, a transient uptake of Na^+ and Cl^- by the perfused liver was induced, which lasted again for a period of 2–3 min (figures 4B and 5B), thus leading to a total uptake of Na^+ and Cl^- of 3.72 ± 0.25 and 4.17 ± 0.47 μmol/g liver, respectively.

3.4 *Changes of pH profile in the effluent following perfusion of liver with anisotonic media*

As shown in figure 6A, the exposure of walking catfish liver to hypotonic (–80 mOsmol/l) medium, following an initial perfusion with isotonic medium, resulted in an increase of pH of the effluent by 0.2 pH unit, which later diminished to an increase of about 0.15 pH unit throughout the period of hypotonic exposure. In contrast, hypertonic (+80 mOsmol/l) exposure of liver resulted in an initial decrease of pH by about 0.2 pH unit, which later restricted to a decrease of 0.17 pH unit throughout the period of hypertonic exposure

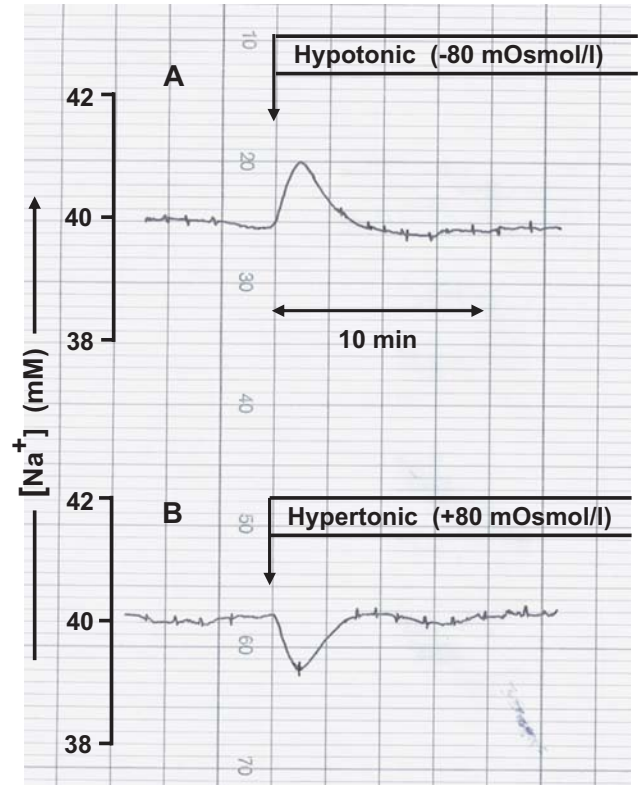

Figure 4. Effects of hypotonicity **(A)** and hypertonicity **(B)** on Na^+ efflux/uptake by the perfused liver of *C. batrachus*. Representative tracings from a series of 3 similar experiments are shown.

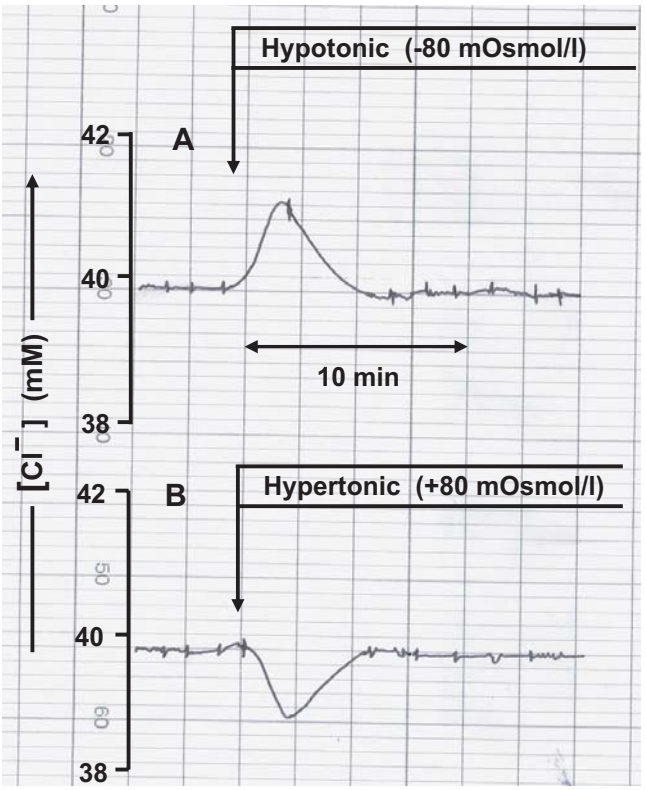

Figure 5. Effects of hypotonicity **(A)** and hypertonicity **(B)** on Cl^- efflux/uptake by the perfused liver of *C. batrachus*. Representative tracings from a series of 3 similar experiments are shown.

(figure 6B). Thus, hypo- and hypertonic exposures resulted in alkalization and acidification of effluents, respectively, which were coming out of the perfused liver.

3.5. *Efflux/uptake of taurine from the perfused liver following anisotonic exposures*

As shown in figure 7A, the perfusion of liver with hypotonic medium (–80 mOsmol/l), following initial perfusion of liver with isotonic medium, caused a transient increase of taurine efflux from the perfused liver for a period of 10–15 min. The total efflux of taurine during hypotonic exposure was calculated to be 5.68 ± 0.38 μmol/g liver (table 1). However, the hypotonically-induced taurine efflux was inhibited almost completely by the presence of DIDS (0.2 mM), which is an anion exchanger blocker, thus restricting the taurine efflux of only 0.41 ± 0.05 μmol/g liver (table 1).

In contrast, when the liver was perfused with hypertonic medium (+80 mOsmol/l) in the presence of taurine (0.2 mM), there was a continuous uptake of taurine, which lasted for about 30–40 min (figure 7B). This uptake of taurine was calculated to be 6.38 ± 0.45 μmol/g liver (table 1). However, the hypertonically-induced uptake of taurine could not be inhibited by the presence of DIDS (0.2 mM) (figure 7B; table 1).

4. Discussion

Cell-volume regulation is critical for aquatic species in dealing with variations in the external environment as well as with changes in the composition of extracellular body fluids. An important aspect of these volume regulation mechanisms appears to be to control the net transport of the intracellular solutes across the cell membrane (Fugelli and Rohrs 1980). The present study demonstrates that the perfused liver of walking catfish (*C. batrachus*) spontaneously undergoes RVD or RVI following exposure to continued hypo- or hypertonic stress, respectively. Hypotonic exposure (–80 mOsmol/l), by lowering the osmolarity of the perfusion medium, caused a transient increase of liver mass, followed by gradual decrease of liver mass within a period of 20–25 min (figure 1A). This was accompanied by the transient release of K^+, Na^+ and Cl^- from the perfused liver (figures 2, 4, 5; table 1) almost parallel to the time required for restoration of liver cell volume near to the basal level

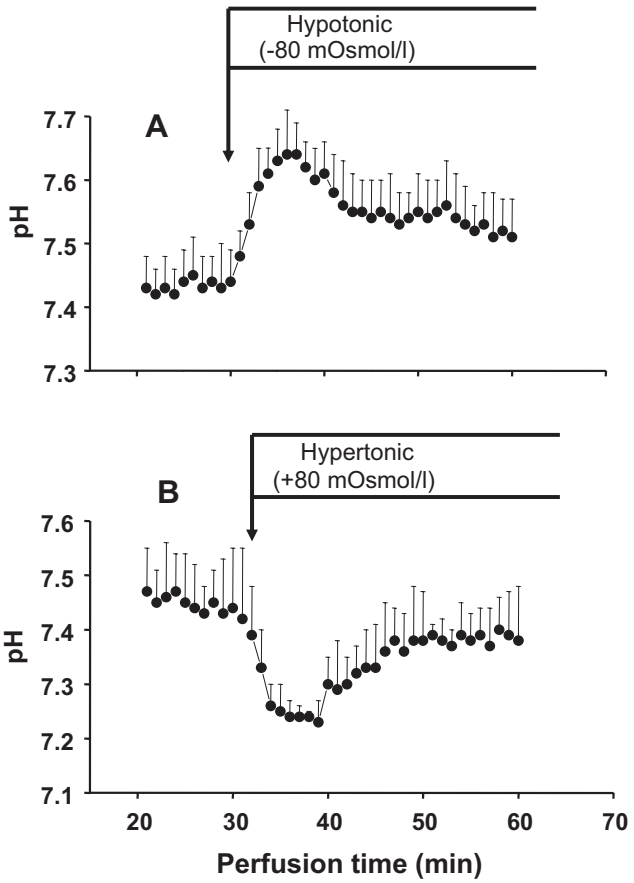

Figure 6. Changes of pH profile in the effluent following perfusion of *C. batrachus* liver with hypotonic **(A)** and hypertonic **(B)** media. Values are plotted as mean ± SEM (n = 3).

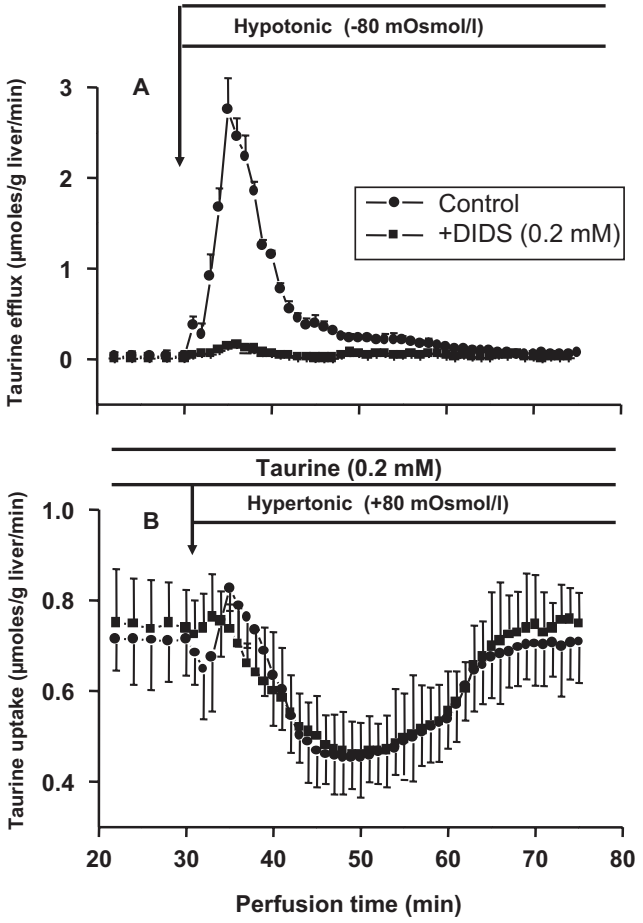

Figure 7. Effects of hypotonicity **(A)** and hypertonicity **(B)** on taurine efflux/uptake by the perfused liver of *C. batrachus*. Values are plotted as mean ± SEM (n = 3).

by releasing excess of water. Marked blunting of K^+ release by the perfused liver during hypotonic perfusion condi-tion was achieved in the presence of Ba^{2+} and more blunting in the presence of quinidine (figure 2; table 1). In contrast, amiloride and ouabain had no effect on K^+ release (table 1). Thus, hypotonicity appears to activate the quinidine- and Ba^{2+}-sensitive K^+ channel as a means of RVD as noticed in other cell types (Larson and Spring 1984; Welling *et al* 1985; Haddad and Graf 1989), and more recently in the isolated intestinal cells of a euryhaline teleost (*Gobius niger*) (Trischitta *et al* 2004). Another possibility of loss of K^+ and Cl^- from the liver cell following hypotonicity could be by activation of K^+-Cl^- co-transporter similar to the observations made in trout hepatocytes (Bianchini *et al* 1988) and the erythrocytes of many species (Häussinger and Lang 1991). However, it needs to be confirmed by using a specific inhibitor for K^+-Cl^- co-transporter.

Conversely, upon hypertonic exposure, there was transient decrease of liver mass (figure 1B), which was accomplished by the transient uptake of K^+, Cl^- and Na^+ ions across the cell membrane as a means of RVI (figures 3–5; table 1). The striking transient uptake of K^+, however, was predominantly inhibited by amiloride and ouabain with no affect of Ba^{2+} and quinidine on K^+ efflux by the perfused liver. This was accompanied with acidification of effluents, which was coming out from the perfused liver. All these observations are clearly indicative of possible involvement of Na^+/H^+ antiporter and Na^+-K^+-$2Cl^-$ symporter systems, and possibly also the Na^+ conductance system in the RVI process in this fish hepatocytes during at least high increase of extra-cellular osmolarity (+80 mOsmol/l). In rat hepatocytes, RVI is mainly achieved by the activation of only Na^+/H^+ antiporter system during a small increase of extra-cellular osmolarity, but during high increase of extra-cellular osmolarity, all the three transporter systems were reported to get activated (Wehner and Tinel 2000). However, it is difficult to say at this moment whether the same strategies have been evolved in our fish, since the uptake of various ions was studied only during high increase of extra-cellular osmolarity.

Volume regulation is often associated with changes in intracellular pH (Gleeson *et al* 1990), as various ion transports, involved in volume regulation, are also of importance for the control of pH. Our observations indicated that following hypotonocity, the pH profile in the effluent showed an increase (figure 6A), causing alkalinization of the effluent and possibly also acidification of intracellular fluid, similar to earlier reports in trout hepatocytes (Krumschnabel *et al* 2003). Part of this could be due to release of bicarbonate parallel to K^+ release as has been suggested in rat liver (Häussinger *et al* 1990), as well as could be due to decreased pyruvate and lactate release as a result of inhibition of glycolytic flux (Goswami and Saha 1998).

On the other hand, hypertonicity resulted in decreased pH of the effluent (figure 6B), thus causing intracellular alkalinization possibly by the activation of coupled Na^+/H^+ and Cl^-/HCO_3^- co-transporters. Such mechanisms have been reported in urodele (*Amphiuma*) erythrocytes (Cala 1985), dog erythrocytes (Parker 1983), human lymphocytes (Grinstein *et al* 1984), rat hepatocytes (Graf *et al* 1988; Corasanti *et al* 1990), Ehrlich ascites tumour cells (Pederson *et al* 1996), and trout hepatocytes (Krumschnabel *et al* 2003). As already stated, uptake of K^+ ions was inhibited by amiloride and ouabain following hypertonicity with possible involvement of Na^+/H^+ antiporter, thus suggesting that Na^+/H^+ exchange is the main mechanism involved in intracellular alkalinization under hypertonicty, along with increased efflux of lactic acid (Goswami and Saha 1998).

In addition to inorganic ions, ability to use amino acids such as taurine in volume regulation has been demonstrated in many species, both invertebrates and vertebrates (for review, see Perlman and Goldstein 1999). In walking catfish, the hepatic taurine concentration was recorded to be 15.61 μmol/g wet wt. and 9.80 μmol/g wet wt., respectively, in the fresh liver (Saha *et al* 2002), and in the liver after perfusion with isotonic medium for 30 min (Saha *et al* 2000), thus constituting about 35–40% of total free amino acids. Such a high physiological hepatic taurine concentration as reported earlier, and our present investigation on taurine efflux/uptake by the perfused liver during anisotonicity suggest that taurine plays an important role in cell volume homeostasis in walking catfish. Hypotonic cell swelling caused prompt transient release of taurine (5.68 μmol/g liver) from the perfused liver within a period of 10–15 min, and hypertonic cell shrinkage caused a transient uptake of taurine (6.38 μmol/g liver) from the extra-cellular fluid within a period of 30–40 min (figure 7; table 1). The role of taurine in cell volume regulation has been greatly emphasized in skate (*Raja erinacea*) (Ballatori and Boyer 1992) and trout (*Oncorhynchus mykiss*) hepatocytes (Michel *et al* 1994), and it has been suggested that taurine efflux plays more significant role in cell volume regulation rather than K^+ during hypotonic cell swelling. However, in the walking catfish liver, in addition to taurine, various ions also appear to play equal roles in cell volume regulation. The taurine efflux by the perfused liver of walking catfish due to hypotonicity was found to be sensitive to DIDS, an anion channel blocker (figure 7A), however, the taurine uptake due to hypertonicity was unaffected by the presence of DIDS (figure 7B). In contrast, in skate erythrocytes, both the efflux and uptake of taurine, due to hypo- and hypertonicity, respectively, were reported to be sensitive to DIDS (Goldstein and Brill 1991). Perlman and Goldstein (1999) suggested that the flux of taurine under anisotonic conditions could be bidirectional and takes place through Na^+-independent process. However, in the walking catfish liver, both the efflux and uptake of taurine during hypo- and hypertonicity, respectively, appear to be unidirectional, i.e., the efflux presumably via the activation of a specific volume regulated anion channel (band 3 protein) (Goldstein and Brill 1991), and the uptake presumably via the activation of a specific taurine transporter, similar to the observation made in rat hepatocytes (Warskulat *et al* 1997). A detailed investigation, however, is needed to be carried out to clarify further the real mechanism(s) of transport of taurine and the mechanism(s) of regulation of transporters under anisotonic conditions in this walking catfish.

In conclusion, it appears that via the involvement of inorganic ions and organic osmolytes, the walking catfish liver possesses various volume regulatory mechanisms. These help it to adapt under anisotonic conditions, and possibly also under other extreme conditions. This is in contrast to the situation in goldfish, which do not possess the proper volume regulatory mechanisms, and try to maintain intra-cellular ionic homeostasis under various extreme environmental conditions including that of anisotonic and hypoxic/anoxic conditions (Espelt *et al* 2003). Although the hepatic cells of the walking catfish possess volume regulatory mechanisms, cells remain in a partly swollen or shrunken state as long as they are exposed to anisotonic conditions. As reported earlier, this causes significant metabolic changes related to carbohydrate metabolism and oxidative stress under anisotonicity (Goswami and Saha 1998; Goswami *et al* 2004; Saha and Goswami 2004).

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