Change in activity of Ornithine-Urea cycle enzymes in *Channa punctatus* under hyperammonia stress

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Abstract: In the present study, the apparent role of ureogenesis in preventing the accumulation of ammonia to a lethal level during hyperammonia stress in the environment was studied in the Indian freshwater air-breathing teleost, *Channa punctatus*, by exposing the fish to different ammonium chloride concentrations experimentally. On assaying two significant urea cycle enzymes, arginosuccinate lyase and arginase, it was observed that these two enzymes were significantly active in the synthesis of urea under hyperammonia stress. So, it was reasonable to predict that *Channa punctatus*, which is otherwise predominantly ammoniotelic, turned totally towards ureotelism to survive under these conditions by converting the high ammonia load into relatively less toxic urea. Thus, due to this unique physiological strategy of turning towards ureotelism from ammoniotelism via the induced urea cycle, this air-breathing teleost is able to survive in very high ambient ammonia conditions.

Keywords: *Ammoniotelism; ammonium chloride; hyper-ammonia stress; urea cycle; ureogenesis.*

Introduction: Nitrogen metabolism is considered as one of the most sensitive physiological systems showing adaptive changes in response to environmental variations. Accordingly, the nature of nitrogen excretory products in animals has changed with the evolution of vertebrates from water to the land habitat [1,2]. The major metabolic end-product produced during the breakdown of nitrogen-containing biomolecules found in various animal tissues is ammonia. The vast majority of the teleost fishes are ammoniotelic, excreting ammonia as the major nitrogenous end product in response to their aquatic habitat. However, under special circumstances, such as high ambient ammonia or aerial exposure, fish can hardly excrete ammonia and the toxic ammonia is concentrated in the blood and body tissues. In general, however, aquatic animals can tolerate more elevated levels of blood ammonia than terrestrial animals. Plasma total ammonia (NH_3+NH_4^+) normally remains between 0.05 and 2 mmol l^{-1} in most teleosts. However, blood ammonia levels greater than 0.05 mM can be toxic to the central nervous system of most mammals [3]. Ammonia is excreted directly if feasible or converted to some less toxic compounds such as urea, uric acid or amino acids in different animals.

Though majority of the teleosts are ammoniotelic, urea also constitutes 10-30% of the total nitrogenous wastes in most of them. The sources of urea and the presence of the ornithine-urea cycle in teleost are still under debate. However, the presence of a functional urea cycle which appears to be the major source of urea formation in higher vertebrates, has been reported in various teleosts such as *Oreochromis albalicus graham*, marine toadfishes *Opsanus tau* etc [4]. Air-breathing teleosts of the Indian subcontinent are unique among

freshwater teleosts in having a very active urea cycle, the capacity to switch from ammoniotelism to ureotelism under hyperammonia stress and during exposure to air, they have the capacity to tolerate very high ambient ammonia [5,6,7].

The Indian freshwater air-breathing teleost used in the present study is *Channa punctatus*, a facultative air breather usually inhabiting stagnant, slow flowing swampy water bodies or wetlands which are usually uninhabitable. When these swamps are covered with macro vegetation like water hyacinth it suffers from low dissolved oxygen and a pH range of 6.5-7.8 with more free carbon dioxide and high ammonia levels as a degradable product of micro and macro vegetation. During summer, when the swamps dry up, fish face more adverse ecological conditions and may burrow inside mud to avoid total dehydration.
The present study has been undertaken to find out the changes in the pattern of enzymatic activity of two of the five enzymes of the ornithine-urea cycle--- arginosuccinate lyase (ASL) used in the penultimate step catalysing the conversion of arginosucinate to arginine and fumarate; and arginase (ARG), the final enzyme converting arginine to ornithine and urea, under hyper-ammonia stress especially in the liver (most ureogenic tissue) and kidney of the much unexplored facultative air-breather *Channa punctatus*.

Materials and Methods

Animals *Channa punctatus* weighing (75±10)gm approximately irrespective of the sex were purchased from Maniktala market, Kolkata for the experiment. *Tubifex* was fed to the fishes and were kept in the laboratory tap water, which was changed regularly.
Experimental Protocol The experiment was conducted in 2 phases:
In the first phase 12 fishes of similar sizes(approximately 18 cm) were distributed equally in 3 aquarium trays containing 2 litres of 25mM, 50mM and 75mM NH_4Cl solution for about 30 days and another aquarium containing water as control. In the second phase 5 fishes were kept in 2 aquarium trays, 4 fishes in 75 mM NH_4Cl solution and 1 fish in water as control. The control fish was sacrificed on 0 day of exposure while the remaining 4 fishes were kept in 75 mM NH_4Cl solution and sacrificed only when they died due to ammonia toxicity.
Both the NH_4Cl solution and water was changed with a fresh medium everyday at a fixed time. The fishes were removed when they died, their lengths were measured and the liver and kidney tissues were dissected out, blotted dry and weighed.

Enzyme Assay 10% homogenate (w/v) of liver and kidney tissues of *C. punctatus* were prepared separately in 0.1% cetyltrimethyl ammonium bromide (CTB) for the assay of activities of ornithine-urea cycle enzymes, ASL and ARG. The homogenates were centrifuged at 600 r.p.m at -2˚C for 15 minutes. The supernatants were kept at -20 ˚C and later thawed for use in enzyme assay.

Chemicals All the chemicals including substrates (L-Arginine-CAS No.74-79-3; Arginino succinic acid-disodium salt hydrate- CAS No. A5707), CTAB (CAS No.57-09-0) were obtained from Sigma Chemical Co. and other chemicals were of analytical grades obtained from indigenous sources.

Results

Fig 1a-1d: Changes in activity of ASL and ARG in the liver and kidney of *C. punctatus* when exposed to different concentrations of NH_4Cl solution

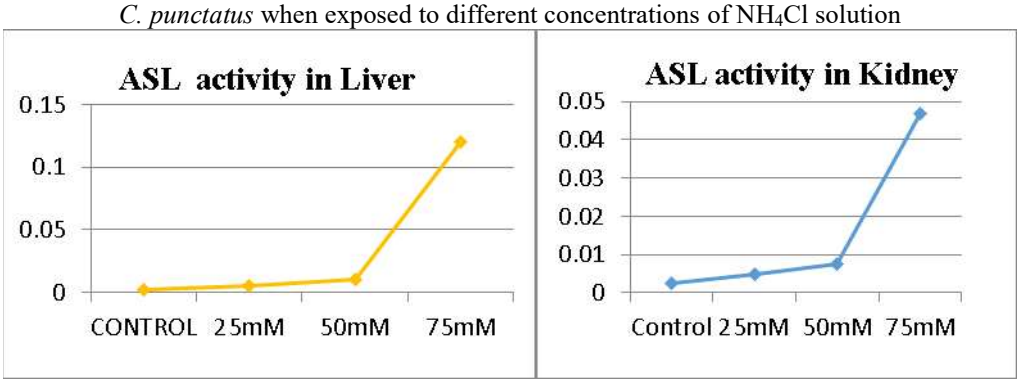

Fig 1a

Fig 1b

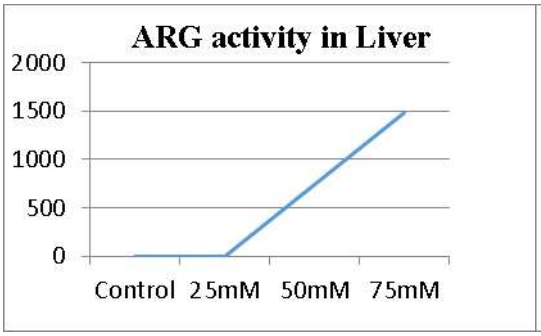

Fig 1c

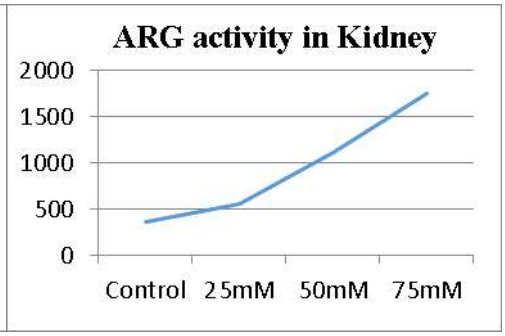

Fig 1d

Fig 2a-2d: Changes in activity of ASL and ARG in the liver and kidney of *C. punctatus* when exposed to 75mM NH_4Cl solution for 10 days.

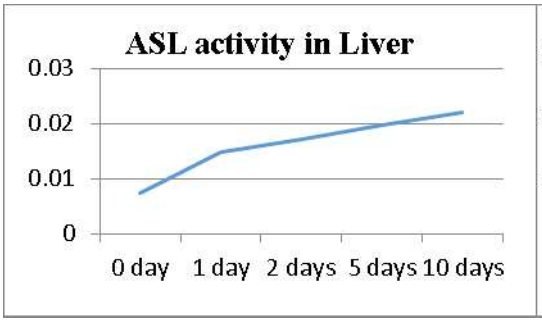

Fig 2a

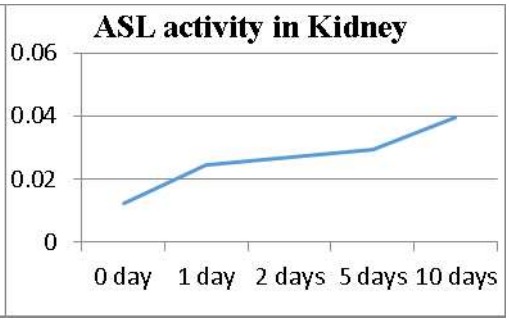

Fig 2b

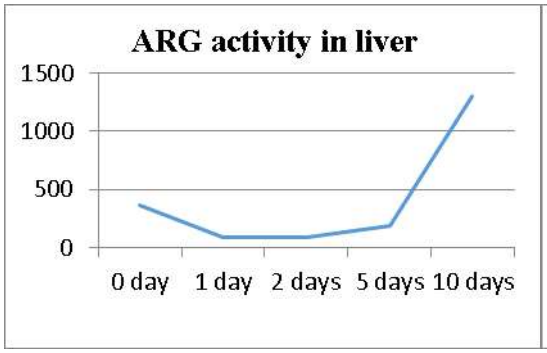

Fig 2c

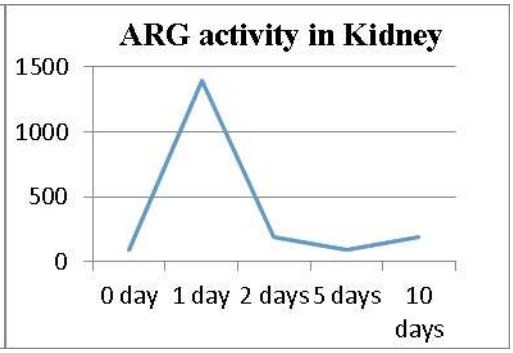

Fig 2d

Discussion and concluding remarks

The given study focuses on the adaptations that *C. punctatus* acquire with the aim of tolerating environmental constraints, particularly hyperammonia stress. One of the most attractive result obtained in this study is a sudden alteration in the activities of the urea cycle enzymes which is normally functional in the fish but at significantly lower levels. The activities of the two enzymes ASL and ARG were found to be considerably higher in the fishes exposed to hyperammonia stress, suggesting that a transition from ammoniotelism to ureotelism takes place to avoid any build up of ammonia in the body tissues of the fish to a toxic level. Most of the Indian air-breathing teleosts appear to have retained the genes for the urea cycle enzymes, since a full complement of urea cycle enzymes have been reported for many of them (Saha *et.al*. 1998).

For the fishes exposed to different concentrations of NH_4Cl (Table 1), the activity of ASL is found to increase with the concentration of NH_4Cl (Fig 1a, 1b). This is particularly due to the fact that as the concentration of NH_4Cl increases, a greater accumulation of ammonia takes place in the tissues which needs to be detoxified, triggering the need for ureotelism and consequent activation of the o-u cycle enzymes. However, in case of ARG, the change in the activity of the enzyme is quite unusual in both liver and kidney of *C. Punctatus* (Fig 1c, 1d). When assessing the enzymatic activity of the fishes exposed to 75mM NH_4Cl, (Table 2) it has been observed that as the number of days of exposure increases, ASL activity also increases proportionately in liver and to some extent in kidney (Fig 2a, 2b). However, the ARG activity shows alterations with increase in days of exposure to 75mM NH_4Cl, in both the tissues of the fish (Fig 2c, 2d). Overall, the ARG activity is found to be higher in the kidney than in the liver.

The accumulation of ammonia in different body tissues is accompanied with a significant stimulation of the activity of certain key enzymes of the urea cycle, such as ASL and ARG in both hepatic and extra-hepatic tissues of *C. punctatus*. The increase in activity of ASL with increase in both concentration of NH_4Cl exposure and days of exposure suggests that this increase might be a chronic adaptation for ammonia detoxification by elevating the rate of urea-nitrogen excretion via the induced urea cycle for the long-term maintenance of nitrogen waste excretion in the form of less toxic urea during exposure to high ambient ammonia (Saha *et.al.* 1998). However, it was observed that the activity of ARG did not increase proportionately with increase in concentration of NH_4Cl exposure or number of days of exposure. The conversion of some part of accumulated ammonia to various non-essential amino acids under high ammonia load (Saha *et. al*. 1999) may be one of the reasons for such anomaly. Initially at lower ammonia load, it is the induced urea cycle which is mainly responsible for the detoxification of ammonia while at a higher ammonia load, other detoxification pathways are also highly involved which may affect the activities of the o-u cycle enzymes. The conversion of accumulated ammonia to various non-essential free amino acids (FAA) is one such alternative pathway that has been reported in the mudskipper, *Periophthalmus cantonensis* (Iwata, 1988). Secondly, the accumulation of ammonia in the liver is a saturable process i.e. at higher rate of ammonia addition, the percentage uptake of

ammonia gradually decreases, which suggests that this low accumulation of ammonia may not serve as a strong modulator to induce the urea cycle. In addition, the physiological level of activity of ARG is quite high, and thus even the highest ammonia load in the tissues did not have any stimulatory effect on this enzyme of the urea cycle for enhanced ureogenesis. Ammonia also interferes with carbohydrate and fat metabolism and ATP levels, both in cerebral and extra cerebral tissues (Wiechetek *et.al.*1979). All these toxic effect of ammonia (both NH_3 and NH_4^-) may lead to convulsion, coma and eventually death.

Thus it appears that *C. punctatus* is capable of stimulating ureogenesis, by inducing the already existing functional urea cycle both in hepatic as well as some non-hepatic tissues, thus turning from ammoniotelism to ureotelism as one of the major physiological strategies to avoid the accumulation of toxic ammonia to a lethal level during exposure to higher ambient ammonia.

Acknowledgement

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