About the Book

Toxicology is a discipline involved adverse effects of chemical susbstances on living organisms, dose of chemical substances and its effect on exposed organisms. With rapid growth in industrialization, urbanization, pesticides usage in irrigation resulted in environmental pollution (air , water, soil pollution).

Due to this, mainly water bodies get contaminated as they receives the effluents from industries, domestic waste, agricultural runoff which directly effects the aquatic organisms. The material in this book brings together all the information on comparative, seasonal estimation of heavy metals and its effects on the aquatic organisms, toxicological studies of detergents on cat fish, ammonia toxicity, effect of medicinal plants against dengue fever vector, natural insecticides influences of organic waste on metabolic activity of aquatic organisms and bioremedial studies.

We are thankful to all the authors and other contributors for giving their valuable time and data. They are responsible for the work presented in the paper and if any plagiarism question arises they are answerable. The 1-author or corresponded author has to take responsibility. The editiors are not responsible for above issues. We are thankful to ISCA, Kolkata, ISCA President, General Secretaries , ISCA Executive Committee Members, and office bearers of the Indian Science Congress held at Manipur University, Imphal, Manipur from March 16 to 20, 2018 for encouraging us to hold symposium with focal themes, "**REACHING THE UNRECAHED**". We extend our sincere thanks to all contributors who have contributed their articles for publications in these proceedings.

This book will be very useful for researchers, scientists, students and all such entrepreneurs who are working in the field of public health.

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Toxicology:
New Research Dimensions

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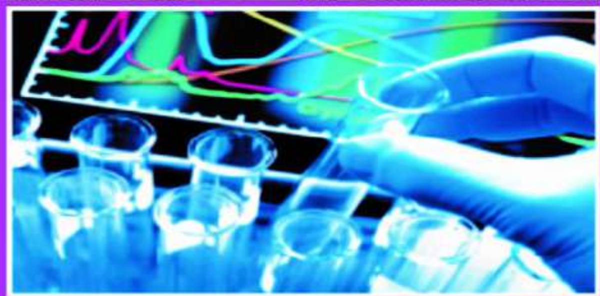

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PASSAGE OF AMMONIA THROUGH THE MITOCHONDRIAL MEMBRANES AND THE BLOOD–BRAIN BARRIER IN *CTENOPHARYNGODON IDELLA* DUE TO AMMONIA TOXICITY

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ABSTRACT

There are many good reasons to study ammonia production and excretion in fish because of its ecological and environmental relevance. Besides, many fish species are of ornamental, aquacultural, and economical values, and ammonia toxicity can be a major issue that leads to mass mortality under unfavourable aqua cultural conditions. However, the intensity of studies on mechanisms of ammonia toxicity in fish is far lower than that in mammals. Interests in studying ammonia toxicity in mammals arise from the fact that liver failure in human leads to the development of neurological abnormalities collectively referred to as hepatic encephalopathy, and ammonia is a key neurotoxin implicated in this condition. The mitochondrial permeability transition involves the opening of a pore in the inner mitochondrial membrane that leads to a collapse of ionic gradients, resulting in mitochondrial dysfunction. In the case of ammonia neurotoxicity, the mitochondrial permeability transition could be related to the permeation of glutamine through the inner mitochondrial membrane and the production of ammonia through glutaminase in the mitochondrial matrix of astrocytes. It has been established that several fish species can tolerate high levels of ammonia and/or glutamine in the brain, but it is uncertain how ammonia and glutamine permeation through the blood–brain barrier, plasma lemma and mitochondrial membranes of brain cells in these fishes are regulated. Furthermore, studies on ammonia genesis of ammonotelic fishes revealed that ammonia production occurred mainly in the liver mitochondrial matrix, but how ammonia exits the membranes of the liver mitochondria is an enigma. It was assumed that ammonia would permeate the inner mitochondrial membrane as NH_3, but such an operation would lead to the disruption of the proton gradient. This could be the reason why ammonia has to be converted into citrulline and glutamine, which are proton-neutral nitrogenous molecules, before exiting the liver mitochondria of ureogenic and uricogenic animals, respectively. On the other hand, excretion of ammonia occurs mainly through the gills of fish, and earlier notions also prescribed that ammonia could permeate the branchial epithelium as NH_3. However, recent studies reveal that ammonia excretion through fish gills can involve transporters, which affirms the possibility of the presence of ammonia transporter in the inner membrane of liver mitochondria of ammonotelic fishes. Therefore, this review focuses on both the earlier literature and the up-to-date information on the problems and mechanisms concerning the permeation of ammonia, as NH_3, NH_4^+ or proton-neutral nitrogenous compounds across mitochondrial membranes, the blood–brain barrier, the plasmalemma of neurons, and the branchial and cutaneous epithelia of fish. Efforts have also been made to examine the relationships between the permeation of ammonia through various types of bio membrane and the high tolerance of certain fish species to ammonia toxicity. It is hoped that this review would revive the interests in investigations on the passage of ammonia through the mitochondrial membranes and the blood–brain barrier of ammonotelic fishes particularly on *Ctenopharyngodon idella* and fishes with high brain ammonia tolerance, respectively.

KEY WORDS: Ctenopharyngodon idella, Ammonia, Mitochondria, Blood Brain Barrier, Glutaminase, Ammonotelic, Permeability

INTRODUCTION

Many fishes are ammonotelic but some species can detoxify ammonia to glutamine or urea. Certain fish species can accumulate high levels of ammonia in the brain or defense against ammonia toxicity by enhancing the effectiveness of ammonia excretion through active NH_4^+ transport, manipulation of ambient pH, or reduction in ammonia permeability through the branchial and cutaneous epithelia. Recent reports on ammonia toxicity in mammalian brain reveal the importance of permeation of ammonia through the blood–brain barrier and passages of ammonia and water through transporters in the plasma lemma of brain cells. Additionally, brain ammonia toxicity could be related to the passage of glutamine through the mitochondrial membranes into the mitochondrial matrix. On the other hand, recent reports on ammonia excretion in fish confirm the involvement of Rhesus glycoproteins in the branchial and cutaneous epithelia. Therefore, this review focuses on both the earlier literature and the up-to-date information on the problems and mechanisms concerning the permeation of ammonia as NH_3, NH_4^+ or proton-neutral nitrogenous compounds, across mitochondrial membranes, the blood–brain barrier, the plasmalemma of neurons, and the branchial and cutaneous epithelia of fish. It also addresses how certain fishes with high ammonia tolerance defend against ammonia toxicity through the regulation of the permeation of ammonia and related nitrogenous compounds through various types of membranes.

Production and Excretion of Ammonia in Fish

Dietary protein is a major source of amino acids in animals. The intestines of carnivorous fishes are adapted to process diets that are high in protein and low in carbohydrate (Buddington *et al.*, 1997). Karlsson *et al.* (2006) determined changes in plasma concentrations of free amino acids and their metabolites in pre- and post-hepatic blood following a single meal in rainbow trout (*Oncorhynchus mykiss*), and confirmed that amino acids could be metabolized in the intestine before they reached the liver. The plasma ammonia level in the hepatic portal vein was higher than that in the dorsal aorta, and the difference between the two blood sampling sites increased during amino acid absorption after a meal. Thus, Karlsson *et al.* (2006) concluded that deamination of certain amino acids occurred in the intestine of the rainbow trout after feeding. In support of the conclusion of Karlsson *et al.* (2006), Tng *et al.* (2008)reported that postprandial amino acid metabolism indeed occurred in the intestine of juvenile *Oxyeleotris marmorata*. The major amino acid accumulated in the intestine and liver of juvenile *O. marmorata* after feeding was glutamate, and feeding led to a significant increase in glutamate dehydrogenase (GDH) activities in the intestine and liver of *O. marmorata*, which could lead to a high retention of the ingested nitrogen for somatic growth. Consequently, only 33% of the ingested nitrogen was excreted during the 24 h post-feeding period, and the brain was effectively prevented from exposure to postprandial ammonia toxicity (Tng *et al.*, 2008).

Animals cannot store excess amino acids, unlike carbohydrates and lipids which can be stored as glycogen and triglycerides, respectively. Thus, dietary amino acids in excess of the amounts needed for growth and maintenance of protein turnover are preferentially degraded over carbohydrates and lipids in the liver (Campbell, 1991). For fishes with high-protein diets, their dietary carbon is extracted from the carbon chain of amino acids after the removal of the α-amino group. Several amino acids including alanine, are converted to glucose by fish hepatocytes (French *et al.*, 1981) and this process is regulated hormonally in much the same way as it is in mammals. Approximately 40–60% of the nitrogen intake from food is excreted within 24 h (Ip *et al.*, 2004c; Lim *et al.*, 2004b). In addition to diet, muscle proteins can act as a source of amino acids, which are catabolized for the production of ATP or carbohydrates, in fasting fishes (Houlihan *et al.*, 1995). Under adverse environmental conditions where ammonia excretion is reduced, some fishes can reduce the rate of ammonia production from amino acid catabolism to slow down the build up of ammonia internally (Ip *et al.*, 2001c, 2004a,b; Lim *et al.*, 2001). During exercise or hypoxia, ammonia can also be produced through the deamination of AMP in the skeletal muscle.

Much of the ammonia produced in fish comes from the α-amino group of amino acids that are catabolized. The rate of alanine and glutamine degradation by catfish hepatocytes can account for 50 and 85%, respectively, of the total ammonia excreted by the fish (Campbell *et al.*, 1983). In addition, the rate of glutamate deamination by intact catfish liver mitochondria can account for 160% of the rate of ammonia excretion (Campbell *et al.*, 1983). For goldfish, the liver is responsible for 50–70% (van den Thillart and van Raaji, 1995) of ammonia production. Ammonia can be produced either directly in the cytosol of hepatocytes by specific deaminases (histidase, asparaginase, serine dehydratase, and threonine dehydratase; Youngson *et al.*, 1982) or via the combined actions (transdeamination) of cytosolic aminotransferases and mitochondrial GDH (Walton and Cowey, 1977; French *et al.*, 1981), but transdeamination is the primary mechanism for catabolism of amino acids in fish liver (Ballantyne, 2001). Since GDH is localized exclusively in the matrix of fish liver mitochondria, it is within this compartment that ammonia is released through the route of transdeamination which involves the deamination of glutamate. Glutaminase, which releases NH_3 from the amide-function of glutamine, is also present in the mitochondrial matrix of some fish species. Thus, ammonia released in the matrix of liver mitochondria has to permeate the mitochondrial membranes before excretion.

Effects of Ammonia on the Mitochondrial Permeability Transition and Oxidative Phosphorylation

Brain edema is a critical component of hepatic encephalopathy associated with acute liver failure and such edema appears to be principally due to astrocyte swelling (cytotoxic edema). Ammonia is believed to represent a major factor responsible for astrocyte swelling, although the mechanisms by which ammonia causes such swelling are not completely understood. It has been hypothesized that in hyperammonemic conditions, glutamine generated in astrocytes from ammonia and glutamate in a reaction catalyzed by glutamine synthetase (GS; Norenberg and Martinez-Hernandez, 1979), could exert osmotic effects and contribute to brain swelling (Brusilow and Traystman, 1986). Treatment of hyperammonemic rats with the GS inhibitor, methionine sulfoximine (MSO), significantly reduced the amount of brain edema, and also diminished the extent of astrocyte swelling (Willard-Mack *et al.*, 1996). The integration of astrocyte swelling with ammonia metabolism and glutamine synthesis leads to the glutamine/osmolyte hypothesis explaining the astrocyte swelling and brain edema in hyperammonemia (Zwingmann *et al.*, 2000). However, recent studies revealed a lack of direct correlation between the extent of cell swelling and cellular levels of glutamine (Jayakumar *et al.*, 2006). Although glutamine may not function simply as an osmolyte, it has been proposed that glutamine-mediated oxidative stress and/or mitochondrial permeability transition may be responsible for the astrocyte swelling by ammonia (Jayakumar *et al.*, 2006). While it is not known how oxidative stress and the mitochondrial permeability transition cause astrocyte swelling, Rama Rao and Norenberg (2007) suggested that these events may affect AQP4, which is abundantly expressed in astrocytes. The mitochondrial permeability transition is a Ca^{2+}-dependent, cyclosporine A sensitive process due to the opening of a pore in the inner mitochondrial membrane that leads to a collapse of ionic gradients and results ultimately in mitochondrial dysfunction. Many of the factors that facilitate the induction of the mitochondrial permeability transition are also known to be implicated in the mechanism of hepatic encephalopathy; these include free radicals, Ca^{2+}, nitric oxide, alkaline pH, and glutamine. Rama Rao *et al.* (2003) have shown that treatment of cultured astrocytes with 5 mmol l^{-1} NH_4Cl resulted in a dissipation of the mitochondrial membrane potential, which was sensitive to cyclosporine A. Further support for the ammonia induction of the mitochondrial permeability transition was obtained by observing an increase in mitochondrial permeability to 2-deoxyglucose-6-phosphate, and a decrease in calcein fluorescence in astrocytes after ammonia treatment, both of which were also blocked by cyclosporine A. Hence, the mitochondrial permeability transition represents an important component of the pathogenesis of hepatic encephalopathy and other hyperammonemic states. It is the key in cell death in excitotoxicity, in which an over-activation of glutamate receptors causes excessive calcium entry into the cell. Indeed, Reddy *et al*. (2009) demonstrated that agents that are able to cross the blood–brain barrier to block the mitochondrial permeability transition significantly reduced ammonia-induced cell swelling.

Passage of Ammonia across the Blood–Brain Barrier and into Brain Cells – Possible Reasons for High Ammonia Tolerance in Certain Fish Species

Once endogenous or exogenous ammonia enters the blood, it would exert toxic effects on all cells, particularly the heart and the brain which are vital organs with excitable cell types. However, at least for rainbow trout, the heart does not seem to be the organ where ammonia toxicity acts (Tsui *et al*., 2004), and that leaves the brain as the main target of ammonia toxicity in fish. Since the blood–brain barrier permeability for NH_4^+ is only <"0.5% that of NH_3 in Rhesus monkey (Raichle and Larson, 1981), the traditional assumption is that NH_3 can pass the blood–brain barrier by diffusion, and NH_4^+ translocation can be neglected (Cooper and Plum, 1987). However, effects of pH on ammonia uptake are often less pronounced than expected, although they are in the direction predicted by the NH_3 diffusion hypothesis. Therefore, it has been proposed recently that NH_4^+ can also permeate the blood–brain barrier with the possible involvement of Rh glycoproteins, Na^+/K^+-ATPase, Barium-inhibitable K^+ channel and bumetanide inhibitable Na^+:K^+:2Cl" cotransporter (Ott and Larsen, 2004; Figure 2). Once NH_3 or NH_4^+ get through the blood–brain barrier, they can permeate the plasma membrane of brain cells through various transporter proteins. It has been demonstrated that astrocytes can down-regulate the gene expression of several transporters, which include the gap-junction channel connexin 43, the water channel aquaporin 4 and the astrocytic inward-rectifying potassium channel genes (*Kir4.1* and *Kir5.1*), in its plasma membrane in response to hyperammonemia (Lichter-Konecki *et al*., 2008).

In mammals, high levels of brain ammonia (1–3 mmol l"¹) lead to glutamatergic dysfunction (Felipo and Butterworth, 2002; Rose, 2002) which remains as the leading candidate in the pathogenesis of hepatic encephalopathy in acute liver failure. However, many tropical air-breathing fishes (see Ip *et al*., 2004b; Chew *et al*., 2006b for reviews) can tolerate high levels of environmental ammonia, and these environmental tolerance correlate well with their high tolerance of ammonia at the cellular and sub-cellular levels (Ip *et al.*, 2005a). This adaptation facilitates the accommodation of relatively high concentrations of ammonia in the blood, which can reduce the net influx of NH_3 by lowering the inwardly directed ΔP_{NH_3} during ammonia-loading. In addition, a build up of ammonia in the body may be a prerequisite for volatilization of NH_3 in certain air-breathing fish species (Tsui *et al.*, 2004). At present, no information is available on the permeability of the fish blood–brain barrier to NH_3 and NH_4^+ but the brain ammonia content of certain fish species can build up to very high levels under certain conditions (see review by Chew *et al.*, 2006). Therefore, future studies should focus on the expression of ammonia transporters in and the regulation of NH_4^+ fluxes across the blood–brain barrier and the plasmalemma of cells in the brain of these fishes.

The administration of (5R,10S)-(+)-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK801), which is an antagonist of NMDA receptors, at a dosage of 2 μg g^{-1} fish has no protective effect on *Periophthalmus schlosseri* and *Boleophthalmus boddaerti* injected with a lethal dose of ammonium acetate, indicating that activation of NMDA receptors is not the major cause of death during acute ammonia intoxication (Ip *et al.*, 2005a). Thus, unlike mammals (Marcaida *et al.*, 1992; Kosenko *et al.*, 2000), activation of NMDA receptors may not be the explanation for acute ammonia toxicity in the brains of *P. schlosseri* and *B. boddarti*. Since membrane depolarization can lead to the removal of the Mg^{2+} block on NMDA receptors and result in their activation (Fan and Szerb, 1993), it would appear that these mudskippers have special abilities to control the intracellular ammonia level in their brains despite drastic increases in brain ammonia contents (intracellular + extracellular). NH_4^+ can replace K^+ in the facilitated diffusion of K^+ through K^+ channels and/or active transport of K^+ through Na^+/K^+-ATPase; both these processes have direct or indirect deleterious effects on the membrane potential of a cell. In view of the high levels of ammonia in the brains of *P. schlosseri* and *B. boddarti* exposed to chronic and acute ammonia toxicity and the lack of protective effect from MK801, it can be deduced that either membrane depolarization occurred but did not lead to activation of NMDA receptors, or membrane potentials were resilient to NH_4^+ interference due to the presence of K^+ channels and Na^+/K^+-ATPase with high substrate specificities for K^+, in the brains of these two mudskippers, the confirmation of which awaits future studies.

CONCLUSION

Ammonia is produced mainly in fish hepatocytes and must exit the mitochondrial matrix to be excreted through the gills. Efforts should be made in the future to elucidate the form and mechanisms involved in the permeation of ammonia through the inner mitochondrial membrane without disrupting the H^+gradient. Ammonia exerts its toxic effects on the brains of vertebrates, but the brains of certain fish species can tolerate high concentrations of ammonia and glutamine, the latter of which is a Trojan horse of ammonia toxicity in mammalian brains. Hence, it would be essential to investigate the permeation of ammonia through the blood–brain barrier of these fishes. Additionally, it would be meaningful to examine how glutamine and ammonia permeation through the mitochondrial membranes are regulated in the brain cells of these fishes, and to determine the level of glutaminase activity present in these mitochondria. Results obtained from these fishes may provide new insights into mechanisms of ammonia toxicity, and shed light on how ammonia toxicity to vertebrate brains can be ameliorated. Defense against ammonia toxicity in fishes under adverse environmental conditions can be achieved through the detoxification of ammonia involving enzymes present in the mitochondria or cytosol and/or the enhancement of effectiveness of NH_3/ NH_4^+ excretion through the branchial and cutaneous epithelia. While the involvement of Rh glycoproteins in ammonia excretion through fish gills and skins has been established, their functional roles in active NH_4^+ excretion in some fish species with high environmental ammonia tolerance are uncertain at present. Future studies on mechanisms involved in active NH_4^+ excretion through the gills and/or skin of these fishes would provide insights into novel therapeutic measure to handle patients with hyperammonemia.

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