BIOCHEMICAL ADAPTIVE RESPONSES IN GLUCOSE METABOLISM OF FISH (*TILAPIA MOSSAMBICA*) DURING AMMONIA TOXICITY

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ABSTRACT

Changes in glucose metabolism were studied in the liver and gill of freshwater teleost, *Tilapia mossambica* exposed to a sub-lethal concentration (0.001 M) of ammonia. A decrease in glycogen content and an increase in phosphorylase and aldolase activity levels suggested rapid mobilization of glycogen through glycolytic pathway. Changes in lactate, pyruvate and lactate dehydrogenase were found to be tissue-specific. Glucose-6-phosphate dehydrogenase activity was increased throughout the time course study suggested enhanced oxidation of glucose by the HMP shunt. The activity levels of citric acid cycle enzymes such as isocitrate, succinate and malate dehydrogenases and cytochrome-C-oxidase were found to be altered as a function of time during ammonia impact. A slight elevation in AMP levels with a significant drop in ATP, ADP and energy charge was observed. The levels of ATP and ADP showed a rapid decrement during the early phase of ammonia stress probably due to their increased utilization than at the later phase of exposure. The metabolic changes induced by ambient ammonia in fish are found to be tissue-specific as well as time-dependent.

INTRODUCTION

AMMONIA has received increasing attention over the past few years as a potentially important pollutant in aquatic systems. It enters natural water systems from several sources including agricultural input, industrial wastes, sewage effluents and animal feedlots. Ammonia (both ionized and unionized) in the aquatic medium causes retardation of growth and deterioration in the calorific value of fish due to its toxicity[1]. It is also known to cause various histopathological changes in different organs of body and resulting in fish kills[2] when it accumulates in higher concentrations. Despite being an ardent pollutant or toxicant ammonia is still being used to increase the body weight of several animals[3]. Recent reports[4] have indicated that ammonia can also act as a growth stimulant in fish when present in lower concentrations. Since contradictory reports exist regarding the impact of ammonia on fish bioconstituents and growth, in the present study, an attempt was made to analyse the influence of mild and moderate doses of ammonia as a function of time on energy metabolism of freshwater teleost *Tilapia mossambica*.

MATERIALS AND METHODS

Freshwater teleost, *T. mossambica* (14 ± 2 g) were collected from local freshwater ponds and fed *ad libitum* with groundnut cake and fried rice. Prior to use they were acclimatized to laboratory conditions for 1 week and starved 24 hr prior to experimentation[5], the LC_{50} (0.0033 M) was determined by the method of Finney[6]. Fish were exposed to a sub-lethal concentration (0.001 M) of ammonium acetate for 2 and 7 days and liver and gill tissues were excised rapidly at the end of experimental period and were processed for the following biochemical estimations, after homogenizing in required media and spinning at 3000 g for 15 min.

Levels of glycogen[7], pyruvate[8] and lactate[9] and activities of phosphorylase[10], aldolase[11], glucose-6-phosphate dehydrogenase (G-6-PDH)[12], isocitrate dehydrogenase (ICDH)[13], lactate dehydrogenase (LDH), malate dehydrogenase (MDH), succinate dehydrogenase (SDH)[14] and cytochrome-C-oxidase[15] were estimated in control and experimental fish tissues. Protein concentration in the enzyme source was analysed following the method of Lowry *et al*[16]. The levels of adenine nucleotides (ATP, ADP and AMP) were determined by ion exchange chromatographic technique[17,18] using an ion exchange resin dowex-1-chloride form, 200–400 mesh. Energy charge (EC) has been calculated according to the formula (EC: ATP + 1/2 ADP/ ATP + ADP + AMP) of Atkinson[19]. Each value in table 1 is the mean ± SD of six different observations. Data were analysed by the student's *t* test to

Table 1 *Changes in glucose metabolism of fish tissues under ambient ammonia stress*

Parameter	Liver			Gill		
	Control	2 days	7 days	Control	2 days	7 days
Glycogen[a]	5.970 ±0.546	2.860* ±0.146 (−52.09)	1.790* ±0.024 (−70.02)	0.265 ±0.009	0.203* ±0.001 (−23.40)	0.122* ±0.002 (−53.96)
Phosphorylase[b]	7.41 ±0.562	10.60* ±0.446 (+43.04)	11.88* ±0.845 (+60.32)	1.94 ±0 096	2.05* ±0.104 (+5.67)	2.14* ±0 143 (+10.31)
Aldolase[c]	13.86 ±0.975	19.76* ±2.214 (+42.56)	21.95* ±2.241 (+58.34)	10 65 ±0 884	13.36* ±1.312 (+25.45)	14.86* ±1.211 (+39.53)
Pyruvate[d]	3 250 ±0.109	5.902* ±0.241 (+81.6)	4.780* ±0.241 (+47.1)	0.678 ±0.008	0.312* ±0.029 (−53.98)	0.498* ±0.002 (−26.54)
Lactate[d]	0.346 ±0.029	0.255* ±0.103 (−26.3)	0.310* ±0.009 (−10.4)	0.204 ±0.001	0.328* ±0.004 (+60.78)	0.205* ±0.017 (+29.92)
LDH[e]	0.738 ±0.005	1.241* ±0.007 (+68.15)	0.991* ±0.011 (+34.28)	0.102 ±0.007	0.047* ±0.001 (−53.90)	0.076* ±0.021 (−25.49)
G-6-PDH[e]	0.786 ±0 014	0.953* ±0.076 (+21.25)	1.302* ±0.010 (+65.65)	0.382 ±0.023	0.454* ±0.029 (+18 85)	0.537* ±0.025 (+40.58)
NAD^+-ICDH[e]	0.729 ±0.056	0.471* ±0.014 (−35.39)	0.548* ±0.066 (−24.83)	0.276 ±0.021	0.152* ±0.016 (−44.93)	0.201* ±0.013 (−27.17)
$NADP^+$ICDH[e]	0.187 ±0.006	0.318* ±0 014 (+70.05)	0.255* ±0.014 (+36.36)	0.052 ±0.004	0.067* ±0.005 (+28.85)	0.060* ±0.003 (+15.38)
SDH[e]	1.43 ±0.012	0.68* ±0.064 (−52.40)	1.02* ±0.057 (−28.57)	0 72 ±0.047	0.24* ±0.017 (−66.66)	0.47* ±0.031 (−34.72)
MDH[e]	0.164 ±0.004	0.099* ±0.034 (−39.60)	0.122* ±0.002 (−25.61)	0.068 ±0.001	0.037* ±0.002 (−45.58)	0.049* ±0.003 (−27.94)
Cytochrome-C-oxidase[f]	126.37 ±9.84	85.27* ±10.81 (−32.52)	100.28* ±9.67 (−20.64)	64.26 ±4.32	42.79* ±7.06 (−33.41)	49.68* ±3.78 (−22.69)
AMP[g]	6.42 ±0.13	8.44* ±0.37 (+31.46)	7.16* ±0.41 (+11.53)	5.28 ±0.15	7.51* ±0.42 (+42.23)	5.98* ±0.21 (+13.26)
ADP[g]	8.97 ±0.19	6.92* ±084 (−22.85)	8.45* ±0.38 (−5.80)	6.43 ±0.18	4.66* ±0.54 (−27.53)	5.85* ±0.47 (−9.02)
ATP[g]	62.84 ±7.26	28.78* ±7.94 (−54.20)	56.98* ±2.38 (−9.33)	45.75 ±6.11	19.13* ±8.76 (−58.19)	38.55* ±3.71 (−15.74)
Energy charge	0.861 ±0 012	0.730* ±0.046 (−15.22)	0.843* ±0 011 (−2.09)	0.852 ±0.015	0.686* ±0.031 (−19.48)	0.823* ±0.014 (−3.40)

Note: Values in parentheses are per cent changes over controls; *Indicates statistical significance at 0.05 level; [a] mg/g wet wt tissue; [b] μmol of Pi formed/mg protein/hr, [c] μmol of FDP cleaved/mg protein/hr; [d] μmol/g wet wt tissue; [e] μmol of formazan formed/mg protein/hr; [f] μmol of diformazan formed/mg protein/hr; [g] μg/g wet wt tissue.

assess the difference between control and experimental treatments. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The changes in glucose metabolism in liver and gill of *T. mossambica* exposed to ambient ammonia were studied (table 1). A significant decrease in the levels of glycogen and increase in phosphorylase and aldolase activities were observed throughout the time course study (up to the 7th day) in liver and gill of ammonia-treated fish (table 1) suggesting increased oxidation of glucose through glycolytic pathway to provide energy for the animal under ambient ammonia stress[20]. The lactate concentration decreased in the liver of ammonia exposed fish with a corresponding increase in NAD^+-dependent LDH activity and pyruvate during short term exposure (up to 2nd day) of ammonia (table 1) indicating rapid conversion of lactate to pyruvate by LDH at the expense of NAD^+ and stepped up pyruvate synthesis in response to the stress induced by ammonia. This finding can be further supported by the studies of Prior *et al*[21] who reported accumulation of pyruvate in the tissues during ammonia intoxication. In contrast, gill experienced high levels of lactate during short-term exposure suggesting the preferential operation of anaerobic glycolysis to maintain osmotic and ionic homeostasis[22].

The pyruvate oxidation through citric acid cycle cannot be envisaged in view of lower oxidative enzyme activities such as NAD^+-dependent ICDH and MDH and FAD-dependent SDH (table 1). The other possible diversion of pyruvate may be through the reactions catalysed by PEP carboxykinase and pyruvate carboxylase to form oxaloacetate and malate to facilitate greater oxidations of triose phosphates[21]. Thus there might be a shift in glucose metabolism towards increased anaerobiosis in gill and wood-werkman reaction in liver during ammonia impact.

The $NADP^+$-specific ICDH activity was increased in both the tissues of ammonia-treated fish during early periods of exposure with a concomitant decrease in NAD^+-specific ICDH (table 1) indicating altered co-enzyme specific 'inhibition-activation' phenomenon in the tissues of fish under induced ammonia stress[23]. $NADP^+$-dependent G-6-PDH activity was also increased significantly in both the tissues of fish throughout the time course study (table 1) suggesting rapid operation of HMP shunt which acts as an alternate source of energy under altered environmental conditions. The increased activity of G-6-PDH also facilitates increased production of $NADPH_2$ for detoxification process[24] and lipid synthesis[25].

The NAD^+-specific ICDH and MDH were found to be decreased during short term in both tissues of ammonia-exposed fish (table 1) and this could be attributed to increased NADH/NAD during ammonia stress[26]. A decrease in SDH activity during short term exposure (table 1) clearly indicates depressed oxidative metabolism at the level of mitochondria leading to an overall depression of citric acid cycle in both tissues of fish during acute ammonia toxicity (up to 2nd day). In support of this, Saheki *et al*[27] and Prior and Visek[28] reported decreased SDH activity in the tissues of rats during ammonia treatment.

As a representative of electron transport system cytochrome-C-oxidase activity was studied and it was also decreased during acute ammonia toxicity (table 1) indicating reduced mitochondrial oxidation of substrates and decreased ATP turnover during early phase of exposure. The drop in cytochrome-C-oxidase activity is in consonance with recent reports[29,30].

To verify diminished oxidative potentials of the fish tissues under induced ammonia stress, the energy states of the tissues have been assessed by estimating the levels of adenine nucleotides and energy charge (table 1). The levels of ATP and ADP were decreased in liver and gill of ammonia-exposed fish during short-term exposure (table 1) suggesting their rapid utilization or diminished synthesis under ammonia impact. This finding is in accordance with the recent studies of Lambert and Wright[31] who demonstrated low hepatic ATP and ADP in the cirrhotic patients. In consonance to the decreased levels of ATP and ADP, a slight increase was noticed in AMP levels confirming the fact that ATP and ADP are being utilized and not replenished during early periods of exposure. The energy charge which indicates the balance between catabolic and anabolic reactions, was found to be decreased up to 2nd day of exposure in liver and gill of experimental fish (table 1) indicating altered energy budget of the tissues during short-term exposure.

However an interesting trend was observed in the present study during long-term exposure (from 2nd day to 7th day) where the levels of pyruvate, lactate, LDH, ICDH, SDH, MDH, cytochrome-C-oxidase, adenine nucleotides and energy charge of liver and gill of ammonia-treated fish were found to increase from 2nd day onwards till 7th day of exposure so as to reach the normal levels. This clearly indicates the

effective operation of detoxification mechanisms in the tissues of fish which increase the survival chance of the animal in ammonical waters.

Basing on these results, it can be concluded that a biphasic adaptive response was observed in glucose metabolism where at early phase of exposure, a steep decrease followed by a slight increase in the activity levels of enzyme systems and energy charge can be witnessed in the tissues of ammonia-treated fish suggesting that within seven days the fish tries to overcome the toxicity to some extent after sub-lethal ammonia stress in aquatic environment.

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1. Bhagowati, A. K. and Ratha, B. K., *Proc. Indian Natl. Sci. Acad.*, 1982, **48**, 67.
2. Kilikidis, S. D., Kamarianos, A. P., Kousouris, T. H. and Singkounakis, T., *Bull. Environ. Contam. Toxicol.*, 1981, **26**, 453.
3. Brandt, R. T., Paterson, J. A. and Bowman, D. K., *Nutr. Res. Int.*, 1983, **28**, 219.
4. Das, N. P. and Das, A. B., *Sci. Rep.*, 1984, **21**, 454.
5. Jones, J. R. E., In: *River population: 2. causes and effects*, (ed.) K. L. Butterworth, Academic Press, New York, 1972, p. 254.
6. Finney, D. J., *Probit analysis*, 2nd edn, Cambridge Univ. Press, London, 1964, p. 104.
7. Carroll, N. V., Longley, R. W. and Roe, J. H., *J. Biol. Chem.*, 1956, **220**, 583.
8. Friedeman, T. E. and Haugen, G. E., *J. Biol. Chem.*, 1942, **144**, 67.
9. Huckabee, W. E., *Am. J. Med.*, 1961, **30**, 833.
10. Cori, G. J., Illingworth, B. and Keller, P. G., *Methods Enzymol.*, 1955, **1**, 200.
11. Bruns, F. H. and Bergmeyer, H. U., In: *Methods of enzymatic analysis*, (ed.) H. U. Bergmeyer, Academic Press, New York, 1965, p. 724.
12. Lohr, G. D. and Waller, H. D., In: *Methods of enzymatic analysis*, (ed.) H. U. Bergmeyer, Academic Press, New York, 1965, p. 96.
13. Kornberg, A. and Pricer, W. E., *J. Biol. Chem.*, 1951, **189**, 123.
14. Nachlas, M. M., Margulius, S. P. and Seligman, A. M., *J. Biol. Chem.*, 1951, **235**, 499.
15. Oda, T., Seki, S. and Okazaki, H., *Acta. Med.*, 1958, **12**, 293.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
17. Cohen, W. E., *Methods Enzymol.*, 1957, 200.
18. Strehler, B. L. and Totter, J. R., *Methods of biochemical analysis*, Interscience, New York, 1954, p. 86.
19. Atkinson, D. E., *Biochem. J.*, 1968, **7**, 4030.
20. Hawkins, R. A., Alexander, L. M., Richard, C. N. and Richard, L. V., *Biochem. J.*, 1973, **134**, 1001.
21. Prior, R. L. and Visek, W. J., *Am. J. Physiol.*, 1972, **223**, 1143.
22. Smith, C. E. and Russo, R. C., *Prog. Fish. Cult.*, 1975, **37**, 150.
23. Stein, A. M., Kirkman, S. K. and Stein, J. H., *Biochemistry*, 1967, **6**, 3197.
24. Kohli, K. K., Bhatia, S. C. and Subramanyam, T. A. V., *Environ. Physiol. Biochem.*, 1975, **5**, 119.
25. Singlevich, T. E., Barboriak, J. J. and Pintar, K., *Eur. J. Toxicol.*, 1972, **4**, 532.
26. Hindfelt, B., Flum, F. and Duffy, T. E., *J. Clin. Invest.*, 1977, **59**, 386.
27. Saheki, T., Towateri, T. and Katunuma, N., *J. Biochem.*, 1971, **70**, 529.
28. Prior, R. L., Zimber, A. and Visek, W. J., *Am. J. Physiol.*, 1975, **228**, 828.
29. McCandless, D. W. and Schenker, S., *Exp. Brain Res.*, 1981, **44**, 325.
30. Brazy, P. C., Gullans, R. S., Mandel, L. J. and Dennis, E., *J. Clin. Invest.*, 1982, **70**, 53.
31. Lambert, D. and Wright, P. D., *Advances in hepatic encephal and urea cycle dis.*, 1984, p. 667.