TRANSAMINATION AND GLUTAMATE DEAMINATION IN *RANA HEXADACTYLA* DURING INDUCED AMMONIA TOXICITY

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Summary : Glutamate dehydrogenase (GDH) and the transaminases namely aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) were estimated in the muscle, liver, kidney, and brain of control and ammonium acetate administered frogs. The results indicated tissue specific responses during induced ammonotoxemia. The inherent endogenous ammonia production decreased in all the tissues. 2-Keto glutarate production appears to be the other main adaptive feature as a result of slightly stepped up transdeamination patterns.

Key words : aspartate and alanine amino transferase glutamate dehydrogenase frog ammonia toxicity

INTRODUCTION

Hyperammonemia is a general condition since it is a consistent factor in a variety of metabolic maladies such as hypoxic hypoxia (13), hypoglycemia (6), hyper capnic acidosis (3, 4), cerebral ischemia (7), and floroacetate poisoning (14). A role of ammonia in all these disorders has, therefore, been implicated and hence investigations are necessary in the direction of elucidating the mechanism of hyperammonemia.

The glutamate-ammonia system (1) is the main process involved in the detoxication of ammonia during ammonia toxicity. Nevertheless, a metabolic situation is considered to arise *in vivo* where, 'some of the factors like relative levels of nucleotides and removal of reaction products favours glutamate oxidation' being catalysed by GDH (16).

The relationship between glutamate-ammonia system and glutamate oxidative deamination, therefore, requires the evaluation of induced ammonia toxicity effect on transdeamination patterns, since this two-phased reaction is involved in the production of glutamate by aminotransferases and subsequent deamination of glutamate by GDH to yield ammonia and 2-keto glutarate.

MATERIAL AND METHOD

Procurement, maintenance of frogs and the standardisation of the dosage of ammonium acetate to induce hyperammonemia were described already (9). Control batch of animals received 1.1 *ml* of physiological saline while test batch of animals received 11.45 m mole of ammonium acetate/*kg* body weight intraperitoneally. One hour after the administration of saline and ammonium acetate the animals were sacrificed and muscle, liver, kidney and brain were isolated in cold and 10% homogenates were prepared in cold 0.25 M sucrose and distilled water separately. The homogenates were centrifuged at 4000 rpm for 10 min and the cell-free extract of sucrose was utilized in the assay of aminotransferases (11) and GDH (5) while distilled water extract was utilized in determining ammonia (17) and glutamine (18) contents. The protein in the enzyme source was determined by Lowry *et al.* (8) method.

RESULTS

The AIAT, AAT activities increased in all the tissues of experimental animals except that brain AAT decreased (Table I). The increase was relatively more in kidney in contrast with other tissues. The GDH activity was also increased in all the tissues of experimental samples except in liver. Ammonia content in all the tissues decreased.

TABLE I : Changes in some of the biochemical components in different tissues of frog during induced ammonia toxicity.

Biochemical component		*Muscle*	*Liver*	*Kidney*	*Brain*
	Control	32.16	22.33	18.39	37.91
		±0.68	±1.70	±0.78	±1.60
AAT	Experimental	37.07	25.96	27.13	30.20
		±2.39	±2.50	±0.61	±3.60
	% difference	15.3	16.2	45.9	—20.4
	't' test	P<0.025	P<0.01	P<0.001	P<0.025
	Control	30.93	17.31	33.35	32.19
		±3.90	±1.50	±1.74	±0.64
AIAT	Experimental	35.89	19.73	44.37	40.70
		±2.97	±2.42	±2.15	±2.75
	% difference	16.1	14.0	33.0	26.5
	't' test	P<0.01	P<0.1	P<0.01	P<0.01
	Control	0.34	3.95	3.42	0.55
		±0.08	±0.12	±0.12	±0.01
GDH	Experimental	0.41	2.96	4.42	0.77
		±0.02	±0.59	±0.35	±0.07
	% difference	22.6	24.9	29.3	39.0
	't' test	NS	P<0.1	P<0.005	P<0.005

Values are mean±SD of 8 observations. AAT and AIAT are expressed as *μmoles* of pyruvate/*mg* protein/h. GDH is expressed as *μmoles* of formazan/*mg* protein/h.

Glutamine content also decreased in all the tissues except muscle while an increased ammonia content (excreted) in the ambient medium was observed (Table II).

TABLE II : Ammonia and glutamine contents in different tissues of frog and ambient medium under ammonia toxicity.

Biochemical component		*Ambient medium*	*Muscle*	*Liver*	*Kidney*	*Brain*
AMMONIA	Control	0.5 ±0.02	3.4 ±0.06	4.2 ±0.07	5.5 ±0.97	5.3 ±0.37
	Experimental	7.1 ±1.35	1.4 ±0.2	2.3 ±0.22	2.7 ±0.2	1.4 ±0.5
	% difference	1466.7	—60.3	—44.2	—51.8	—74.5
	't' test	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
GLUTAMINE	Control	Not detected	3.3 ±0.81	23.4 ±2.75	9.7 ±1.32	8.1 ±0.80
	Experimental	"	3.8 ±0.46	5.1 ±1.82	8.7 ±1.32	7.0 ±2.4
	% difference	—	16.2	—78.4	-9.8	—13.8
	't' test	—	NS	P<0.001	P<0.01	P<0.01

Values are mean ± SD of 8 observations. Values are expressed as *µmoles/gm.* wet/wt. of tissue while in ambient medium as *µmoles*/100 *ml* of the medium.

DISCUSSION

An increased activity levels of AAT and AlAT was observed in all the tissues except brain AAT after ammonium acetate administration. Similar reports in other animals agree with the present findings (10, 12, 15). Elevated AAT and AlAT activity levels suggest an increased formation of oxaloacetate, pyruvate and glutamate. In the case of brain, AAT activity registered significant fall suggesting non-availability of substrate, perhaps, depletion of 2-keto glutarate for other transamination reactions. An assay of GDH levels was also done in order to understand the utilisation of glutamate during the ammonia toxicity. The GDH activity showed an increase in all the tissues except liver. As liver is the main centre for ammonia removal, further production of ammonia through deamination of glutamate by GDH would have been prevented for the already existing high ammonia levels. Hence, a decreased GDH activity was observed. In confirmation, Dikshitulu (2) also reported an increased Km and lesser affinity of the GDH towards the substrate in cell free extracts of goat liver during *in vitro* ammonia toxicity. While in the other tissues, since the GDH activity was elevated, the slightly stepped up oxidative deamination of glutamate bringing about 2- keto glutarate and ammonia would have occurred. The stepped up GDH activity observed in the present study did not bring about increased formation of ammonia, since the ammonia levels were found to decrease in all the tissues. In fact, the GDH activity was increased, while ammonia levels were decreased suggesting that there might be the removal of ammonia through other ways.

The exact pathway of ammonia can be given only by levels of glutamic acid and glutamine synthetase and needs further work which is under investigation. But the glutamine levels except muscle showed a decrease. Probably the excess ammonia to some extent was excreted into the ambient medium as an immediate response to ammonia toxicity. In accordance, the ammonia content in the ambient medium showed an increase.

The overall changes observed in AlAT. AAT. GDH. ammonia and glutamine levels after the ammonium acetate administration would suggest that transdeamination reactions continue to operate slightly increased, but not vigorously facilitating the formation of 2-keto glutarate. A clear picture however is still essential with regard to the exact ways of disposal of excessive ammonia, the mechanism of its action and tissue specific responses and retention capabilities to ammonia load.

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