ARGININE PROTECTION AGAINST AMMONIA TOXICITY IN EXHAUSTED RAT

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Summary : Arginine administration *(5 m moles/kg/day)* to albino rats for 7 days, revealed that this vital basic amino acid possesses latent potentiality for the accentuation of urea cycle or atleast for arginase activity. The mitigation of ammonia toxicity was observed to be more effective in the case of gastrocnemius and red vastus as compared to white vastus. Further, ammonia and lactate levels were also decreased by arginine in blood and thereby delaying the onset of fatigue by preventing ammonotoxemia and lactic acidemia.

Key words : fatigue muscles arginine muscular efficiency

INTRODUCTION

The etiology of fatigue is still unclear as the causative factors depend on duration and intensity of work, fiber type, delivery of substrates, oxygen, intracellular enzyme and co-enzyme activities and milieu interior (3,8,13.) The increase in muscle and blood ammonia (5, 6, 7), has been implicated in the vitiation of aerobic metabolism, leading to functional inefficiency (6). Mutch and Banister (19) suggested that a reduction in blood amonia can increase an individual's capacity for exhausting activity. Arginine, a vital basic amino acid is a specific activator of N-acetyl glutamate synthetase (23), which catalyses the synthesis of N-acetyl glutamate. Walser (24) unequivocally proved that arginine is particularly effective in combating hyperammonemia in a variety of clinical situations and he also established that some animal species are especially susceptible to the hyperammonemia during arginine deficiency. In the present study, the role of arginine on ammonia metabolism was investigated to understand the salutary effect of arginine in alleviating ammonia toxicity and in activating lactate oxidation and to improve metabolic efficiency in working muscles.

MATERIAL AND METHODS

Healthy, adult male albino (Wistar) rats (150 ±5 g) maintained under laboratory conditions (temp. 25 ± 5°C, RH 75% and LP 12 h) were fed with equal amount of standard diet (Hindustan Lever Ltd., Bombay) and provided drinking water *ad libitum*.

The animals were divided into two groups of sixteen each and one group was given arginine ip (*5 m moles/kg* body wt/day) for 7 days continuously (15). Fatigue was induced in treated and untreated rats by subjecting them to prolonged swimming (8). Suitable controls were maintained.

The control and experimental animals were autopsied immediately and gastrocnemius (Fast-twitch red, FTR), red vastus (fast-twitch mixed, FTM) and white vastus (fast-twitch glycolytic, FTG) (17, 18) were isolated, supercooled immediately to prevent autolysis and used for biochemical analysis.

Lactate was estimated by the modified method of Huckabee (11). Ammonia content was analysed as described by Sadasivudu *et al.* (22). The diacetyl monozime method as decribed by Natelson (20) was followed for determining urea levels. Arginase activity was assayed following the method of Beruter *et al.* (2) by determining the urea with diacetyl monoxime and protein content in the enzyme source was estimated by the method of Lowry *et al.* (16).

RESULTS AND DISCUSSION

From the studies, it was observed that there was a significant exponential accumulation of lactate and ammonia in the muscle and blood of exhausted rats (Table I and II). Increase in lactic acid (3) and ammonia (19) and their role in the development of fatigue has been well documented. Greater increase in lactate and ammonia levels in white vastus suggests predominant anaerobiosis, limited oxygen reserves and less oxidative red fiber population as compared to gastrocnemius and red vastus. The higher rate of utilization of ATP and lower rate of ATP synthesis in white vastus might be responsible for the increase in ammonia content during intense contractile activity (18). Gastrocnemius and red vastus due to high oxidative fiber population might have resisted the production of ammonia and lactate than the fast glycolytic white vastus. Moreover, increased AMP and adenosine deamination (14) results in incresed ammonia production

TABLE I : Modulatory potential of Arginine on certain metabolites and enzymes in different muscles of albino rat during exhaustion.

Parameter	*Treatment*	*Gastrocnemius*		*Red vastus*		*White vastus*	
Lactate (*mg/g* tissue)	UC	3.88 ± 0.29		3.94 ± 0.38		4.16 ± 0.33	
	UF	6.79* ± 0.84	(+75.26) (a)	6.81* ± 0.32	(+71.76) (a)	9.52* ± 0.61	(+128.60) (a)
	TC	3.50 ± 0.22		3.59 ± 0.28		3.81 ± 0.32	
	TF	6.28* ± 0.53	(— 7.48) (b)	6.41* ± 0.53	(—5.92) (b)	8.94* ± 0.73	(—6.15) (b)
Ammonia (*μmoles/g* tissue)	UC	1.34 ± 0.12		1.26 ± 0.13		1.08 ± 0.09	
	UF	1.78* ± 0.21	(+32.40) (a)	1.69* ± 0.15	(+34.17) (a)	1.84* ± 0.19	(+70.55) (a)
	TC	1.10 ± 0.09		1.01 ± 0.07		0.94 ± 0.09	
	TF	1.47* ± 0.11	(−17.32) (b)	1.42* ± 0.10	(—16.12) (b)	1.58* ± 0.11	(—13.73) (b)
Urea (*μmoles/g* tissue)	UC	4.54 ± 0.38		4.21 ± 0.41		3.82 ± 0.26	
	UF	5.49* ± 0.27	(+21.02) (a)	5.14* ± 0.39	(+22.17) (a)	4.26* ± 0.29	(+11.68) (a)
	TC	5.43 ± 0.41		5.03 ± 0.46		4.41 ± 0.51	
	TF	6.83* ± 0.39	(+24.38) (b)	6.58* ± 0.43	(+28.04) (b)	4.71* ± 0.32	(+15.27) (b)
Arginase (*μmoles* of urea*/mg* protein/h)	UC	0.12 ± 0.01		0.11 ± 0.01		0.08 ± 0.01	
	UF	0.17* ± 0.01	(+38.98) (a)	0.16* ± 0.01	(+46.67) (a)	0.10* ± 0.01	(+27.85) (a)
	TC	0.17 ± 0.01		0.16 ± 0.01		0.12 ± 0.01	
	TF	0.21* ± 0.01	(+22.67) (b)	0.20* ± 0.01	(+20.00) (b)	0.12* ± 0.01	(+14.85) (b)

The values are mean and ± S. D. of eight experiments. *Significantly different from control $P<0.05$. Values in perenthesis are % changes over (a) % changes of Untreated Fatigued (UF) over Untreated Control (UC)
(b) % changes of Treated Fatigued (TF) over UF.

which consequently interfers with oxidative metabolism and promots anaerobiosis (18) and proteolysis (9) in white vastus as compared to gastrocnemius and red vastus.

TABLE II : Lactate and ammonia levels in the blood of control and arginine treated rats during exhaustion.

Parameter	*Treatment*	*Blood*				
Lactate (*mg/100 ml*)	UC	8.47	±	0.69		
	UF	14.82	±	1.26	(+74.97)	(a)
	TC	8.10	±	0.92		
	TF	12.94	±	0.89	(—12.68)	(b)
Ammonia *μmoles*/100 *ml*	UC	12.61	±	1.32		
	UF	18.84	±	1.57	(+49.40)	(a)
	TC	11.55	±	0.81		
	TF	16.04	±	1.37	(—14.86)	(b)

Values are mean and ± S. D. of eight experiments. All values are significantly different from control $P<0.05$. Values in parenthesis are % changes (a) = % changes of UF over UC, (b) = % change of TF over UF.

Above results indicate that the synergistic action of lactate and ammonia was mainly responsible for the metabolic disturbances in exhausting muscles. So an attempt has been made to promote ammonia detoxication through urea cycle operation or atleast through arginase activity by treating the rats with arginine which is known to play an indispensable role in buffering ammonia levels in the intracellular atmosphere.

The administration of arginine reduced the ammonia level in all the three muscles and blood (Table I and II) to a significant extent. Greater mitigation of ammonia toxicity was observed in gastrocnemius and red vastus as compared to white vastus, suggesting that the amelioratory potential of arginine on ammonia toxicity seems to vary with muscle fiber type and functional efficiency. Owing to greater vascular nature and oxidative potential of red muscles (5,6,12), a major portion of ammonia might have been transported for its disposal or for efficient conversion to urea, under the influence of arginine. In support of this contention, the gastrocnemius and red vastus recorded greater urea levels as compared to white vastus during exhaustion in the arginine treated animals (Table I).

The increased urea levels in the muscles during exhaustion might be due to activation of carbamoyl phosphate synthetase, by increase in N-acetyl-glutamate content by the administered arginine (10). Moreover, even if arginine did not have that potentiality, the ornithine derived from the injected arginine must have effected the carbamoyl phosphate synthetase reaction. One mechanism by which ornithine could have influenced the carbamoyl phosphate synthetase reaction would be as a direct stimulator of the enzyme as suggested by Cohen *at al.* (4) and thereby ornithine carbamoyl transferase activity through increased ornithine availability (10). The treatment of arginine might be viewed as a protective mechanism as this basic amino acid would counteract the acidic effects of lactate (Table I and II) and thereby mitigating the ammonia production, since the lactate induces more deamination (7).

The increase in urea level along with arginase activity during exhaustion in arginine treated animal suggest that skeletal muscles possess latent potentiality for the accentuation of urea cycle or atleast for arginase activity as the presence of complete complement of urea cycle enzyme is doubtful in extra hepatic tissues (13, 21).

In consonance to this, amonia level decreased in the blood of arginine treated exhausted rats, indicating the mobilization of ammonia into the tissues from the blood for the biosynthesis of urea and glutamine (1). The increased urea levels in muscles of arginine treated exhausted rats add credence to the above contention.

Arginine treatment was found to decrease muscle and blood lactate content in exhausted rats which might be due to rapid transport of lactate to liver (3, 15, 19) or due to reduced rate of lactic acid biosynthesis in view of low glycogenolysis (7). Hence the administration of arginine might help in alleviating the synergistic action of fatigue toxins (lactate and ammonia), which was contemplated to be majorly responsible for the metabolic disturbances and impairment in energy releasing systems during muscular fatigue leading to less efficient mechanical work.

Though the presence of complete complement of urea cycle enzymes is doubtful, the inducibility of atleast a few enzymes like arginase in working muscles was reported (21). Further detailed studies are required on this aspect for arriving at definite conclusions about the existence of full complement of ornithine cycle in muscle. In addition to this, estimation of urea in urine may also throw some light on these aspects as the administration of arginine stimulates urine flow (10).

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