EFFECT OF ELECTRICAL STIMULATION ON AMMONIA DETOXIFICATION POTENTIAL IN AMPHIBIAN SKELETAL MUSCLE

Introduction

MUSCLE, after prolonged electrical stimulation fails to contract due to progressive decrease of oxidative and glycolytic activities[1] and increase in proteolytic activities[2]. Elevated proteolysis increases the free amino acid levels[3] and these amino acids are actively subjected to oxidative deamination releasing large quantities of ammonia. Although ammonia production in muscle is well established, its functional significance has remained obscure. Hence an attempt has been made in the present investigation to analyse the changes in muscle constituents such as ammonia, urea and related ammonogenic enzymes such as AMP deaminase and glutamate dehydrogenase so as to assess the possible role of these enzyme systems in the production, and mobilization of ammonia during prolonged periods of contractile activity.

Materials and Methods

Rana hexadactyla were double pithed and the gastrocnemius muscles of both the legs were isolated with least injury, washed in amphibian Ringer's medium[4] for three times and placed in the same solution for at least 10 minutes to recover from shock effects. One of the muscles was placed in amphibian Ringer's medium and subjected to repeated biphasic electrical stimuli of 10 volts at a pulse frequency of 60/min. using Inco/CSIO Research Stimulator model MRC (Ambala-3, India) for different periods ranging from 1 min to 40 min. The contralateral normal muscles were also maintained under similar conditions but not subjected to electrical stimulation. Stimulated and unstimulated muscles were chilled immediately by placing them on a watch glass, prechilled to 0° C in an ice-chamber of refrigerator to arrest the residual metabolism. The tissues were homogenized in suitable media (distilled water/sucrose solution), centrifuged and the supernatants were used for the estimation of AMP deaminase and glutamate dehydrogenase activities. Glutamate dehydrogenase (GDH) activity was estimated by the method of Lee and Lardy[5] with slight modifications as described by Pramilamma *et al*[6]. AMP deaminase activity was assayed by the method of Weil-Malherbe and Green[7] with slight modifications as described by Wagelin *et al*[8]. Ammonia and urea levels were determined by the methods of Bergmeyer[9] and Natelson[10] respectively.

Results and Discussion

GDH and AMP deaminase showed an increment in their activity levels at first minute of stimulation and decreased thereafter upto fatigue (Table I). The initial rise in the activity levels may be due to the activation of these enzymes which is in agreement with the findings of Kendric-jones and Perry[11], who also noticed similar increase in the activities of several enzymes in the muscle during initial phases of prolonged contractile activity.

TABLE I

Activity levels of glutamate dehydrogenase (GDH) and AMP deaminase at different periods of stimulation of gastrocnemius muscle of frog

Sl. No.	Components	Duration of stimulation (minutes) USC	1	5	10	20	30	(45 fatigue)
1.	GDH (μM of formazan/mg protein/(hr)	0·210 ±0·012	0·251 ±0·012 P<0·001	0·221 ±0·007 NS	0·171 ±0·013 P<0·001	0·145 ±0·010 P<0·001	0·137 ±0·016 P<0·001	0·123 ±0·018 P<0·001
	% deviation over USC	..	+19·5	+5·2	−18·6	−30·9	−34·8	−41·4
2.	AMP deaminase (μM of ammonia/ mg protcin/hr).	1·127 ±0·044	1·315 ±0·045 P<0·001	1·259 ±0·078 P<0·01	1·159 ±0·043 NS	1·103 ±0·025 NS	0·999 ±0·077 P<0·01	0·684 ±0·056 P<0·001
	% deviation over USC	..	+16·7	+11·7	+2 8	−2·1	−11·4	−39·3

All the values are means ± S.D. of six observations.
N.S. = Not significant. USC = Unstimulated control.

TABLE II

Levels of ammonia and urea at different periods of stimulation of gastrocnemius muscle of frog (Values expressed in μgms/gm wet wt of tissue).

Sl. No.	Components	Duration of stimulation (minutes)						
		USC	1	5	10	20	30	45 (fatigue)
1.	Ammonia	0·019 ±0·001 ..	0·034 =0·005 P<0·001	0·027 ±0·0005 P<0·001	0·025 ±0·003 P<0·01	0·020 ±0·0004 P<0·05	0·018 ±0·0006 NS	0·008 ±0·003 P<0·001
	% deviation over USC	..	+78·9	+42·1	+31·6	+5·3	−5·3	−57·9
2.	Urea	0·024 +0·002 ..	0·037 +0·001 P<0·001	0·053 ±0·05 P<0·001	0·063 ±0·004 P<0·001	0·084 ±0·007 P<0·001	0·106 ±0·016 P<0·001	0·106 ±0·016 P<0·001
	% deviation over USC	..	+54·2	+120·8	+162·2	+250·0	+341·7	+341·7

All the values are means ± S.D. of six observations.
N.S. = Not significant. USC = Unstimulated control.

Among the two enzymes studied the activity levels of AMP deaminase were found to be higher than that of GDH in both control and experimental muscles suggesting greater involvement of this enzyme in ammonia production, since GDH activity is very low in skeletal muscles[12]. Increased AMP deaminase activity levels observed in the muscles in the present study are well in line with the reports of Wajzer *et al*[13], who noticed increased conversion of adenosine into inosine compounds during muscle contraction.

The continuous decrement of GDH and AMP deaminase after 1st minute upto fatigue may be due to increased proteolysis[2,3], which lowers catalytic potential of these two enzymes. Accumulation of fatigue substances[14] such as lactic acid, oxaloacetate, hydroxybutyrate and acetate may alter the ionization pattern of the enzyme molecule[15,16] resulting in denaturation or inactivation of the enzymes[17,18].

The decreased GDH activity during prolonged periods of stimulation is in consonance with the lowered oxidation rates of pyruvate, malate and succinate by isolated skeletal muscle mitochondria during exhaustive exercise or fatigue[19].

There exists a positive correlation between phosphorylase activity and AMP levels[20,21] in the muscle. In view of decreased phosphorylase activity[2] during prolonged contractile activity one can expect low content of AMP. Decreased AMP deaminase activity observed in the present context may be correlated to the low levels of substrate (AMP) in the muscle during prolonged contractions[22,23].

Elevated activity levels of both GDH and AMP deaminase in the early phase of contractile activity may release more quantities of ammonia, which will be immediately converted into urea in the later phase as evinced by decreased ammonia and increased urea contents at this period of stimulation (Table II). The increased production of urea is in accordance with the increased quantities of non-protein nitrogen in the muscles after stimulation[24–26]. These results suggest a possible shift in ammonia metabolism towards *ureogenesis*, a means to dispose off the ammonia. The detoxification mechanism seems to involve an effort on the part of the tissue to maintain metabolic homeostasis by combating the toxic effect of ammonia during prolonged periods of contractile activity.

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STUDIES ON CESTODE MIGRATION AND pH CHANGES IN *CLARIAS BATRACHUS* (LINN) INFECTED BY *LYTOCESTUS INDICUS* (MOGHE)

EFFECTS of *Lytocestus indicus* (Moghe) on the hydrogen ion concentration and migration of the cestode in the intestine of *Clarias batrachus* (Linn) have been studied.

L. indicus are localized in the intestine of *C. batrachus*. It has been observed that the cestode migrates to the duodenum after feeding of their host. Before feeding of the fish, 50% of the Cestodes are in the first part of intestine, 20% in the second part, 20% in the third part and 10% in the fourth part. The fifth and sixth parts of the intestine are free from the infection. One hour after feeding of the host, 80% of *L. indicus* are in the first part, 15% in the second part and 5% in the third part of intestine. The fourth, fifth and sixth parts of intestine are free from the infection after feeding. The authors believe that the migration of the cestode after feeding of the host is for absorbing digested food materials from the duodenum.

It has been observed that the pH changes after feeding of the fish. The pH of healthy intestine is 8·2 before feeding and 7·8 one hour after feeding of the fish. The pH of the infected intestine is much lower. Before the feeding of the host, the pH of mildly infected intestine is 7·0, of moderately infected intestine 6·4 and of heavily infected intestine 4·5. One hour after feeding of the host, the pH of mildly infected intestine is 6·7, of moderately infected intestine 6·0 and of heavily infected intestine 4·0.

This is the first report of cestode migration and pH changes in helminthic infection of fishes. However, Mettrick[1] has reported worm migration in rats. Mettrick[1], Podesta and Mettrick[2] and Titchener *et al*[3] have reported pH changes in rats and pigs respectively after helminthic infections.

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ON THE OCCURRENCE OF *BULLIA TRANQUEBARICA* (RODING) NASSARIDAE (GASTROPODA) IN KAVARATTI ATOLL (LAKSHADWEEP)

DURING the course of the investigation of the natural history of Lakshadweep island, a live buccinid gastropod *Bullia tranquebarica* (Roding) belonging to the family Nassaridae under series Buccinacea was collected from Kavaratti atoll (Fig. 1). Kavaratti is situated along latitude 10° 33′ north and longitude 72° 36′ east and has a total area of about 3·629 sq. km. Specimen was collected from the lagoon on the west