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INTRACELLULAR CHARGES AND REGULATION OF PHOSPHORYLASE ACTIVITY IN THE GASTROCNEMIUS MUSCLE OF FROG

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ABSTRACT

The levels of protein, glycogen, phosphorylases *'a'* and *'b'* have been investigated in the control cathodal and anodal halves of the gastrocnemius muscle of the frog. The cathodal half contains high protein and phosphorylase activity as compared with control and anodal halves. Glycogen content does not show significant change between the control and experimental halves. The elevated activity of phosphorylases is attributed to the altered levels of calcium ions and adenine nucleotides in the respective muscle halves.

PREVIOUS investigations in this laboratory on subcellular electrokinetics in muscle cells have shown that the major cell contents in general possess a net positive charge at physiological pH, and when subjected to a field of direct current tend to show cathodal migration[1,2]. In addition, the control and experimental muscle halves are known to have distinct and different metabolic potentialities[3]. In view of this, it was considered necessary to gain some information on the glycogen metabolism of the muscle subjected to subcellular electromigration. A preliminary communication has been presented[4].

MATERIAL AND METHODS

The two gastrocnemius muscles of *Rana hexadactyla* were isolated with least injury from a freshly pithed animal. One of the pair was subjected to subcellular electromigration by exposing to long axis voltage gradient for 15 min. The control, cathodal and anodal halves were obtained as described previously[1].

Glycogen was estimated[5] from the control and the muscle halves subjected to electromigration. The activities of phosphorylases *'a'* and *'b'* were estimated in the direction of glycogen synthesis[6].

A 5% homogenate was prepared in aqueous medium containing 0·037 M ethylene diamine tetra acetic acid (EDTA), pH 6·5 and 0·1 M sodium fluoride, pH 6·5, as recommended by Guillory and Mommaerts[7]. After centrifugation for 15 min at 1000 × g, the supernatant was diluted four times with cysteine (0·03 M)-β-glycerophosphate (0·015 M) buffer, pH 6·5. The diluted enzyme (0·4 ml) was added to 0·2 ml of 2% glycogen and incubated for 20 min at 35° C. The reaction was started by the addition of 0·2 ml of 0·016 M glucose-1-phosphate (G-1-P) to one tube (phosphorylase *'a'*), 0·2 ml of G-1-P and 0·004 M adenosine '5-monophosphate to the other (phosphorylase *'b'*). After incubation for 15 min. for phosphorylase-*b*, and 30 min for phosphorylase *'a'* activities, the reaction was stopped by the addition of 10% sulphuric acid. Inorganic phosphate (Pi) liberated was estimated[8]. Phosphorylase activity was expressed as μ moles of Pi liberated/mg protein/hr. Protein was estimated by Biuret method[8].

RESULTS AND DISCUSSION

Average values of soluble proteins in the control muscles (C 1 and C 2) are given in Table I and they were found to be similar. The soluble protein content is different in the two halves of the experimental muscle (Table I). There is no difference in the protein contents of the cathodal half and the control, whereas the anodal half had 40·1% less protein compared to the cathodal half. The difference in the distribution between the KH and the AH is attributed to a net positive charge density of the major sarcoplasmic proteins and their consequent cathodal migration[10-13].

Glycogen content also shows variation in its level in the experimental halves, even though the difference is not statistically significant. Since glycogen does not possess any charge, the change in its level may not be due to electromigration. Glycogen is a rapidly mobilizable and labile energy fuel and it is possible that there may be a passive mobilization from the anodal to cathodal half due to increased energy demands in the cathodal half[1,9-13].

The activities of phosphorylase *'a'*, as well as *'b'* also showed variations in the experimental halves (Table I). The difference in the activity of phosphorylase *'a'* between KH and AH (43·2%) is significant. A similar trend was observed for phosphorylase *'b'* also. It is of interest to note that the elevated glycogen content and phosphorylase activity in the KH as compared to AH are similar to that of white (fast) muscle which has higher glycogen content and glycogenolysis[14].

Phosphorylase kinase, a key enzyme known for its regulation of the activity of phosphorylase and consequent glycogenolysis, is known to be calcium (Ca^{++}) dependent[15,16]. Elevated levels of Ca^{++} activate this kinase which in turn converts phosphorylase *'b'* to *'a'*. In the present study the KH has higher activity of phosphorylase *'a'*. This

TABLE I

The levels of proteins (mg/gm wet weight), glycogen (mg/gm wet weight), phosphorylase 'a' and 'b' (μ moles of inorganic phosphate/mg protein/hr) in control, cathodal and anodal halves of the amphibian gastrocnemius muscle

	Control $\frac{C1 + C2}{2}$	KH	% change over control	AH	% change over control	% change KH over AH
Protein	23·0± 2·0	24·2± 1·1	+5·4 N.S.	17·3± 3·1	−21·8 $p < 0.05$	+40·1 $p < 0.01$
Glycogen	1·4± 0·2	1·5± 0·3	+2·0 N.S.	1·2± 0·4	−1·6 N.S.	+20·9 N.S.
Phosphorylase '*a*'	31·8± 2·3	33·4± 2·5	+4·7 N.S.	21·5± 3·4	−21·6 $p < 0.05$	+43·2 $p < 0.05$
Phosphorylase '*b*'	77·1± 12·0	79·2± 9·0	+2·6 N.S.	51·2± 4·6	−35·5 $p < 0.01$	+11·4 $p < 0.05$

Values are mean ± S.D. of 6 observations; + or − indicates increase or decrease respectively.

may be due to higher calcium content and calcium precipitable proteins in this muscle half[17], and may not be due to electromigration of the enzyme as such since the pI of phosphorylase is 6·8 which is the same as that of the homogenate pH. It has been shown that high levels of AMP and low levels of ATP enhance the activity of phosphorylase '*a*'[18]. The KH was shown to have high levels of AMP and low levels of ATP compared to the AH[18] and these factors may contribute to the high level of activity of this enzyme in the KH. The AH has high levels of neutral protease and its associated lysis[11] when compared to the KH. Such increased proteolytic activity may bring about a degradation of cellular proteins and enzymes which may result in a decreased phosphorylase activity.

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