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NEURAL REGULATION OF PROTEINS IN THE SKELETAL MUSCLES OF FROG, *RANA HEXADACTYLA* (LESSON)

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ABSTRACT

Electrophoretograms of normal and denervated gastrocnemius and peronius muscles of frog showed the existence of 6 bands, which are discernable into four relatively slow moving and two relatively fast moving types. Following neurectomy, the changes in the protein fractions were conspicuous in both the muscles. The atrophy effects were more prominent in the peronius than in the gastrocnemius muscle. The probable significance of the protein characters on neurectomy have been discussed.

THE effect of innervation on the biochemical, metabolic and physiological properties of the muscle has received considerable attention[1-7]. However there are only few reports on the changes in the electrophoretic pattern of cell proteins following peripheral nerve section. The present study of the denervation induced changes in the protein fraction of the muscle was undertaken to have a better understanding of the range of control exerted by the nervous system on the innervating tissues, since this will throw light on the mechanism of the neural regulation of the biochemical attributes of skeletal muscle[8,9].

Two muscles were chosen for the present study, namely, (1) the gastrocnemius, which is a mixed muscle with high proportion of slow fibres and (2) the peronius, which is essentially a fast muscle, both being innervated by the common sciatic nerve[10].

MATERIALS AND METHODS

Rana hexadactyla were subjected to unilateral denervation by severing about one centimeter of the sciatic nerve from its origin on one side of the leg, while the contralateral muscle was considered as the control. They were fed *'ad lib'* with earthworms and water was changed regularly. One month post-operatively, they were sacrificed by pithing, the gastrocnemius and the peronius muscles were isolated with least injury and washed in amphibian Ringers' medium[11]. The cell proteins were extracted as previously described[12].

Polyacrylamide disc electrophoresis was conducted by the method of Davis and Orstein[13]. 0·1 ml protein extract was directly applied, followed by a small quantity of 40% sucrose solution. A direct current of 1·5 m Amps per tube was applied for 60 minutes at 4° C in 0·05 M tris-glycine buffer at pH 8·9. After electrophoretic run, the gels were removed and stained in 1% amido black in 7% acetic acid. The excess stain was removed by repeated washings with 7% acetic acid until the non-protein part of the gel became transparent.

RESULTS AND DISCUSSION

The electrophoretic mobility patterns of cell proteins of the gastrocnemius muscle revealed the existence of 6 bands. 3 of the bands are relatively slow moving type (*a, b* and c), one is intermediary (*d*) and the remaining two are fast moving types (Fig. 1). The pattern remains similar in the denervated muscle suggesting that the major classification of the cell proteins remains unchanged. However some of the protein bands indicate physical changes involving electromobility and the quantitative aspects. The bands *'d'*, *'e'* and *'f'* in the denervated peronius showed a tendency of increased electromobility while in the gastrocnemius similar changes could not be evinced on denervation. However on the quantitative point of view, as visualized in terms of the density of the band it is likely that bands *'d' 'e'* and *'f'* could be increasing on denervation in both the muscles. Thus the increase in

fast moving fractions in both types of muscles on denervation atrophy compared to the respective controls seemed to be an essential consequence of neurectomy, and similar changes have been reported previously[9, 12]. The apparently negligible change in the protein level of the *'a'*, *'b'* and *'c'* bands may indicate the lack of neurotrophic control on them and as suggested by Guth and Watson[9] they may be under the control of intrinsic factors present in the muscle[9].

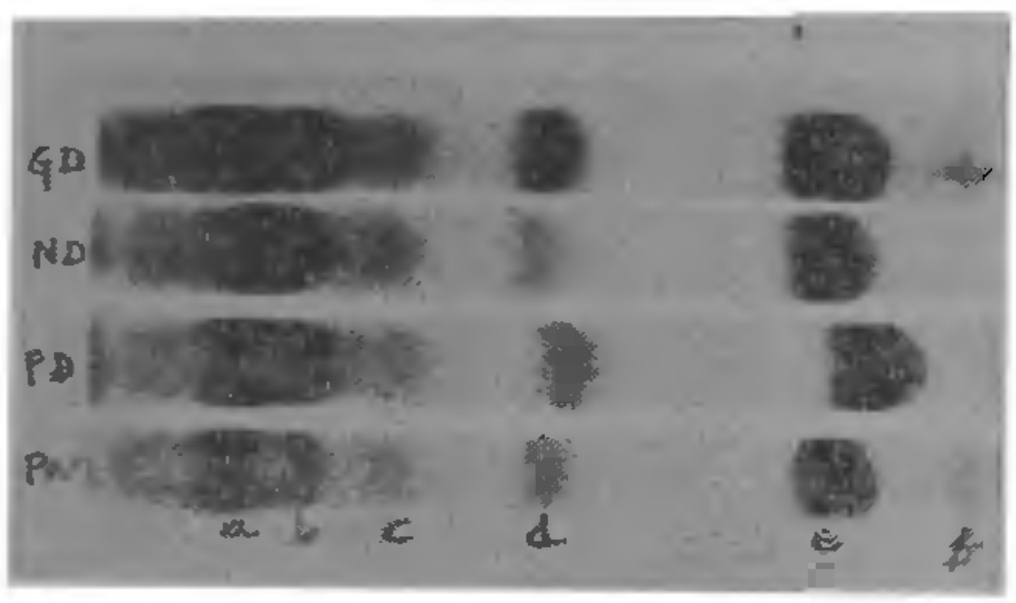

FIG. 1. Polyacrylamide disc electrophoretograms of soluble protein fractions of normal and denervated skeletal muscles, *viz.*, Gastrocnemius denervated (G.D.), Gastrocnemius normal (G.N.), Peronius denervated (P.D.), and Peronius normal (P.N.). The markings *a, b, c, d, e* and *f*, indicate protein fractions.

By comparison with the electromobility pattern of the frog serum it is likely to consider bands *'e'* and *'f'* to belong to the albumin and pre-albumin-types while *'a'*, *'b'*, *'c'* and *'d'* belong to that of globulin-type protein. The band *'d'* is comparable to the globulin-type of protein. The increase in the globulin-type of cell proteins especially that of *'d'* band may correspond to the increased proteolytic enzyme content which is globulin in nature[14]. Similar positive modulation of proteolytic enzymes by globulin-type proteins was reported in the amphibian gastrocnemius muscle[15] and the elevated protease activity in the denervated muscle could be under similar situation.

The elevated albumin and prealbumin-types as revealed by the density of *'d'* and *'f'* bands may probably contribute to the divalent ion complex and the increased Ca^{++} content reported in the denervated muscle is in agreement[16]. Even though the sciatic nerve innervates both the peronius (fast) and gastrocnemius (mixed) muscles, the denervation-induced response appear to be different in the fast muscle as compared to the mixed muscle.

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SHORT TERM GEO-STIMULATION AND P^{32} DISTRIBUTION IN ALASKA PEA SEEDLINGS

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PLANTS exhibit two growth responses—geotropism and gravimorphism, when their 'preferred' orientation with respect to gravity is disturbed. A number of changes in the cell and tissues probably underlie these responses.

Auxin moves to the lower side of the stimulated organ[1, 2] and increases the extensibility of the lower epidermal cells[3] with a concomittant enhancement of cellulase activity[4]. Induction of differences in the electrical potential[5] and redistribution of growth