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Phosphodiesterases in Normal and Dystrophic Human Muscle[1] (38677)

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Enzymes capable of hydrolyzing the *p*-nitrophenyl esters of thymidine 5′-phosphate (phosphodiesterase I) and thymidine 3′-phosphate (phosphodiesterase II) are widely distributed in mammalian tissues including skeletal muscle (1). These phosphodiesterases act on both ribo- and deoxyribo-internucleotide bonds at exposed ends of polynucleotide chains, but do not cleave RNA or DNA into large fragments. It seems possible that these enzymes by their ability to remove units sequentially from the ends of nucleic acid chains can control the fine structure of nucleic acids and thereby of synthetic processes in the cell. Because of the central role the nucleic acids play in protein metabolism and since there is a net loss of tissue protein in human muscular dystrophies, it was thought worthwhile to investigate the phosphodiesterases in muscle of these patients.

Materials and Methods. Chemicals. *p*-Nitrophenyl thymidine 5′-phosphate, *p*-nitrophenyl thymidine 3′-phosphate and bis-(*p*-nitrophenyl) phosphate were obtained from Sigma Chemical Company, St. Louis, MO. All other chemicals were of reagent grade and obtained locally.

Muscle. Muscle specimens were usually biopsies of gastrocnemius, quadriceps or deltoid obtained from 42 patients with various muscle and neuromuscular diseases. Histologically normal gastrocnemius and quadriceps muscles were obtained from five patients who showed no evidence of muscle disease. Specimens were immediately cooled and then stored at −18° for a maximum period of 2 wk. There was no significant difference between fresh and stored muscle phosphodiesterase activity.

The muscle was carefully trimmed of fat and connective tissue. It was then minced and homogenized with 9 vol of ice-cold distilled and deionized water in an all-glass homogenizer for about 2 min with intermittent cooling in ice. An aliquot of the tissue homogenate (0.1 ml) was used directly for enzyme assays.

Histological grouping. A fragment of the muscle tissue was routinely examined histologically and then broadly grouped as mildly or severely affected according to the degree of muscle fiber and interstitial tissue involvement as well as other criteria (2). The severely affected muscles were visibly abnormal and shown to contain markedly reduced noncollagen protein content (3).

Enzyme Assays. Phosphodiesterase I and phosphodiesterase II were assayed according to the method of Razzell (1). The reaction mixture for phosphodiesterase I contained 100 m*M* Tris–Hcl buffer, pH 8.9, 1 m*M* *p*-nitrophenyl thymidine 5′-phosphate, 10 m*M* $MgCl_2$ and 0.1 ml muscle homogenate in a total volume of 1 ml. After incubation at 37° for 1 hr the reaction was stopped with 4 ml of 0.1 *N* NaOH. The tubes were centrifuged and the absorbance of the clear solutions at 420 nm was determined and referred to *p*-nitrophenol standards treated in the same manner. Controls were kept in which the enzyme was added after the incubation. The assay system for phosphodiesterase II contained 100 m*M* citrate buffer, pH 6, 1 m*M* *p*-nitrophenyl thymidine 3′-phosphate and 0.1 ml muscle homogenate in a total volume of 1 ml. Other conditions were the same as employed for phosphodiesterase I.

The noncollagen protein (NCP) was determined as described previously (3).

Results. Factors influencing the activity. The results of a study of varied Mg^{2+} concentrations on phosphodiesterase I activity at pH 8.9 are shown in Table I. Maximal activity of phosphodiesterase I was obtained

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TABLE I. EFFECT OF Mg^{2+} ON PHOSPHODIESTERASE I ACTIVITY IN HUMAN MUSCLE HOMOGENATES.

Mg^{2+} (m*M*)	Phosphodiesterase I (nmoles/mg NCP/hr)
0	38.8
5	50.4
10	74.0
20	43.2

TABLE II. EFFECT OF CERTAIN FACTORS ON PHOSPHODIESTERASE II ACTIVITY IN HUMAN MUSCLE HOMOGENATES.

Addition (m*M*)	Phosphodiesterase II (nmoles/mg NCP/hr)
None	10.0
Mg^{2+} (10)	9.7
EDTA (1)	10.1
EDTA (10)	9.8

with a 10 m*M* concentration of Mg^{2+}. Magnesium ions or EDTA had no effect on phosphodiesterase II activity (Table II).

Variation of activity with pH. With homogenates of normal human quadriceps muscle, the activity against *p*-nitrophenyl thymidine 5′-phosphate in the presence of 10 m*M* Mg^{2+} had a pH optimum of 9, and hydrolysis of *p*-nitrophenyl thymidine 3′-phosphate showed a pH optimum of 6 (Fig. 1).

Phosphodiesterase I in normal and dystrophic human muscle. It can be seen that the activity of phosphodiesterase I is essentially in the normal range in mildly affected muscles obtained from patients with Duchenne dystrophy, other major forms of muscular dystrophies, inflammatory myopathies and spinal muscular atrophy (Fig. 2). On the other hand, the activity was increased three- to fourfold in severely abnormal muscles obtained from patients with different disease conditions (Fig. 2).

Phosphodiesterase II in normal and dystrophic human muscle. The results presented in Fig. 3 show that phosphodiesterase II behaves almost similarly to phosphodiesterase I in various pathologic muscles. There was, however, a slight increase in its activity in mildly affected muscles in Duchenne

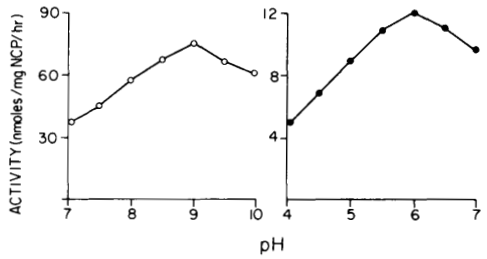

FIG. 1. Variation of activity with pH. Incubation systems were as described in the text. (○, phosphodiesterase I; ●, phosphodiesterase II.)

dystrophy and a slight decrease in muscles in myotonic dystrophy at an early stage of this disease. Otherwise, like phosphodiesterase I, phosphodiesterase II also showed greatly elevated activity in severely deranged muscles irrespective of disease etiologies. Highest increase (fivefold normal) in its activity was observed in muscles obtained from patients with advanced polymyositis or dermatomyositis (Fig. 3).

It is apparent that changes in the activity of phosphodiesterases seen in degenerating muscles bear a secondary re lationship to pathologic processes. These changes are not peculiar to Duchenne dystrophy or any other major form of dystrophy, but are found in a variety of muscle wasting conditions in man.

Discussion. The results reported here demonstrate that two phosphodiesterases with markedly different substrate specificities occur in human skeletal muscle homogenates. The pH optima for phosphodiesterase I and phosphodiesterase II in human muscle are approximately nine and six respectively, which are similar to rat spleen or kidney enzymes (1). Lack of consistent stimulation of phosphodiesterase I by Mg^{2+} and inhibition of phosphodiesterase II by EDTA in rat spleen homogenates have been reported (1). In the present work, human muscle phosphodiesterase I is shown to be optimally stimulated by 10 m*M* Mg^{2+} and phosphodiesterase II is not inhibited by 1 or 10 m*M* EDTA. The reason for this difference is not clear but it could be due to tissue or species difference. It has been found that muscle phosphodiesterase I hydrolyzes *p*-nitrophenyl thymidine 5′-phosphate about ten times faster than

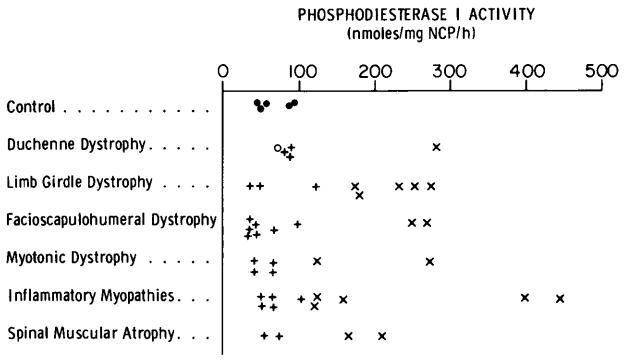

FIG. 2. Phosphodiesterase I activity of human skeletal muscles. (●, controls; ○, manifesting Duchenne dystrophy female carrier; +, mildly affected muscles; ×, severely affected muscles.)

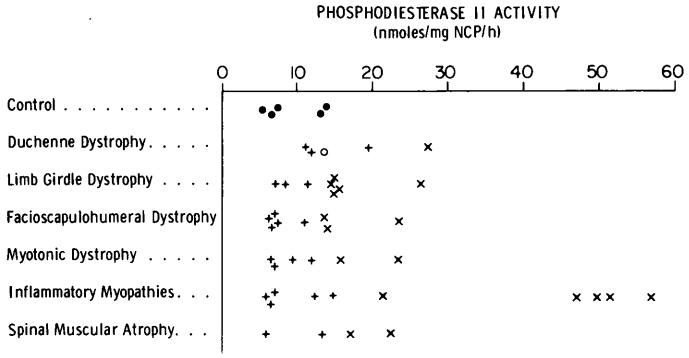

FIG. 3. Phosphodiesterase II activity of human skeletal muscles. Symbols are the same as in Fig. 2.

bis-(*p*-nitrophenyl) phosphate, another commonly used substrate for nonspecific phosphodiesterases.

In other tissues like liver or kidney of rats phosphodiesterase I has been shown to be localized predominantly in microsome fraction whereas phosphodiesterase II is largely soluble (1). The physiological roles of these enzymes are not known. However, location of phosphodiesterase I in microsomes, the site of protein synthesis, has led others to postulate that this enzyme, by its ability to control the fine structure of nucleic acids, may exert a regulatory role in protein syntheses (1). The acid pH optimum of phosphodiesterase II suggests that it might be primarily associated with other acid hydrolases in lysosomes. Indeed, an acid phosphodiesterase has been demonstrated in rat liver lysosomes (4).

The results of this study show that the activities of both phosphodiesterases are greatly elevated in severely abnormal muscles from patients with Duchenne dystrophy, other major forms of dystrophies, inflammatory myopathies and spinal muscular atrophies. The significance of the observed increases in phosphodiesterase I and phosphodiesterase II activities is unknown. Elevation of several acid, neutral and alkaline hydrolases also have been reported in severely diseased muscles from patients with various muscle wasting conditions (2, 3, 5–15). The increases noted in phosphodiesterase II as well as other acid hydrolases may reflect an increased lysosomal activity in response to cell injury. It is also possible that some of these increases may be related to a relatively greater loss of other sarcoplasmic enzymes and proteins from the muscle fibers (3).

Together with previous evidence on the presence of a number of ribonucleases in human muscle (8), the presence of the phosphodiesterases as well indicates a potential for nucleic acid hydrolysis which has not

yet been integrated with other cellular functions. It is of interest to note that ribonuclease activity at acid, neutral and alkaline pH ranges are reported to be markedly increased in muscles from patients with Duchenne dystrophy (8). However, it is not known whether these changes are early or specific features of this disease.

The data, showing increased levels of the phosphodiesterases in the degenerating muscle tissue in different clinical disease conditions, suggest that such enzymes are involved in and possibly responsible in part for muscle wasting in human muscle diseases. On the other hand, in contrast to our initial hypothesis, it now seems unlikely that these enzymes play any significant role in controlling the fine structure of nucleic acids in dystrophic muscle and thus in their subsequent control of protein synthesis in muscle fibers, especially in the early periods of the dystrophies and other diseases of striated muscle.

Summary. The levels and some properties of phosphodiesterase I and phosphodiesterase II were determined in biopsied muscles from normal persons and the results were extended to diseased muscles. The activities of both enzymes were generally normal or minimally elevated in mildly affected muscles from patients with Duchenne dystrophy, other forms of dystrophies and related neuromuscular diseases. However, in severely abnormal muscles, with advanced dystrophy or advanced polymyositis, the levels of these enzymes were greatly elevated.

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