5′-Nucleotidase Activity of Normal and Dystrophic Human Muscle[1] (37483)

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Histochemically it has been first shown by Bourne and Golarz (1) that the proliferating endomysium in dystrophic human muscle displayed significantly increased 5′-nucleotidase activity compared to controls. In a brief biochemical study Pennington (2) demonstrated the increase in muscle from patients with Duchenne muscular dystrophy (DMD). However, since no comparative data is available on this enzyme in other muscle and neuromuscular disorders we decided to measure its activity in muscle from patients with major forms of muscular dystrophies and with selected myopathies and neuropathies. We have also correlated the enzyme activity with histopathological abnormalities of the affected muscle.

Materials and Methods. Muscle specimens were gastrocnemius, quadriceps or deltoid obtained at surgery from 36 patients with various muscle diseases. Normal samples of the same muscles were also obtained at surgery from 6 persons who had no muscle disease. The preparation of muscle homogenate and the method of histologically classifying the muscle biopsies as "mildly" or "severely" affected were described earlier (3, 4). 5′-Nucleotidase activity was estimated at pH 8.5 with AMP as substrate as described by Heppel and Hilmoe (5) and the noncollagen protein was determined as described previously (3).

Results. 5′-Nucleotidase activity was significantly increased in mildly affected muscles from patients with DMD (Table I). The increase was 5.8-fold compared to normal. On the other hand, mildly abnormal muscles from patients with the limb girdle, facioscapulohumeral and myotonic forms of muscular dystrophies, polymyositis and spinal muscular atrophies showed values essentially in the normal range. High enzyme activity was, however, found in severely abnormal muscles from patients affected with diseases of various etiologies: limb girdle dystrophy, myotonic dystrophy, congenital myopathy, polymyositis and certain denervating diseases. The enzyme activity was 12.8-fold higher than normal in degenerating muscles from patients with polymyositis and only moderately increased in severely atrophic muscles from patients with spinal muscular atrophy and peripheral neuropathy.

Discussion. The results of this study confirm the earlier histochemical (1) and biochemical (2) findings of increased muscle 5′-nucleotidase activity in DMD. They also demonstrate that the increase is found in early stages of this disease. Such observations, however, fit with the pathological evidence that indicates that at least in some cases an increased amount of collagen is found in the early stages of this disease (6). Collagen nitrogen is also shown to be increased in dystrophic muscle by biochemical studies (7) with a concomitant decrease in the noncollagen protein content (3, 7). Recently Bradley *et al.* (8) have observed significant endomysial and perimysial fibrosis in muscle biopsies from very young patients with DMD. These findings led the authors to postulate that these changes are not secondary to muscle fiber necrosis, phagocytosis and regeneration, but rather represent a primary response. This concept is supported by the recent *in vitro* experiments of Ionasescu, Zellweger and Conway (9) which have demonstrated that muscle polyribosomes from patients with DMD can synthesize an increased amount of protein especially of collagen compared to normal.

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TABLE I. 5′-Nucleotidase Activty of Human Muscle Homogenates.[a]

Disease	No. of cases	Age (yr)	Degree of tissue damage	5′-nucleotidase
None	6	5–60	—	1.74 ± 0.269[b]
DMD	4	5–8	Mild	10.16 ± 2.035[c]
Myotonic dystrophy	6	24–61	Mild	1.95 ± 0.375
Facioscapulohumeral dystrophy	4	15–44	Mild	1.88 ± 0.264
Limb girdle dystrophy	1	39	Mild	1.36
Polymyositis	7	14–64	Mild	2.69 ± 0.586
Spinal muscular atrophy	4	22–54	Mild	2.25 ± 0.415
Limb girdle dystrophy	2	22/29	Severe	10.95 / 18.42
Myotonic dystrophy	1	38	Severe	11.05
Polymyositis	4	23–67	Severe	22.28 ± 5.430[c]
Congenital myopathy, undefined	1	23	Severe	9.36
Spinal muscular atrophy	1	22	Severe	5.94
Peripheral neuropathy	1	64	Severe	6.48

[a] Activity is expressed as nanomoles of phosphate released per milligram of noncollagen protein/minute.

[b] Mean ± standard error.

[c] Significantly different from normal by the t test ($p < 0.001$).

It is of interest to note that in contrast to 5′-nucleotidase nonspecific alkaline phosphatase (assayed with *p*-nitrophenyl phosphate as substrate at pH 10.3) activity of muscle remains essentially unchanged in early DMD (4). The two enzymes also differ in their histochemical localizations. Unlike 5′-nucleotidase which occurs in the proliferating endomysium (1), the nonspecific alkaline phosphatase has been found in certain abnormal muscle fibers themselves (10). These observations suggest that the increased 5′-nucleotidase activity in homogenates of diseased muscles may be largely contributed to by the relatively greater complement of collagen nitrogen in them. This could explain the findings of an elevated level of 5′-nucleotidase in degenerating muscles from patients affected with limb girdle and myotonic forms of muscular dystrophies and in polymyositis since extensive fibrosis is also noticed in advanced stages of these disorders (6).

Summary. 5′-Nucleotidase activity of muscle in various muscle diseases was determined and the results were correlated with the histological abnormalities. Among various muscle diseases, Duchenne muscular dystrophy at an early stage showed significantly increased enzyme activity. Increased activity was, however, observed in severely abnormal muscles from patients with other major forms of muscular dystrophies, polymyositis and also certain neurogenic diseases. The increase in the activity of 5′-nucleotidase in diseased muscle appears to be related to the pathological evidence of fibrosis in it.

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