Disruption of the Purine Nucleotide Cycle

A POTENTIAL EXPLANATION FOR MUSCLE DYSFUNCTION IN MYOADENYLATE DEAMINASE DEFICIENCY

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ABSTRACT A patient with symptoms of easy fatigability, postexercise myalgias, and delayed recovery of muscle strength after activity is described. Skeletal muscle from this patient had <1.0% normal myoadenylate deaminase activity and NH_3 was not released from muscle after ischemic exercise. In association with this enzyme deficiency, exercise led to a >90% reduction in muscle content of adenine nucleotides. No inosine monophosphate accumulated after exercise and total purine content of the muscle fell to 21% of control. Repletion of the adenine nucleotide pool in this patient was delayed compared to controls, and ATP content had only returned to 68% of control at 165 min after exercise. These studies demonstrate that disruption of the purine nucleotide cycle as a consequence of myoadenylate deaminase deficiency results in marked alterations in ATP content of muscle, and potentially, these changes in ATP content could account for muscle dysfunction in this patient.

INTRODUCTION

Myoadenylate deaminase (EC 3.5.4.6) catalyzes the deamination of AMP to inosine monophosphate (IMP),[1] and regulation of the activity of this enzyme by purine nucleotides and inorganic phosphate (Pi) may play an important role in controlling the purine nucleotide cycle in skeletal muscle (1). The purine nucleotide cycle in turn may control several different aspects of muscle metabolism, such as glycolysis, adenylate kinase activity, NH_3 production, and generation of fumarate from amino acids for oxidative metabolism (2). Deficiency of myoadenylate deaminase has been reported in association with skeletal muscle dysfunction (3, 4). The clinical picture of muscle dysfunction in subjects with myoadenylate deaminase deficiency has been variable (3, 4), and it has been suggested that the clinical symptoms reported by patients harboring this enzyme deficiency may be unrelated to the absence of myoadenylate deaminase activity (5). If the latter conclusion were correct, one would not expect the deficiency of myoadenylate deaminase to produce major alterations in muscle content of nucleotides, such as ATP, which are critical for muscle contraction. On the other hand, if this enzyme and a functioning purine nucleotide cycle were important in skeletal muscle metabolism, one would expect to be able to demonstrate significant alterations in muscle content of purine nucleotides in patients with myoadenylate deaminase deficiency during exercise. We hypothesized that the deficiency of myoadenylate deaminase would result in skeletal muscle with features of purine metabolism similar to that of myocardium, a tissue with low intrinsic activity of this enzyme (6). When work outstrips energy

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[1] *Abbreviation used in this paper*: IMP, inosine monophosphate.

supplies in the heart there is rapid depletion of the purine nucleotide pool, nucleosides and bases are lost from the muscle, and repletion of the nucleotide pool is slow, requiring several hours to restore adenine nucleotide content to control levels (7, 8).

METHODS

Ischemic exercise test. A blood pressure cuff was placed around the biceps with the subject supine and resting. After obtaining a base-line anticubital venous sample for lactate and ammonia, the cuff was inflated above systolic pressure, and the patient instructed to rapidly open and close the fist until muscle fatigue set in. The cuff was released and venous samples obtained at 0, 2, 6, and 10 min after release for lactate and ammonia determinations.

Muscle metabolites. After infiltrating the skin with 1% xylocaine, a 5–15-mg biopsy of vastus lateralis was obtained with a Travenol Tru-Cut needle (Travenol Laboratories, Inc., Morton Grove, Ill.) and immediately plunged into liquid nitrogen. Biopsies were obtained at zero time, immediately after 15 min of step-up exercise on a 12-in. platform at 17–19 steps/min, and again after the patient had recovered for 45 and 165 min postexercise. The biopsies were extracted with 12% trichloroacetic acid and neutralized with an equal volume of 0.5 M tri-*N*-octylamine in freon. Nucleotides were quantified on a Waters high performance liquid chromatography (HPLC) instrument (Waters Associates, Inc., Milford, Mass.) using a Whatman Partisil-SAX anion exchange column (Whatman Inc., Clifton, N. J.) (9); nucleosides and bases were quantified on this instrument using a Waters μBondapak C^{18} reverse-phase column (Waters Associates, Inc., Milford, Mass.) (10).

Enzyme assays. Vastus lateralis biopsies were frozen in liquid nitrogen in <2 min and homogenized in water with a glass mortar and pestle. Myoadenylate deaminase activity was quantified in 30 mM triethanolamine-HCl buffer, pH 6.8, that contained 300 mM KCl, 0.2 mM [^{14}C]AMP (2,150 dpm/nmol), and 15–50 μg of muscle extract. After incubation at 37°C for 30 min, the reaction was stopped by boiling for 2 min, and 5 μl of the supernate were spotted on polyethylene imine (PEI)-cellulose thin-layer chromatography plates. Plates were developed in 1.5 M monobasic potassium phosphate and the IMP and AMP spots visualized with ultraviolet light. Creatine phosphokinase activity was quantified with an assay kit obtained from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. All assays were linear with respect to time of incubation and protein concentration, the latter determined by the method of Lowry et al. (11).

Histochemical stain. 10-μm transverse sections of frozen muscle were incubated on coverslips in triethanolamine buffer, pH 6.1, that contained 3.2 mM dithiothreitol, 1.2 mM nitroblue tetrazolium, 0.2 M KCl, and 1.2 mM AMP. The release of NH_3 into the immediate area of the tissue section with myoadenylate deaminase activity led to the formation locally of the insoluble diformazan. In 43 biopsies studied, the only one that failed to give a positive stain was from this patient.

Informed consent was obtained from the patient before muscle biopsy, and the studies described in this report met the institutional guidelines for experimentation in human subjects (Baylor College of Medicine).

RESULTS

Patient description. The patient, a 36-yr-old female, has noted easy fatigability and exercise-induced muscle aches since childhood, but first developed significant impairment of her daily activities at age 21. Her symptoms have predominantly affected the proximal muscles and have been characterized by rapid fatigability, weakness, and myalgias. There has been a steady progression of these symptoms over the last 15 yr. She now fatigues and develops myalgias from activities such as dressing or walking from one room to another. The patient cannot climb stairs or walk more than one block. She requires a rest period of several hours after mild activity before her strength returns to base line. Because she experiences no symptoms at rest, most of her time is spent in bed. She has adapted to her illness by alternating periods of activity with rest to complete simple household chores.

Right grip strength measured 66 lb initially and declined on successive grips to 55, 45, and 36 lb. After 1 min of rest it recovered to 55 lb. Serum creatine phosphokinase and aldolase activities have been

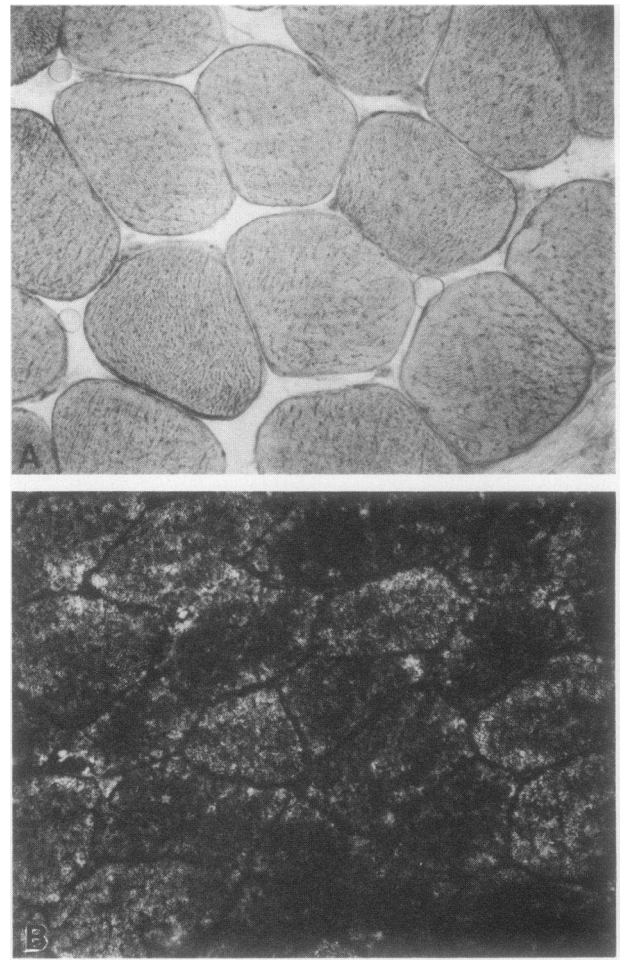

FIGURE 1 (A) Myoadenylate deaminase histochemical stain from the patient. (B) Myoadenylate deaminase histochemical stain from a control.

TABLE I
Results of Exercise Testing and Enzyme Assays

	NH_3 production		Lactate production		Enzyme activity	
	Patient	Controls*	Patient	Controls*	Patient	Controls‡
	μmol/liter		*meq/liter*		*nmol/min/mg*	
Ischemic exercise testing						
Base line	20	31±12	1.7	1.5±0.5		
0 min	30	111±68	4.2	5.4±2.5		
2 min	32	117±50	3.4	5.1±1.5		
6 min	28	98±49	2.7	4.0±1.5		
10 min	32	70±35	2.6	3.3±1.6		
Enzyme assays in muscle extract						
Myoadenylate deaminase					0.18	58±35
Creatine phosphokinase					15	20±9

Results are presented as the mean±1 SD.
* *n*, 20 normal volunteers.
‡ *n*, biopsies from 44 patients with muscular symptoms and normal NH_3 production on ischemic testing were used as controls. Myoadenylate deaminase activity ranged from 14–134 nmol/min per mg in this population.

normal throughout the course of her illness. Biopsy of the biceps examined by routine stains (trichrome, DPNH-TR reductase, ATPase) was also normal except for scattered small, angular fibers 3 μm Diam. Needle electromyogram and nerve conduction studies gave normal results, but repetitive stimulation showed a 65% incrementing response at 10 Hz and an 11% post-exercise increment followed by a 9.3% decrement at 2 Hz.

At age 33 yr the patient was diagnosed as having myasthenia gravis, received prednisone for 6 mo, and underwent a transternal thymectomy. However, her symptoms of easy fatigability and postexercise myalgias became progressively worse over the next 3 yr, and a diagnosis of myoadenylate deaminase deficiency was made based on the data presented in Table I. On ischemic exercise testing the patient produced normal amounts of lactate but essentially no ammonia. Direct assay of muscle extract revealed <1% of control myoadenylate deaminase activity with normal activity of creatine phosphokinase. Histochemical stain of muscle for NH_3 production from AMP was also negative (Fig. 1).

Changes in purine content of muscle after exercise. Results of muscle analyses for purine nucleotides, nucleosides, and bases at rest, immediately following exercise, and after 45 and 165 min of recovery from exercise are recorded in Table II. At rest, muscle from the patient contained adenine nucleotides in concentrations comparable to those found in biopsies from two patients with other types of myopathies (4.20 and 4.85 μmol of ATP/g), as well as controls described in the literature (12). In one other patient with myoadenylate deaminase deficiency purine nucleotide content of resting muscle was also reported to be normal (3). However, after the exercise protocol described above, ATP and ADP content fell to <10% of the resting values with essentially no change in AMP content. IMP was not detectable (<0.01 μmol/g) in any of the muscle biopsies from this patient. Depletion of the adenine nucleotide pools was associated with an increase in muscle content of adenosine, inosine, and hypoxanthine, but this amounted to only 10% of the purine lost from the adenine nucleotide pools. After exercise total purine content of the muscle (adenine and guanine nucleotides, nucleosides, and bases) was reduced to 21% of control.

During the recovery period there was an increase in the adenine nucleotide content of the muscle over that found at the completion of exercise. However, after

TABLE II
Effect of Exercise on Nucleotide Content of Muscle from Myoadenylate Deaminase Deficient Patient

	Rest	Post-exercise	45-min recovery	165-min recovery
	μmol/g			
Adenine nucleotides and catabolites				
ATP	4.35	0.29	2.57	2.94
ADP	0.67	0.06	0.43	0.43
AMP	0.01	0.03	0.02	0.01
IMP	<0.01	<0.01	<0.01	<0.01
Adenosine	<0.005	0.079	<0.005	<0.005
Inosine	0.012	0.181	0.123	0.017
Hypoxanthine	<0.01	0.216	<0.01	<0.01
Other nucleotides				
GTP	0.14	<0.01	0.09	0.07
NAD	0.21	0.02	0.16	0.15
UTP	0.08	<0.01	0.05	0.05
CTP	0.06	<0.01	0.03	0.04

45 and 165 min of complete rest the ATP content had only returned to 59 and 68% of control, respectively.

Adenylate energy charge only fell from 0.93 to 0.84 after exercise. This is similar to the fall in energy charge noted in muscle of normal subjects undergoing vigorous exercise (12).

Changes in muscle content of NAD, GTP, UTP, and CTP paralleled the changes in ATP content both during exercise and during the recovery period (Table II). Reduction in muscle content of NAD with exercise has also been noted by others (13). Changes in GTP, UTP, and CTP with exercise have not been reported previously. One might speculate that the reduction in muscle content of these nucleoside triphosphates is a reflection of the decrease in ATP, since ATP is the immediate energy donor for synthesis of the other nucleoside triphosphates.

DISCUSSION

The patient described in this report has a functional deficiency of myoadenylate deaminase activity as demonstrated by a failure to produce IMP and NH_3 after exercise. This enzymatic deficiency has been documented by direct radiochemical assay of muscle extract and histochemical stain of muscle tissue for myoadenylate deaminase activity. These data establish that this patient has a high-grade deficiency of myoadenylate deaminase activity in skeletal muscle. Additional studies will be needed to determine if this functional deficiency of myoadenylate deaminase is the primary defect in this patient or whether it is a secondary manifestation of a more fundamental alteration in the muscle.

This deficiency of myoadenylate deaminase is associated with a number of changes in purine nucleotide content of muscle after exercise and during the post-exercise recovery period that are different from the changes observed in normal subjects. First, in normal human volunteers exercise that is more strenous and prolonged than that performed by this patient resulted in a modest drop in the ATP content of the vastus lateralis to 56–86% of control values (12, 14). In this patient, undergoing less strenuous exercise, muscle ATP content fell to <10% of control. The more pronounced drop in ATP content of muscle in this patient during exercise may be explained in part by the hypothesis that increased flux through the purine nucleotide cycle during exercise generates energy for muscle contraction by converting amino acids to fumarate and malate (2). Second, in normal subjects, the exercise-induced decrement in ATP is matched by an almost stoichiometric rise in IMP via deamination of AMP by myoadenylate deaminase (14). As a consequence, there is little change in total purine nucleotide content of muscle even during vigorous exercise. In this patient there was no accumulation of IMP. The depletion of ATP was associated with an increase in muscle content of purine nucleosides and bases, but quantitatively this did not match the loss in adenine nucleotides. Because cells are more permeable to nucleosides and bases than phosphorylated compounds such as IMP, one might postulate that the loss in total purine content of muscle in the myoadenylate deaminase-deficient patient is the result of diffusion of nucleosides and bases out of the cell into the vasculature during exercise. This sequence of events has been documented to take place in cardiac muscle (a tissue relatively deficient in myoadenylate deaminase activity) during perfusion of an ischemic area of myocardium (7). Results of the present study do not exclude the possibility that a defect in membrane permeability to nucleotides could have contributed to the loss in total purine compounds during exercise. Third, in normal subjects repletion of the ATP pool following vigorous exercise is complete in 15–30 min and is temporarily associated with a reduction in the IMP content of muscle (14). Thus, in normal subjects the IMP that accumulates during exercise is used to replenish the ATP pool during recovery from exercise. In the patient with myoadenylate deaminase deficiency disruption of the purine nucleotide cycle is associated with a slower rate of repletion of the ATP pool, requiring several hours for restoration of ATP to control concentrations. These results suggest that the rate of IMP synthesis from the salvage and *de novo* pathways may be limiting for adenine nucleotide synthesis in skeletal muscle after exercise.

Because ATP is the only direct source of energy for muscle contraction, alterations in skeletal muscle content of this metabolite in the myoadenylate deaminase-deficient patient could provide an explanation for some of the clinical symptoms observed in this disorder. The profound drop in ATP after moderate exercise could account for easy fatigability. The prolonged time required for repletion of the ATP pool could account for the delay in return of muscle strength after physical activity.

Results of these studies suggest a previously unrecognized function for the purine nucleotide cycle. This series of reactions provides a mechanism for preserving the purine nucleotide content of exercising muscle through accumulation of IMP, a nondiffusable nucleotide, which can be used to rapidly restore the ATP pool during periods of rest.

REFERENCES

1. Wheeler, T. J., and J. M. Lowenstein. 1979. Adenylate deaminase from rat muscle: regulation by purine nucleotides and orthophosphates in the presence of 150 mM KCl. *J. Biol. Chem.* **254**(18): 8994–8999.
2. Lowenstein, J. M. 1972. Ammonia production in muscle

and other tissues: the purine nucleotide cycle. *Physiol. Rev.* **52**(2): 382–414.
3. Engel, A. G., C. S. Potter, and J. W. Rosevear. 1964. Nucleotides and adenosine monophosphate deaminase activity of muscle in primary hypokalaemic periodic paralysis. *Nature (Lond.).* **202:** 670–672.
4. Fishbein, W. N., V. W. Armbrustmecher, and J. L. Griffin. 1978. Myoadenylate deaminase deficiency: a new disease of muscle. *Science (Wash. D. C.)* **200:** 545–548.
5. Shumate, J. B., R. Katnik, M. Ruiz, K. Kaiser, C. Frieden, M. H. Brooke, and J. E. Carroll. 1979. Myoadenylate deaminase deficiency. *Muscle and Nerve.* **2:** 213–216.
6. Solano, C., and C. J. Coffee. 1978. Differential response of AMP deaminase isozymes to changes in the adenylate energy charge. *Biochem. Biophys. Res. Commun.* **85**(2): 564–571.
7. Parker, J. C., E. E. Smith, and C. E. Jones. 1976. The role of nucleoside and nucleobase metabolism in myocardial adenine nucleotide regeneration after cardiac arrest. *Cir. Shock.* **3:** 11–20.
8. Swain, J. L., R. L. Sabina, P. A. McHale, J. C. Greenfield, and E. W. Holmes. 1980. Prolonged myocardial ATP depletion after brief ischemia in the open-chested dog. *Clin. Res.* **28:** 473A. (Abstr.)
9. Rose, L. M., and R. W. Brockman, 1977. Analysis by HPLC of 9-β-D-arabino-furanosyladenine-5′-triphosphate levels in murine leukemia cells. *J. Chromatogr.* **133:** 335–343.
10. Schweinsberg, P. D., and T. L. Loo. 1980. Simultaneous analysis of ATP, ADP, AMP and other purines in human erythrocytes by high-performance liquid chromatography. *J. Chromatogr.* **181:** 103–107.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
12. Sutton, J. R., C. J. Toews, G. R. Ward, and I. H. Fox. 1980. Purine metabolism during strenuous muscular exercise in man. *Metab. Clin. Exp.* **29**(3): 254–260.
13. Graham, T., G. Sjogaard, H. Lollgen, and B. Saltin, 1978. NAD in muscle of man at rest and during exercise. *Pflueg. Arch. Eur. J. Physiol.* **376:** 35–39.
14. Sahlin, K., G. Palmskog, and E. Hultman. 1978. Adenine mucleotide and IMP contents of the quadriceps muscle in man after exercise. *Pflueg. Arch. Eur. J. Physiol.* **374:** 193–198.