SHORT COMMUNICATION

The purine nucleotide profile in mouse, chicken and human dystrophic muscle: an abnormal ratio of inosine plus adenine nucleotides to guanine nucleotides

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Summary

1. AMP, ADP, ATP, IMP, GDP, GTP and adenylosuccinate have been measured by high pressure liquid chromatography in three types of animal muscular dystrophy and in a human patient with Duchenne muscular dystrophy.

2. Abnormalities in nucleotide content varied from one dystrophy to another.

3. In each case, however, the ratio [total adenine nucleotide + IMP]/[total guanine nucleotides] was lower in dystrophic muscle, even when severely exercised or ischaemic muscles were used.

4. The practical advantages of this assay for diagnosis of muscular dystrophy are discussed.

Key words: adenine nucleotides, guanine nucleotides, muscular dystrophy, purine nucleotides.

Abbreviations: GDP, GTP, guanosine di- and tri-phosphate; IMP, inosine 5ʹ-phosphate.

Introduction

The abnormal purine nucleotide concentrations found in dystrophic muscle [1–5] are not useful in diagnosis because even the most clear-cut change, a 20–60% lower ATP content, is too variable and non-specific. Also, the high fat and collagen content of dystrophic muscles makes it necessary to relate nucleotide concentrations to a factor such as non-collagen nitrogen. Finally, the biopsy must be rapidly freeze-clamped in liquid nitrogen-cooled tongs to prevent nucleotide interconversion. It is difficult to obtain such specimens from dystrophic children; only a single human biopsy meeting these criteria has been made available to us.

We now report a number of abnormalities in the muscle purine content in four types of animal dystrophy and in the human case mentioned above. One demonstrable abnormality may prove diagnostically useful.

Methods

The experimental details were as previously given [6] except where indicated. The dystrophic mice were from the 129 ReJ dy/dy strain (from a colony maintained by Mrs R. Watts, Guy's Hospital Medical School, London) and the C57 Bl/6J dy/dy strain were from our own colony; chickens were of line 304 (early onset of dystrophy [7]) from a colony maintained by Dr P. Barnard (Imperial College, London). Mice were anaesthetized by intraperitoneal injection of sodium pentobarbitone (80 mg/kg) and chickens were anaesthetized by halothane. Freeze-clamped muscle specimens were taken from the posterior latissimus dorsi of chickens and the hamstrings group of mice. Spontaneous twitching of the hind limbs of dystrophic C57 Bl/6 mice was suppressed by intraspinal injections of lignocaine.

Human dystrophic muscle was obtained from...
the biceps muscle of a boy of 4 years who had Duchenne muscular dystrophy. Control muscle was obtained from the rectus abdominis of an adult undergoing laparotomy. This is an unsatisfactory control specimen but it would rarely be possible to obtain suitable specimens from the biceps of healthy children.

Extraction of muscle biopsies was performed by the method of Lush et al. [6] except that CMP was used as the internal standard. Portions (25 μl) of the extracts were analysed by high pressure liquid chromatography with a 100 mm × 5 mm Hypersil-APS weak anion-exchange column and a Waters Instruments (Cheshire, U.K.) HPLC system. A 9 min linear gradient was used, prepared from potassium phosphate (1-2 g/l), pH 2-5 (Aristar grade; BDH Chemicals, Poole, Dorset, U.K.) and Arister potassium phosphate (100 g/l), pH 2-5, both made up with fresh doubly glass distilled water and AnalaR grade hydrochloric acid.

Nucleotide concentrations were expressed in terms of non-collagen protein, which was measured by a modification of Lilienthal's assay [8] in which the delipidated protein pellet from the extraction [6] was dissolved in sodium hydroxide (50 mmol/l) and measured by the biuret method [9].

**Results**

The two types of dystrophic muscles from mice and that from the single human patient showed a low ATP content, whereas dystrophic chicken muscle ATP was normal (Table 1). Adenylosuccinate levels were elevated in dystrophic C57 Bl/6 muscle and dystrophic chicken muscle but normal in dystrophic 129 ReJ mouse muscle. In all dystrophic groups the total guanine nucleotide (GDP + GTP) concentration was raised.

In view of the raised guanine nucleotide levels and generally lowered ATP levels in dystrophic muscle one would expect that the ratio [adenine nucleotides]/[guanine nucleotides] would be low in dystrophic muscle, and this is indeed the case in relaxed, rapidly freeze-clamped muscle (Table 1). During severe work (not shown) or ischaemia (not shown), however, there is a marked conversion of ATP into IMP so that the relevant ratio is [adenine nucleotides + IMP]/[guanine nucleotides] ([A + I]/[GI] ratio). The value of this ratio in freeze-clamped muscle specimens is shown in Table 1; since there was no significant difference between the ratios in relaxed and working muscle (87.7 ± 4.4 and 80.3 ± 3.0 respectively, P = 0.2) these data have been combined.

| Table 1: Muscle nucleotide concentration in freeze-clamped muscle samples |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | C57 mouse, normal (7) | C57 mouse, dystrophic (6) | 129 ReJ mouse, normal (10) | 129 ReJ mouse, dystrophic (2) | Chicken, normal (10) | Chicken, dystrophic (4) | Human, normal (10) | Human, dystrophic (10) |
| AMP                | 0.09 ± 0.02       | 0.10 ± 0.05       | 0.13 ± 0.04       | 0.13 ± 0.05       | 0.05 ± 0.02       | 0.04 ± 0.02       | 0.02 ± 0.04       | 0.04 ± 0.02       |
| ADP                | 0.03 ± 0.01       | 0.03 ± 0.02       | 0.03 ± 0.02       | 0.03 ± 0.02       | 0.03 ± 0.02       | 0.03 ± 0.02       | 0.03 ± 0.02       | 0.03 ± 0.02       |
| IMP                | 0.01 ± 0.01       | 0.02 ± 0.02       | 0.02 ± 0.02       | 0.03 ± 0.02       | 0.02 ± 0.03       | 0.03 ± 0.02       | 0.02 ± 0.02       | 0.03 ± 0.02       |
| GDP                | 0.12 ± 0.01       | 0.15 ± 0.03       | 0.16 ± 0.03       | 0.18 ± 0.04       | 0.20 ± 0.03       | 0.24 ± 0.04       | 0.25 ± 0.03       | 0.25 ± 0.03       |
| GTP                | 0.12 ± 0.01       | 0.14 ± 0.02       | 0.13 ± 0.02       | 0.16 ± 0.03       | 0.16 ± 0.02       | 0.20 ± 0.03       | 0.15 ± 0.02       | 0.20 ± 0.03       |
| [A + I]/[GI] ratio | 0.32 ± 0.01       | 0.32 ± 0.02       | 0.32 ± 0.02       | 0.34 ± 0.03       | 0.41 ± 0.02       | 0.41 ± 0.02       | 0.42 ± 0.03       | 0.43 ± 0.02       |
To see whether rapid freeze-clamping was essential we measured the \([A + I]/[G]\) ratio in muscles of C57 Bl/6 mice after 5 min ischaemia at 25°C; it was 62.5 ± 2.6 (n = 4) in normal muscle and 46.5 ± 2.0 (n = 4) in dystrophic muscle. These results are significantly different (P < 0.006). All the 14 dystrophic C57 Bl/6 muscles (from relaxed, exercised or ischaemic groups) had lower \([A + I]/[G]\) ratios than any of the 15 specimens from normal animals.

Discussion
Our findings on adenine and guanine nucleotide concentrations agree broadly with those of other workers [1–5]; values for adenylosuccinate and IMP have not hitherto been reported. The only ‘universal’ abnormality was the decreased \([A + I]/[G]\) ratio, which was approximately halved in all the four dystrophies studied. This abnormality could be caused by a defect in the control of adenine and guanine nucleotide synthesis since both these pathways begin with IMP. In this context it is of interest that there was a significant fall (P = 0.005) in \([A + I]/[G]\) ratio when normal muscle was made ischaemic whereas in dystrophic muscle there was no significant change (P = 0.6). The fall in normal muscle \([A + I]/[G]\) ratio probably results from partial conversion of IMP into guanine nucleotide.

Sanada & Yamaguchi [10] have observed a lowered (i.e. 3% of control) adenylosuccinase activity in C57 Bl/6J dy/dy mice; this appears to be the same dystrophy as we investigated on the 129 ReJ background. It would be reasonable to surmise that partial loss of adenylosuccinase activity might cause the observed decrease in the \([A + I]/[G]\) ratio.

The adenylosuccinase activity in a variety of human muscular dystrophies has been found to be normal [11]; however, adenylosuccinase is only one of a number of enzymes whose activity or control would have an effect on the adenine nucleotide/guanine nucleotide ratio.

The \([A + I]/[G]\) ratio provides a convenient marker for muscular dystrophy. In the animal results there was no overlap between the normal and dystrophic ranges. The particular advantage of this measure of purine nucleotide abnormality is that small amounts of tissue are required and the method does not rely on the measurement of the amount of tissue or, within limits which will need to be determined for human specimens, rapid excision and freezing of the muscle sample.

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References