Non-invasive ^{31}P magnetic resonance spectroscopy revealed McArdle disease in an asymptomatic child

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Abstract. A Turkish couple suffering from McArdle disease (myophosphorylase deficiency) and their two sons aged 5 and 9 years, respectively, were studied using ^{31}P phosphorus magnetic resonance spectroscopy (^{31}P MRS). During exercise both sons showed the same pathological pattern as their parents. In contrast to healthy volunteers, intracellular pH (pH_i) as measured by ^{31}P MRS every 10 s during exercise, never fell below the lower 95% confidence limit (6.94) of pH_i at rest (7.06), but tended to be raised. It is of special interest that the 5-year-old boy was completely asymptomatic, although the enzyme defect seems to be fully expressed at the cellular level.

Key words: Glycogen storage disease type V – Myophosphorylase deficiency – Nuclear magnetic resonance

Introduction

McArdle disease (myophosphorylase deficiency, glycogenosis type V) is caused by the deficiency of the enzyme glycogen phosphorylase in skeletal muscle (myophosphorylase) leading to a blockage of the enzymatic cleavage of muscle glycogen, which is essential for the energy supply of the muscle cell during exercise. This disease is a rare inborn error of metabolism transmitted as an autosomal recessive trait and is characterized by premature muscle fatigue, stiffness and painful muscle cramps provoked by moderate exercise. At rest, patients are asymptomatic [8]. The onset of clinical symptoms is usually not before adolescence [3, 8].

The pathophysiology of McArdle disease has been investigated intensively by classical methods [7, 9, 10, 14] as well as by ^{31}P phosphorus magnetic resonance spectroscopy (^{31}P MRS) [1, 3–5, 12]. The latter has become a well established non-invasive method for investigating defects of glycolysis such as myophosphorylase deficiency. However, it has been rarely used as a diagnostic tool [12], although in asymptomatic children of patients with this myopathy invasive investigations such as muscle biopsies are questionable. We demonstrate that ^{31}P MRS is quick and effective to reveal the characteristics

of myophosphorylase deficiency noninvasively in symptomatic patients and in asymptomatic children.

Patients and methods

Patients

Both parents and the two sons of a Turkish family were examined by ^{31}P MRS. The family history showed multiple consanguinity: the mother's and the father's grandfathers were brothers and their grandmothers were sisters. The biochemical analysis of a muscle biopsy from the mother (38 years) showed total absence of the muscle phosphorylase activity, proving the diagnosis of McArdle disease. The father (35 years) exhibited the full symptoms of myophosphorylase deficiency which could not be confirmed biochemically because he refused a muscle biopsy. Detailed clinical information has been reported by Frick et al. [6].

Son 1 (9 years) was suffering from muscle cramps during moderately strenuous everyday activities for the last 4 years. He underwent no previous clinical or biochemical investigations. Son 2 (5 years) was completely asymptomatic.

Volunteers

Three healthy persons, a 26-year-old male, a 10-year-old girl and a 7-year-old boy volunteered for control measurements. Parental consent was obtained for the examinations of the children.

Examinations

Patients and volunteers performed submaximal work by moving a foot against resistance at a frequency of 1 cycle/s timed by a metronome. With a special treadmill the applied force was adapted to the subjects age and physical training condition so that exercising could be sustained for at least 1–2 min. Including the time needed for instrument set-up, the whole examination was performed within 30 min.

^{31}P MRS investigations were carried out with a Bruker 2.35-Tesla 40-cm-bore magnet (Karlsruhe, FRG) using a surface coil of 5.5 cm in diameter, tuned to 40 MHz for ^{31}P and 100 MHz for ^1H [2]. The calf muscle was positioned on the surface coil for the examination. After a quick field optimization (shimming) on the ^1H -signal, ^{31}P -spectra were obtained every 10 s as a sum of four signal acquisitions repeated every 2.5 s. Radiofrequency pulses lasted 80 μs and the spectral width was

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Abbreviations: ADP = adenosine diphosphate; ATP = adenosine triphosphate; Mg = magnesium; P_i = inorganic phosphate; P_{tot} = Sum of peak areas ($\text{PME} + \text{P}_i + \text{PCr} + \gamma\text{ATP} + \alpha\text{ATP} + \beta\text{ATP}$); PCr = phosphocreatine, pH_i = intracellular pH; PME = phosphomonoesters; ^{31}P MRS = ^{31}P phosphorus magnetic resonance spectroscopy

Fig. 1a, b. The dynamics of the intracellular high energy phosphates in human calf muscle as measured by ^{31}P MRS during rest, work and recovery in a healthy volunteer (**a**) and a patient (**b**). Each of the shown spectra consists of 8 signal acquisitions. Peak assignments: 1 P_i ; 2 PCr; 3–5 $\gamma/\alpha/\beta$ -phosphate of ATP; 6 PME. The intracellular pH_i is marked at the left of each spectrum

4065 Hz (100 ppm). Prior to Fourier transformation the data were treated by a gaussian line-broadening and zero-filled. Spectra were scaled in parts-per-million (ppm) of the resonance frequency (1 ppm = 40.5 Hz) with the peak of phosphocreatine (PCr) arbitrarily set to zero ppm.

Evaluation of data

The sum of the areas under the peaks (obtained for each peak by integration) of phosphomonoesters (PME), inorganic phosphate (P_i), PCr, adenosine triphosphate (γATP , αATP , βATP) were defined as P_{tot} (peak assignments in Fig. 1). Based on the fact that the ATP concentration does not change during short submaximal work in healthy persons [13] or in patients [10, 12] we considered a measurement to be reliable as long as the ratio of $\beta\text{ATP}/\text{P}_{\text{tot}}$ during work did not change more than two standard deviations of the mean value at rest. The intracellular pH (pH_i) was calculated from the chemical shift of the P_i peak relative to the PCr peak [11]. To obtain reliable values for pH_i whenever P_i or PCr were very low ($\text{PCr}/\text{P}_{\text{tot}} > 0.4$ or $\text{PCr}/\text{P}_{\text{tot}} < 0.1$, respectively) three spectra (corresponding to 12 accumulations) were summed resulting in a reduced time resolution of 30 s. We used the ratio of $\text{PCr}/\text{P}_{\text{tot}}$ as an indicator of the work performed by muscle cells [5]. A selection of typical ^{31}P MR spectra obtained at rest, during exercise and recovery is presented in Fig. 1a for the healthy volunteers and in Fig. 1b for the patients.

Results

In Fig. 2 both pH_i and the ratio of $\text{PCr}/\text{P}_{\text{tot}}$ are plotted against time. This graph shows the correlation of the time course of the intracellular pH_i (top) to the actually performed muscle

work during exercise, as indicated [5] by the decreased ratio of $\text{PCr}/\text{P}_{\text{tot}}$ (bottom).

Volunteers

At rest the pH_i value was 7.04 ± 0.04 (mean $\pm$ 1 SD) and the ratio of $\text{PCr}/\text{P}_{\text{tot}}$ was 0.45 ± 0.04 (mean $\pm$ 1 SD). Immediately after the beginning of exercise—marked by a drop in the ratio of $\text{PCr}/\text{P}_{\text{tot}}$ —the pH_i rose by more than 0.1 units in all volunteers, followed by a steady decrease. The end of work is indicated by an abrupt rise in the ratio of $\text{PCr}/\text{P}_{\text{tot}}$.

Patients

The complete data are presented in the right half of Fig. 2. The pH_i at rest was 7.06 ± 0.06 (mean $\pm$ 1 SD) and the ratio of $\text{PCr}/\text{P}_{\text{tot}}$ was 0.47 ± 0.05 (mean $\pm$ 1 SD). Although muscle work was performed by all four patients (indicated by the drop of $\text{PCr}/\text{P}_{\text{tot}}$), the pH_i rose and remained above the value at rest until exercising was stopped.

Discussion

Our observations correspond well to those of other investigators [1, 3, 5, 12].

Healthy volunteers

The initial rise in intracellular pH_i at the beginning of work is caused by the creatine kinase reaction which is responsible for the energy supply of the cell before the onset of glycogenolysis [4]. This reaction is proton consuming according to Eq. (1):

Creatine kinase

With the onset of glycogenolysis and with increasing pyruvate pool the intracellular pH_i decreases and the lactate dehydrogenase catalysed reaction is producing lactate according to

Fig. 2a, b. Time course of the intracellular pH_i in correlation to $\text{PCr}/\text{P}_{\text{tot}}$ as an indicator of the performed muscle work (start and end indicated by arrows) for healthy volunteers (**a**) and patients with myophosphorylase deficiency (**b**). The shaded areas mark the normal range (mean $\pm$ 1 SD) of the values at rest in volunteers. During work the pH_i in patients never dropped below the 95% confidence limit (6.94) of the pH_i at rest (7.06) in contrast to healthy volunteers

LDH

At the beginning of recovery the resynthesis of PCr by the reversed creatine kinase reaction (1) is liberating protons, causing an additional drop of pH_i [13].

Patients

As a consequence of diminished glycogenolysis, the concentration of pyruvate is extremely low in working muscles of patients with McArdle disease [10, 14]. Therefore, lactate production is practically absent, while the creatine kinase reaction (1) is raising the pH_i values for the whole duration of exercise.

In myophosphorylase deficient muscles the myokinase reaction may increase pH_i even further during prolonged hard exercise by producing ammonia [7, 10]. The PME signal in ^{31}P MR-spectra of skeletal muscle arises mainly from phosphorylated glycolytic intermediates (sugar phosphates). The absence of glycogenolysis maintains very low concentrations of these glycolytic intermediates [9, 14]. Thus, the low level of PME even during maximal effort allowed to differentiate myophosphorylase deficiency from phosphofructokinase deficiency and phosphoglycerate kinase deficiency which both show clearly increase PME signals during exercise [5].

The only disease showing similar ^{31}P MR-spectra during rest, work and recovery is the debranching enzyme deficiency, but the clinical findings are different [5, 8]. The diagnosis of McArdle disease was therefore confirmed in both parents and is highly suggestive in the two children. Although son 2 was fully asymptomatic, we observed the same typical ^{31}P MR spectra of myophosphorylase deficiency. This indicates that this enzyme deficiency is already fully expressed at the cellular level, but the uptake of glucose as an alternative source of

energy into muscle cells seems to be more efficient in asymptomatic children than in symptomatic adults explaining the late onset of symptoms [3].

It was impossible to use a standardized exercise protocol especially in the children (patients as well as volunteers), but we noted that the pH_i in all healthy subjects decreased as long as a marked decrease in $\text{PCr}/\text{P}_{\text{tot}}$ (approx. $\frac{2}{3}$ of the value at rest) was achieved during submaximal work.

Conclusions

^{31}P MRS is noninvasive and thus a very attractive, if not the only applicable means of diagnosing McArdle disease in cases where invasive diagnostics are inappropriate (e.g. asymptomatic children) or refused by the patient. Because the whole examination can be performed within 30 min using a simple protocol, we suggest ^{31}P MRS as an easy means of diagnosis in suspected cases of myophosphorylase deficiency. To establish ^{31}P MRS as a diagnostic tool in McArdle disease more comprehensive clinical studies should be performed.

Acknowledgements. We thank Dr. E. Frick (Kantonsspital St. Gallen, Switzerland) for allowing us to study his patients. Grants from the Swiss National Foundation (3.941-0.84 and 4.894-0.85.18), the committee for the Promotion of Applied Scientific Research, the Kamillo-Eisner-Stiftung and Spectrospin AG, and a special grant (R.G.) of the Eid. Techn. Hochschule (ETH), Zürich, are gratefully acknowledged.

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Received May 22, 1989 / Accepted September 4, 1989

THE CHILD is the father of the man.

Wordsworth, 1770–1850