Using phosphorus nuclear magnetic resonance spectroscopy, we studied the muscle metabolism in four patients with paramyotonia congenita as reflected in the PC/P_i and PC/ATP ratios. At normal body temperature, all results were indistinguishable from controls. After cooling to 29°C muscle temperature and a single 2-sec maximum contraction, the forearm muscles were stiff. At this stage, no decrease in the ATP content could be detected. In >50% of the experiments with patients, an additional peak occurred transiently at the resonant frequency for monophosphates. After a working period at 29°C muscle temperature, the paramyotonic muscles were paralyzed, but displayed spectra similar to those obtained at normal body temperature and full working capability. Intracellular pH was close to 7 in paramyotonic muscle under all conditions examined.

IN VIVO P-NMR SPECTROSCOPY: MUSCLE ENERGY EXCHANGE IN PARAMYOTONIA PATIENTS

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Paramyotonia congenita is a dominantly inherited muscle disease in which the main symptoms, slowed muscle relaxation after voluntary contractions and a long-lasting muscle weakness, are brought on by low temperature.[1] Although all skeletal muscles are affected, the long flexors of the forearm have been proven to be most suitable for clinical investigation and diagnosis.[16] The immediate cause of the muscle weakness has been shown to be a lasting depolarization of the muscle fiber membranes to about −40 mV, which makes the fibers inexcitable.[14] The reason for the extreme prolongation of the relaxation phase is not fully understood (Ricker K, Rüdel R, Lehmann-Horn F, Küther G: Muscle stiffness and electrical activity in paramyotonic congenita, submitted to Muscle &

Nerve). A defect in the pathways providing high energy phosphorus-containing metabolites has not been excluded as a contributing factor to the pathomechanisms. Phosphorus nuclear magnetic resonance (P-NMR) measurement is a noninvasive technique for the determination of intracellular adenosine triphosphate (ATP) and phosphocreatine concentration changes.[2,3,8 – 10,1] This technique also permits an evaluation of the intracellular pH.[8,9,11]

MATERIALS AND METHODS

We have studied P-NMR spectra at body temperature and after cooling to 29°C from the forearms of four patients whose clinical condition had been well established (patient 1 of Lehmann-Horn et al.[13]; patient 11 of Haas et al.[12]; patient 2 of Ricker K, Rüdel R, Lehmann-Horn F, Küther G: Muscle stiffness and electrical activity in paramyotonic congenita, submitted to Muscle & Nerve) and his brother. Control spectra were recorded from four healthy subjects. The force of finger flexion was determined before each NMR measurement with the apparatus described by Ricker et al.[16] The supinated forearm of a proband was fixed in a water bath with the fingers of the half-opened fist pressing against an isometric force transducer. The proband, who could observe the force signal on an oscilloscope screen, was asked to exert maximal strength for a predetermined period of time and then to relax the fingers as fast as possible.

During 30 minutes of cooling of the forearm in water of 14°C, the proband was asked to avoid voluntary contractions. At the end of the cooling period, the intramuscular temperature of the forearm flexors had dropped to 29°C, as probed by a thermoneedle inserted 15 mm into the muscle belly. The probands were then subjected to one of the following three regimens: (1) they had to avoid any muscle exertion, permitting the study of the influence of low temperature by itself; (2) they had to execute one or two 2-second contractions, permitting the study of the state of muscle stiffness; or (3) they had to execute repeated or long-lasting contractions until paresis set in, permitting the study of muscle weakness. Following the completion of the respective task, the forearm was quickly released from the water bath, dried, and placed into a superconducting magnet with a 30-cm bore. During preliminary experiments, a 1.9 Tesla BNT 80, and later a 2.4 Tesla Topikon 24/300 (Bruker Oxford Instruments, Karlsruhe, West Germany), were used. A surface coil, 4.0 cm in diameter, probed about 15 ml of tissue within the flexor compartment. The spectra were recorded at 32.5 and 40.5 MHz, respectively. Radiofrequency pulses with a sweep width of 2 kHz were applied at intervals of 2 seconds (only about 1.6 kHz spectral width is displayed in the figures). From 10 to 100 spectra were accumulated and analyzed with a Fourier transform spectrometer (Bruker Medizin-technik, Karlsruhe, West Germany). The homogeneity of the magnetic field was repeatedly adjusted by means of the ^{1}H-NMR signal from the tissue water. The intensities of the various phos-phorus compounds were determined by integrating the areas under the respective peaks.

RESULTS AND DISCUSSION

Spectra recorded at body temperature showed the normal pattern of relative signal intensities for inorganic phosphate (P_i), phosphocreatine (PC), and adenosine triphosphate (ATP) in the patients and in the controls. Table 1 contains the mean values of all tests for the PC/Pi and PC/ATP ratios, the pH calculated from the chemical shift, S, in parts per million (ppm) of the inorganic phosphate peak with respect to the phosphocreatine peak (ppm = 0) according to pH = $6.8 + \log_{10}(S - 3.31)/(5.845 - S)$,[8] the maximum force, and the relaxation time (the time between 90% and 10% maximum force amplitude). Representative examples of spectra are shown in Figure 1A, and A', for a control person and a patient, respectively. The force records taken immediately before measuring the spectra are given as insets.

After cooling, the amplitudes of the first contractions were only slightly reduced in the control, whereas in the patients, the force amplitude was reduced by 20% and the relaxation time was very much prolonged. The accompanying spectra showed little difference between controls and patients. In the patients, the PC/P_i and PC/ATP ratios were slightly reduced (Fig. 1B, and B', and Table 1). This could have been caused by PC splitting during the prolonged contractions. The absolute intensity of the ATP peaks was not reduced. The largest difference between patients and controls occurred when both had exercised their muscles in

Table 1. Intensities of the inorganic phosphate and the ATP resonances (integrated areas in relation to the area under the phosphocreatine peak), intracellular pH, maximum force amplitude (in percent of the value obtained at body temperature), and absolute relaxation time in seconds (measured during the drop of the respective force amplitude from 90% to 10%).

Experimental condition	PC/P_i	PC/ATP	pH	Force (%)	Relaxation (sec)
Controls					
36°C	6.9 (4)	4.3 (4)	7.03 (3)	100.0 (6)	0.10 (3)
First contraction at 29°C	7.1 (5)	4.3 (5)	7.05 (5)	94.4 (6)	0.14 (3)
29°C plus work	3.7 (5)	3.7 (5)	6.80 (3)	87.8 (2)	0.18 (3)
Paramyotonia					
36°C	6.4 (11)	4.4 (11)	6.96 (9)	100.0 (9)	1.32 (4)
First contraction at 29°C	4.5 (10)	4.1 (10)	6.98 (9)	80.8 (8)	24.80 (4)
29°C plus work	4.5 (6)	4.2 (6)	6.97 (4)	11.2 (6)	0.23 (4)

Mean values from four controls and four patients. The figures in parentheses give the numbers of successful evaluations used for averaging.
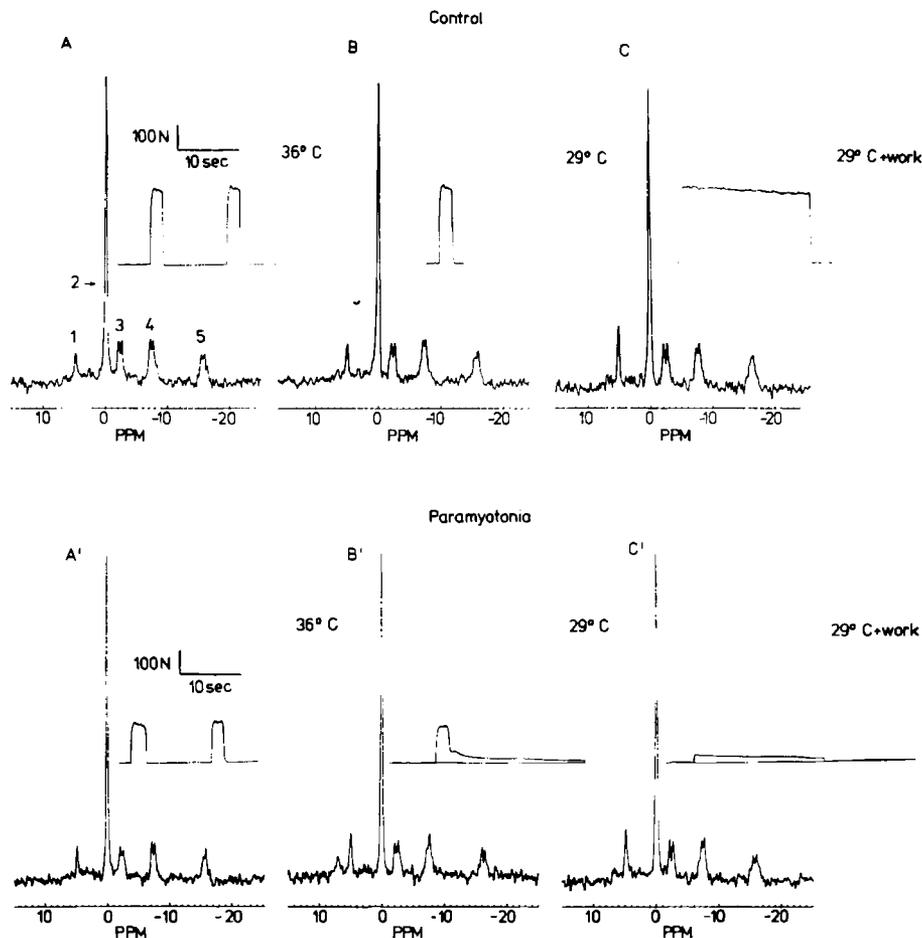
FIGURE 1. Phosphorus nuclear magnetic resonance (P-NMR) spectra from a control subject (above) and the paramyotonic case 1 (below). The first spectra (**A** and **A'**) were recorded at 36°C muscle temperature. The subsequent spectra (**B** and **B'**) were recorded after cooling the forearm to 29°C muscle temperature, and after execution of a 2-second maximum isometric force exertion shown in the inset. The final spectra (**C** and **C'**) were recorded after cooling and work-out. The resonances in **A** are assigned as follows: 1, inorganic phosphate; 2, phosphocreatine; 3, 4, and 5 the γ, α, and β phosphates of ATP.

the cold. The controls were still able to produce 88% maximum force, showed a slight decrease in pH, and displayed a marked decrease in the PC/P_i ratio and a small decrease in the PC/ATP ratio. The patients were only able to produce 11% maximum force, and their spectra were not different from those obtained before exercise (Fig. 1C, and C'). In some cases, the PC/P_i ratio after work was even larger than before (Fig. 2). These results show that paramyotonic stiffness is not caused by an ATP deficiency and that paramyotonic weakness is not accompanied by a fall in the PC/P_i ratio, in contrast to what has been found during fatigue of normal muscle.[6,7,21] The pH was always close to 7 in paramyotonia, i.e., not different from control.

A special feature in the cold was the transient appearance of a peak of +6.7 ppm (Figs. 1B' and 2A). This peak was noticeable in spectra from

three of four patients in five of a total of nine tests. It was only present immediately after the first contractions in the cold as long as stiffness prevailed and was never detected without muscular work in the cold. During the period of cold- and work-induced paresis, it was absent or hardly conspicuous (Figs. 1C' and 2B). However, a clear connection between the appearance of this peak and muscle stiffness cannot be postulated, because in four tests, the peak was absent, although the muscles were stiff, and in one of seven tests with healthy persons in the cold, the peak at +6.7 ppm transiently appeared after three contractions lasting 20 seconds each, although muscle stiffness was not apparent.

The unidentified peak appears with a chemical shift typical for monophosphates. Peaks with similar shifts have been found in toad muscle at rest
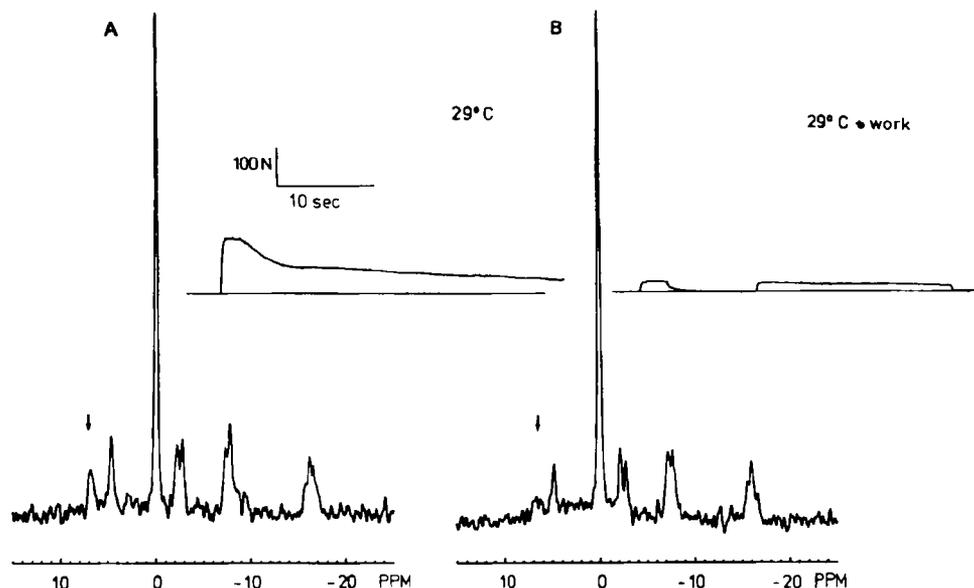
FIGURE 2. P-NMR spectra from the paramyotonic case 2. The first spectrum **(A)** was obtained at 29°C muscle temperature after execution of a single contraction, shown as inset. Note the additional resonance at +6.7 ppm (arrow), the chemical shift for monophosphates. Spectrum **B** was recorded after a work-out in the cold. The forearm muscles are paretic, as shown in the inset; the additional peak at +6.7 ppm (arrow) is hardly conspicuous. The PC peak is even larger than immediately after cooling; the ATP peaks are normal.

Table 2. Chemical shift of some pure phosphorus-containing substances.

Substance	Chemical shift (ppm)	
	Present study (at pH 7.3)	Shaw D, 1983 (at pH 7.0)
Ribose-5-P	—	+7.0
AMP	+6.5	+6.8
IMP	+6.3	—
GMP	+6.3	—
Glucose-6-P	—	+5.6
Glucose-1-P	+4.8	—
cAMP	+1.2/+1.6	—
PC	0.0	0.0
ATP (γ-P)	−5.0/−5.6	−2.7
ADP (β-P)	−4.5	−3.1
ADP (α-P)	−7.8	−7.3
ATP (α-P)	−8.1/−8.6	−7.8
ATP (β-P)	−19.2/−19.6	−18.3

and after work, in frog muscle after work,[4,5] and also in patients suffering from a phosphofructokinase deficiency.[8] For all these cases, it was suggested that such peaks indicated the presence of large amounts of sugar monophosphates. However, there is also the possibility that a peak with a shift of 6–7 ppm is caused by nucleotide monophosphates. To identify the peak in the spectra from paramyotonia patients, we have investigated a series of relevant pure substances in our set-up.

The results are presented in Table 2, together with similar results reported by Shaw.[19] Our unidentified peak is indeed closest to the position where nucleotide monophosphates resonate. The concentration of free adenosine monophosphate (AMP) in muscle is always <0.1 μM,[20] therefore, it is unlikely that it could be detected by P-NMR. The concentration of inosine monophosphate (IMP) may increase very much during muscle exertion,[15,18] so that possibly our unidentified peak is caused by IMP.

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