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Muscle Na^+ channelopathies

MRI detects intracellular ^{23}Na accumulation during episodic weakness

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Abstract—*Background:* Muscle channelopathies such as paramyotonia, hyperkalemic periodic paralysis, and potassium-aggravated myotonia are caused by gain-of-function Na^+ channel mutations. *Methods:* Implementation of a three-dimensional radial ^{23}Na magnetic resonance (MR) sequence with ultra-short echo times allowed the authors to quantify changes in the total muscular ^{23}Na signal intensity. By this technique and T2-weighted ^1H MRI, the authors studied whether the affected muscles take up Na^+ and water during episodes of myotonic stiffness or of cold- or exercise-induced weakness. *Results:* A 22% increase in the ^{23}Na signal intensity and edema-like changes on T2-weighted ^1H MR images were associated with cold-induced weakness in all 10 paramyotonia patients; signal increase and weakness disappeared within 1 day. A 10% increase in ^{23}Na, but no increase in the T2-weighted ^1H signal, occurred during cold- or exercise-induced weakness in seven hyperkalemic periodic paralysis patients, and no MR changes were observed in controls or exercise-induced stiffness in six potassium-aggravated myotonia patients. Measurements on native muscle fibers revealed provocation-induced, intracellular Na^+ accumulation and membrane depolarization by -41 mV for paramyotonia, by -30 mV for hyperkalemic periodic paralysis, and by -20 mV for potassium-aggravated myotonia. The combined in vivo and in vitro approach showed a close correlation between the increase in ^{23}Na MR signal intensity and the membrane depolarization ($r = 0.92$). *Conclusions:* The increase in the total ^{23}Na signal intensity reflects intracellular changes, the cold-induced Na^+ shifts are greatest and osmotically relevant in paramyotonia patients, and even osmotically irrelevant Na^+ shifts can be detected by the implemented ^{23}Na MR technique.

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Paramyotonia congenita (PC), hyperkalemic periodic paralysis (HyperPP), and potassium-aggravated myotonia (PAM) are channelopathies caused by mutations in the *SCN4A* gene coding for the $Na_v1.4$ muscle sodium channel.[1-3] In PC, muscle exertion in cold environment causes muscle stiffness, which is usually followed by flaccid weakness lasting for up to 12 hours. Rest after exhausting exercise or potassium-rich food causes muscle stiffness in PAM and flaccid weakness in HyperPP. In these disorders, a gating defect of the Na^+ channels, which are essential for the generation of the muscle action potential, destabilizes the inactivated state. The incomplete channel inactivation results in a persistent inward Na^+ current and causes the muscle fibers to depolarize and to generate repetitive action potentials. During an attack of weakness, the persistent inward current is so large that the progressing membrane depolarization leads to loss of membrane excitability because it renders the population of normal sodium channels inactivated. Since the mutant channels exert an effect on cell excitability, the mutations produce a gain of function leading to a dominantly inherited disease.

Studies on heterologously expressed channels have revealed that the persistent current is large in HyperPP, moderate in PAM, and small in PC, which typically shows slowing of fast inactivation instead.[4-9] However, these patch clamp studies gave no information whether the persistent current leads to an intracellular Na^+ accumulation or if $[Na^+]_i$ is normal due to activated ion transporters or the Na^+ pump. A slight Na^+ accumulation has been described in few HyperPP fibers as measured with Na^+ sensitive microelectrodes.[10] No Na^+ concentration values

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are available for PC and PAM since the membrane hyperexcitability complicates the intracellular measurements.

Noninvasive assessment of the Na^+ content of muscle tissue is difficult. The in vivo ^{23}Na NMR signal is 22,000 times smaller than that of ^1H and the extremely short T2 relaxation times of ^{23}Na in tissue lead to very low signal-to-noise images in clinically feasible measurement times.[11] Specific hardware and MR sequences with ultra-short echo times are needed for ^{23}Na MRI. Only with clinical MR units with broadband capability ^{23}Na MR protocols for the visualization of the tissue's total Na concentration in humans could be developed,[12] e.g., in a few patients with myotonic dystrophy, a progressive muscle dystrophy.[13,14]

Since the muscle channelopathies offer the chance to observe ^{23}Na MRI before, during, and after an episode of weakness or stiffness we implemented a three-dimensional radial $^{23}Na^+$ MR sequence with ultra-short echo times for imaging of the lower leg muscles. In this study, we sought to assess whether the muscles exposed to typical triggers take up ^{23}Na. For this purpose, we examined 23 patients with PC, HyperPP, and PAM in whom diagnosis was genetically confirmed by ^{23}Na and conventional ^1H MRI. The ^{23}Na MRI results were checked with intracellular recordings of resting membrane potentials and Na^+ activities in muscle samples of eight patients.

Methods. *Patients and volunteers.* The study was approved by the institutional review boards in Heidelberg and Ulm and conducted according to the declaration of Helsinki. Written informed consent was obtained from all volunteers and patients (6 women, 17 men) after the nature of the examination had been fully explained. Serum K^+ levels could be immediately determined by a chip (i-Stat Corporation, East Windsor, NJ) and K^+ salts and glucose-insulin infusion solutions were available for immediate use. Ten patients with PC (median age 45 years), seven with HyperPP (median age, 42 years), and six with PAM (median age, 43 years) were included in this study. For comparison, we included 10 volunteers with no evidence or history of muscular or cardiovascular disease and no family history of channelopathies (all with normal muscle strength and normal ^1H MRI findings; median age, 27 years). The 23 patients and 10 volunteers were all examined by ^{23}Na MRI. Eight of the patients underwent a muscle biopsy in addition to MRI.

Patient examination protocol. ^{23}Na MRI was performed on both lower extremities before and after provocation of one lower extremity. The provocation tests were performed by the senior author, an experienced neurologist and muscle physiologist, who did not participate in MRI data analysis to avoid a reading bias. Five to 10 minutes elapsed between the end of the provocation test and the start of ^{23}Na MRI sequences. The provocation methods were cooling for PC,[15] and exercise for HyperPP and PAM. In addition, cooling was also applied to HyperPP[16] and PAM patients[17] for better comparison of the effects on ^{23}Na MRI for the various diseases. The cooling test consisted of ice-water bags wrapped around the non-dominant lower leg for 20 minutes while the subject rested on a stretcher. Immediately after cooling, the subjects had to dorsiflect the foot of the non-dominant leg against resistance 30 times and to stand on the tiptoes 30 times. The exercise test was performed on a cycle ergometer for 20 minutes followed by rest for 5 minutes. The load was adjusted to a maximum pulse rate of 160/minute.

Muscle strength grading. The muscle strength before and immediately after provocation, as well as 30 minutes after provocation, i.e., after the second part of the MRI examination, were scored according to the linear grading system proposed by the British Medical Research Council (MRC): 0, complete paralysis; 1, minimal contraction; 2, active movement with gravity eliminated; 3, weak contraction against gravity; 4, active movement against gravity and resistance; and 5, normal strength. Examination of the lower limb comprised strength testing of the following: dorsiflexion, plantarflexion, and eversion of the foot, toe dorsiflexion, and toe plantarflexion. In addition, voluntary and involuntary contractions of foot dorsiflexion and plantarflexion were measured by use of a noncommercial isometric force transducer and an EMG apparatus (Medelec, Woking, UK). The patient was asked to activate the respective muscle maximally for several seconds and to then relax. In some cases, also the EMG was recorded with two platinum wire electrodes (insulated except 1.5 mm at the tip) placed at a distance of <2 cm within the respective muscle belly. In contrast to conventional concentric EMG electrodes, this arrangement allowed us to record from large muscle areas and to record electrical activity as much as possible.

^{23}Na MRI technique. The study was performed with a 1.5 Tesla clinical MR system (MAGNETOM Symphony, Siemens AG Medical Solutions, Erlangen, Germany) equipped with hardware for broadband spectroscopy using a CE certified double-resonant (16.84 MHz/63.6 MHz) birdcage coil, a unique specimen (Rapid Biomed Inc., Wuerzburg, Germany) for the ^{23}Na and ^1H measurements. All 23 patients were examined with MRI before provocation. ^{23}Na MRI was repeated in 21 patients after provocation of the nondominant lower leg. A 63-year-old PC and a 45-year-old PAM patient were unavailable for provocation.

^{23}Na MRI protocol. The ^{23}Na signal in vivo decays biexponentially, with a fast (T_{2fast} = 0.5 to 3 msec) and a slow (T_{2slow} = 15 to 30 msec) component of the T_2 relaxation time. In order to include the intracellular sodium signal, it was proposed that sequences with ultra-short echo times of less than a millisecond are needed.[13] In this case, a weighted average of $[^{23}Na]_i$ and $[^{23}Na]_e$ is observed. However, as long as the tissue is adequately perfused, $[^{23}Na]_e$ will remain constant, so that changes in ^{23}Na MR signal intensity will directly relate to changes in $[^{23}Na]_i$.[12] Therefore, a ^{23}Na three-dimensional-radial gradient echo sequence was implemented which scans k-space from the center to the surface of a sphere in a star-like fashion immediately after slice selection. After a 300 μs rectangular radiofrequency (RF) pulse and a 50 μs delay, the radial readout gradients and signal acquisition started simultaneously (repetition time [TR] = 4 msec; echo time [TE] = 0.2 msec, field of view [FOV] = 500 mm; BW = 500 Hz/pixel; 5,000 projections × 64 samples per projection; Nacq = 10; Tacq = 10 minutes). An online gridding reconstruction re-gridded the radially acquired data using a Kaiser-Bessel window and a rho filter modified to correct for undersampling onto a Cartesian grid followed by a conventional three-dimensional fast-Fourier transform (FFT) producing an isotropic data set.[18]

Analysis of ^{23}Na MRI data. In order to quantify the signal enhancement on the ^{23}Na MR images after provocation of the non-dominant lower leg, three-dimensional-radial images were evaluated by regions of interest (ROI) analysis using 0.3% NaCl solution phantoms placed between both lower legs as reference. ROIs had a size of 100 pixels and were placed by an experienced radiologist in the field of musculoskeletal MRI in consensus with the physicist who developed the ^{23}Na MR sequence to recognize sequence specific image artifacts that might interfere with ROI analysis.

A ROI was placed on the soleus muscle of each lower leg using the ^1H MR images as reference and a third ROI was placed on the phantom. The signal intensity of three-dimensional-radial MRI was normalized to the phantom for interindividual and intraindividual comparisons, i.e., the values of the ROIs placed on the soleus muscles were divided by the values of the phantom. The signal intensities before and after provocation were analyzed separately for each lower leg. Signal intensity alterations were considered to reflect changes of muscular Na^+ concentration. Then the percent change between the normalized muscular ^{23}Na MRI signal (S) before and after provocation was calculated and findings were expressed according to the following equation:

$$\text{percent change} = \frac{S \text{ after} - S \text{ before}}{S \text{ before}} 100$$

Analysis of ^1H MRI. ^1H MRI was performed in addition to the ^{23}Na MRI protocol before and after provocation. The protocol comprised an axial T1-weighted spin-echo (SE) sequence (TR =

Table 1 *Analysis of muscular ^{23}Na MRI signals*

	Signal intensity before provocation		Percent change after cooling		Percent change after exercise	
	Provoked lower leg	Reference lower leg	Provoked lower leg	Reference lower leg	Provoked lower leg	Reference lower leg
PC	0.80 ± 0.15	0.80 ± 0.16	22 ± 6*	−1 ± 5	—	—
HyperPP	1.05 ± 0.22	1.03 ± 0.23	11 ± 2*	−2 ± 1	10 ± 3*	0 ± 7
PAM	0.88 ± 0.11	0.88 ± 0.11	17 ± 2*	−1 ± 2	−2 ± 3	−2 ± 0
Volunteers	0.99 ± 0.12	1.00 ± 0.11	−1 ± 2	−2 ± 3	0 ± 4	−3 ± 2

The muscular 23Na MRI signal normalized to the 0.3% saline solution reference before provocation and the percent change after provocation are given. Negative changes in percent correspond to a signal reduction after provocation.

* Significant difference between values before and after provocation.

516 msec, TE = 15 msec, matrix 308 × 512, slice thickness = 6 mm) and an axial T2-weighted turbo-SE sequence (TR = 3,000 msec, TE = 104 msec, matrix 308 × 512, slice thickness = 6 mm) and a fat-suppressed T2-weighted short tau inversion recovery (STIR) sequence (TR = 5,670 msec, TE = 50 msec, inversion time 150 msec, matrix 256 × 256, slice thickness = 6 mm). Image interpretation was performed in consensus by two experienced readers in the field of musculoskeletal MRI. A muscle edema was defined as an area of localized hyperintensity on T2-weighted images. Fatty infiltration was defined as areas of signal intensity equivalent to subcutaneous fat on T1- and T2-weighted images. Analysis of all MRI data was performed without knowledge of the exact provocation scheme and the side of provocation.

Intracellular recordings from native muscle fiber segments. Muscle specimens were removed from quadriceps muscle under regional anesthesia from three PC, three HyperPP, and two PAM patients, and from three adult individuals who had undergone muscle biopsy for exclusion of malignant hyperthermia susceptibility. The specimens were about 28 mm in length and 5 mm in diameter. As described previously,[19] the fiber segments resealed over 2 hours in a solution containing (in mM): NaCl 108, KCl 3.5, $CaCl_2$ 1.5, $MgSO_4$ 0.7, $NaHCO_3$ 26.2, NaH_2PO_4 1.7, Na-gluconate 9.6, glucose 5.5, sucrose 7.6, 290 mosmol/L, and maintained at 37 ºC if not indicated otherwise. The pH was adjusted to 7.4 by gassing with 95% O_2 and 5% CO_2. In some experiments, extracellular K^+ was increased to 7 mM. Resting membrane potentials were measured by use of microelectrodes, which were filled with 3 M KCl and had an input resistance of 5 to 10 MOhm, and a voltage amplifier (Turbo TEC-05, NPI Electronic Instruments, Tamm, Germany). Ion-sensitive microelectrodes were made from single- or double-barreled glass pulled to a tip diameter of smaller than 0.5 μm. One barrel was filled with the "Na cocktail" Fluka 71176, the other electrode with 1 M KCl as reference. Input resistances of the electrodes were about 2×10^{10} Ohm and that of the amplifier 10^{14} Ohm. The values of intracellular Na^+ are given in activities, aNa_i. The concentration is somewhat higher assuming an intracellular activity coefficient of 0.74.[20]

Detection of channel mutation. To confirm typical mutations, whole EDTA blood (20 mL each) was taken for *SCN4A* mutation analysis. Mutation screening was performed using PCR amplification of *SCN4A* exons 13, 22, and 24 as described previously.[21] PCR products were loaded on a 2% agarose gel, stained with ethidium bromide, and the band cut out under UV light. Bands were purified using the Amersham Pharmacia kit, and cycle-sequenced with 1 pmol primer using the dye terminator kit (Applied Biosystems, Foster City, CA). Sequencing was performed on 6% denaturating polyacrylamide gels in an ABI 377 HT automated sequencer. All sequences with base exchanges were verified by reverse sequencing of a new PCR product of the same DNA sample.

Statistics. Data entry procedures and statistical analysis were performed with a statistical analysis software system (SPSS for Windows, version 11.5.1, 2002, SPSS Inc., Chicago, IL). Data were analyzed using a Student *t* test (level of significance, $p = 0.05$). Results of ^{23}Na MRI quantification were expressed as means ± SD and results of MRC score were presented as medians.

Results. *Molecular genetics.* In all 10 PC patients, typical mutations were identified: 9x T1313M,[22] 1x R1448C.[23] In all but one HyperPP patient, frequent mutations were found: 4x M1592V,[24] 2x T704M,[25] 1x unidentified $Na_v1.4$ mutation (the disease in the family of this patient is linked to *SCN4A*). Also in the six PAM patients, typical mutations were discovered: 2x V1589M,[21] and 4x G1306A.[26]

Muscle strength. Prior to provocation, strength of the lower leg muscles was normal in all patients and volunteers (MRC 5.0). Cooling (followed by short exercise) of the nondominant lower leg caused paresis of ankle dorsi- and plantar-flexors in PC (median MRC 3.5; n = 9; see figure E-1 on the *Neurology* Web site at www.neurology.org) and less pronounced in HyperPP patients (MRC 4; n = 2). No paresis was induced by cold in volunteers and PAM patients except one of the G1306A carriers (MRC 4). The weakness lasted several hours in PC and almost an hour in HyperPP patients and this PAM patient. After exercise, all PAM patients presented with normal muscle strength and severe muscle stiffness whereas HyperPP patients developed slight flaccid weakness (MRC 4; n = 5).

^{23}Na MR images. Since the muscular ^{23}Na signal intensity was normalized to a reference which contained the same sodium concentration as normal muscle tissue (0.3% NaCl), volunteers showed a mean ratio of about 1.0 (table 1, figure 1). Prior to provocation, significantly lower ^{23}Na signal intensity ratios were observed in PC (mean signal intensity, 0.8, $p = 0.0001$) and PAM patients (0.88, $p = 0.0054$), whereas HyperPP patients had ^{23}Na signal intensity ratios comparable to volunteers (1.05, $p = 0.211$). Cooling and exercising of the nondominant lower leg caused, on average, an increase in the relative ^{23}Na signal intensity in the order of PC > HyperPP > PAM (table 1, figure 2, figure E-2). Since removal of the ice-water bags induced a transient reddening of the skin, we re-examined two PC patients after the perfusion of the skin had normalized for almost 2 hours (figure 2C) to be sure that the circulation of the lower leg was unaltered by cooling. The percent change of the ^{23}Na signal intensity in the rewarmed leg was even higher than immediately after cooling in both patients. This result may serve as further evidence that the increased ^{23}Na signal is not a perfusion-dependent artifact. On the next day, the ^{23}Na signal intensity returned to the pre-provocation level as measured in three PC patients.

Unilateral exercise on a bicycle induced not only episodic weakness in the exercised leg of HyperPP patients but also a significant increase in the muscular ^{23}Na signal intensity (table 1). In contrast, this type of exercise did not cause a significant increase in the muscular ^{23}Na signal

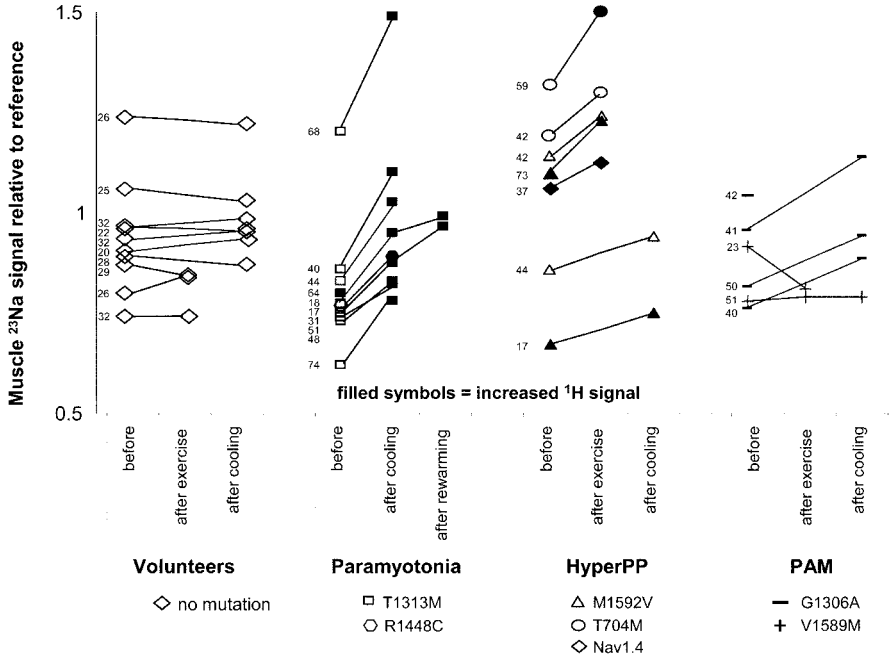

Figure 1. Muscular ^{23}Na signal intensity relative to reference prior to and after provocation. Muscular ^{23}Na MRI signal intensities of regions of interest from the lower legs are shown in relation to the signal of the phantom prior to and after provocation. The age of the individual is given in years to the left of the symbols. PC and HyperPP patients exhibited a striking increase in the muscle ^{23}Na signal intensity after provocation whereas volunteers showed no significant changes. This increase could also be observed in all PAM patients after cooling. A muscle edema was caused by cooling in all PC patients. The 17-year-old HyperPP patient presented with a unilateral calf edema after he had experienced an attack of paresis and aching muscles of this calf 2 days before.

intensity in PAM patients (table 1) although their muscles had become extremely stiff. In all volunteers, the muscular ^{23}Na signal intensity was not significantly different after cooling or exercise (table 1).

^{1}H magnetic resonance images. Increased ^{1}H signal intensities on T2-weighted ^{1}H MRI were visible in the triceps surae muscles of 1 of 10 PC and 3 of 7 HyperPP patients prior to provocation whereas all PAM patients and all healthy volunteers showed normal images before and after provocation (figure 1). The remaining nine PC patients showed increased ^{1}H signal intensities after cooling (figure 1, figure E-3). Whereas these edema-like changes did not markedly expand after provocation in the three HyperPP patients, a ^{1}H signal increase occurred after exercise in an additional HyperPP patient, a T704M carrier (figure 1).

Intracellular recordings from resealed native muscle fiber segments. In a solution containing 3.5 mM K^+ and a temperature of 37 °C, PC, HyperPP, PAM, and control fibers had resting membrane potentials of approximately −82 mV (table 2). Upon cooling, most PC fibers showed repetitive activity. An increase in extracellular K^+ to 7 mM caused most HyperPP fibers (except those from T704M carriers) and all PAM fibers to fire repetitive action potentials. The repetitive activity lasted several seconds up to minutes. After this activity, PC muscle fibers had resting membrane potentials of approximately −42 mV, HyperPP fibers about −54 mV, and PAM fibers −61 mV whereas control fibers showed no substantial depolarization. At these values, PC and HyperPP were electrically inexcitable whereas action potentials could be still elicited in PAM fibers (figure E-4). The depolarization was not reversed by rewarming or when $[K^+]_e$ was set back to 3.5 mM, but tetrodotoxin (TTX), a specific Na^+ channel blocker, was always able to repolarize the fibers to the potential expected according to the Nernst equation.

The sodium, which is conducted through non-inactivating Na^+ channels, could accumulate in the myoplasm or be extruded again by pumps and transporters. To test these possibilities, we measured intracellular Na^+ activities aNa_i with Na^+ sensitive microelectrodes. The values of patients and controls showed no significant difference at $[K^+]_e$ of 3.5 mM. At cooling or $[K^+]_e$ elevation, most PAM and many PC fibers spontaneously fired repetitive action potentials and twitched. The measurements were often interrupted by microelectrode displacement but, in some fibers, the steady-state potential was almost or completely reached. Also, HyperPP fibers from M1592V carriers frequently twitched, but T704M-HyperPP fibers usually showed no spontaneous activity and could therefore be studied for a longer time period.

In contrast to the activities at 37 °C or $[K^+]_e$ of 3.5 mM, aNa_i was higher in PC and HyperPP patients than in controls when the fibers were provoked by cooling or $[K^+]_e$ of 7 mM (table 2). As shown in figure 3, an elevation of $[K^+]_e$ to 7 mM caused a larger depolarization than in controls, indicating that 7 mM K^+, in addition to the shift of the K^+ reversal potential, had another depolarizing effect. A Na^+ inward current was activated as soon as the threshold for the Na^+ channel activation was exceeded, and aNa_i increased and reached a maximum. The following slight aNa_i reduction could reflect a Na^+ dilution due to osmotic water influx. Addition of TTX in the presence of 7 mM K^+ repolarized the membrane almost to the corresponding Nernstian potential and decreased aNa_i to subnormal levels, most likely by a high-K^+ induced stimulation of the Na^+ pump. In agreement with this hypothesis, lowering $[K^+]_e$ to 3.5 mM reduced pump activity which increased aNa_i to normal values. Despite this increase in aNa_i, the membrane repolarized, suggesting that the depolarization was caused by a pathologically increased Na^+ open probability now abolished by TTX.

Correlation between in vivo and in vitro recordings. For the three diseases, the provocation-induced increase in the ^{23}Na MRI signal intensity has been correlated to the membrane potentials which results from the typical provocation (figure 4, closed symbols). This correlation yields a

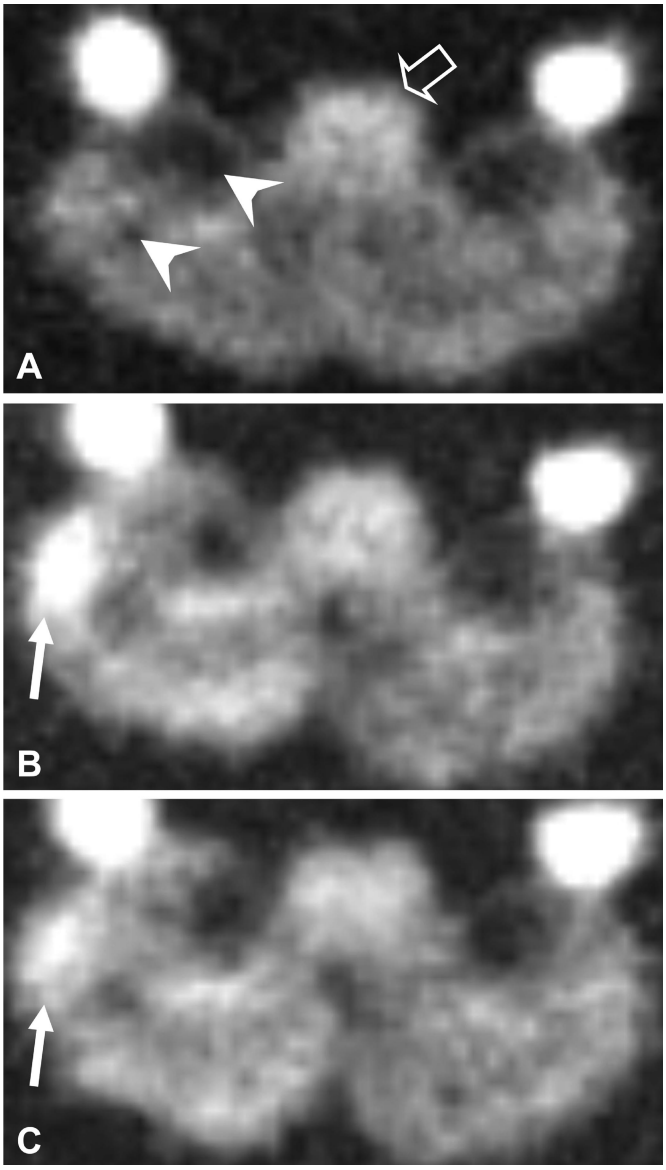

Figure 2. Effects of unilateral cooling and rewarming on ^{23}Na MR images in a PC patient. Compared to state prior to provocation (A), ^{23}Na signal intensity increased in a 17-year-old man with PC directly after cooling plus short exercise (B) of the right lower leg and further increased after rewarming (C) (arrows). The paresis according to MRC score was 2 for foot dorsiflexion and 3 for foot plantarflexion directly after cooling and after rewarming, i.e., about 2 hours after cooling. Reference phantoms with various NaCl concentrations or densities are positioned: 0.3% NaCl solution between the legs (open arrow), 0.6% NaCl next to the left, and 0.3% NaCl in agarose next to the right lower leg. Bones appear as areas of low signal intensity (arrowheads in A). The ^{23}Na signal is probably higher in gastrocnemius than soleus muscle after provocation because the ice-water bags wrapped around the nondominant lower leg for 20 minutes cooled more intensively the superficial than the deeper located muscles. The inherent inhomogeneity of the ^{23}Na MR images is a technical consequence resulting from limited resolution of the ^{23}Na sequence and blurring artifacts due to the decay of the short T_2 component during data acquisition.

coefficient of r = 0.92 after Pearson, suggesting that the ^{23}Na signal intensity increase reflects an intracellular Na^+ accumulation. In addition, the increase in the ^{23}Na MRI signal intensity has also been correlated to the reduction in muscle strength caused by the provocation (figure 4, open symbols). This correlation is much weaker (r = 0.48) which could be based on difficulties with scoring the muscle strength of the ankle plantar flexors.

Discussion. In vivo ^{23}Na MRI visualized an episodic Na^+ accumulation in the sodium channelopathies caused by non-inactivating muscular Na^+ channels. The use of ultra-short echo times allowed us to measure the total ^{23}Na content of skeletal muscle. More than half of the signal is due to intracellular ^{23}Na if the partial volume of the extracellular compartment is not greater than 7%.[12] Since the extracellular space of skeletal muscle is less than 8%[27] including the transverse tubular system that is 0.32% of the fiber volume,[28] this prerequisite is fulfilled for normal muscle. Vacuoles, the results of a substantial proliferation of the transverse tubular system,[29] were absent in the patients who underwent a muscle biopsy. Therefore, the prerequisite should also be met for the diseased muscle of this study.

The ^{23}Na signal is composed of the weighted average of extra- and intracellular ^{23}Na. $[Na^+]_e$ is about 10-fold higher than $[Na^+]_i$ both in brain[12] and muscle according to our study. $[Na^+]_i$ depends on the cell's ability to extrude Na^+ and on the function of the Na^+ channels to conduct Na^+ along the concentration and electrical gradient. As the Na^+ channel open probability and thus the conductance is increased by non-inactivating channels, $[Na^+]_i$ might be increased. In contrast, $[^{23}Na]_e$ will remain virtually constant since there are no hints that perfusion is altered in muscle channelopathies. However as cooling could alter the perfusion we measured the total ^{23}Na content in PC muscle at two times, immediately after cooling and several hours after rewarming. Since cold-induced weakness in PC lasts up to 12 hours, the late measurements were performed without that changes in the degree of weakness had occurred. At both times, the total muscular ^{23}Na signal was almost the same. Therefore, we believe that $[^{23}Na]_e$ is virtually constant and any changes of the total ^{23}Na signal of skeletal muscle reflect alterations of the intracellular ^{23}Na content.

Our data show that, in HyperPP patients, cooling in addition to K^+ ingestion and exercise is a reproducible and reliable trigger for weakness. In the past, a significant decrease of the cMAP amplitude as an objective parameter of cold-induced weakness has been reported only in a few, partly atypical families.[30-33] Thus, a decreased cMAP amplitude with cooling has been considered to indicate PC and not HyperPP.[34] In our study, the HyperPP patients presented with typical clinical features. They carried the most frequent HyperPP mutations, T704M and M1592V, showing that they are typical HyperPP

Table 2 *Resting potentials E_m and intracellular sodium activities $[Na^+]_i$*

Type of value		PC, n = 3	HyperPP, n = 3	PAM, n = 2
Before provocation				
E_m (mV)	−83.3 ± 4.8 (35)	−82.5 ± 5.3 (58)	−84.4 ± 3.9 (28)	−81.0 ± 5.8 (21)
aNa_i (mM)	7.7 ± 0.9 (14)	5.8 ± 0.8 (3)	6.4 ± 0.6 (9)	6.9 ± 1.0 (2)
After provocation				
E_m (mV) at 27 °C	−75.7 ± 3.6 (27)	−41.9 ± 6.5 (50)*	—	—
E_m (mV) at 7 K^+	−70.1 ± 3.9 (29)	—	−53.8 ± 8.9 (96)*	−60.6 ± 8.0 (26)*
aNa_i at 27 °C	10.2 ± 1.4 (10)	14.5 ± 1.9 (5)	—	—
aNa_i at 7 K^+	7.4 ± 1.2 (10)	—	10.2 ± 0.8 (8)	—

The standard solution contained 3.5 mM K^+ at 37 °C. Provocation was performed by cooling to 27 °C or elevation of $[K^+]_e$ to 7.0 mM at 37 °C. Number of fibers is in parentheses.

* Due to the high numbers, the values differ from the corresponding controls at a significance level of $<< 0.01$ and from each other at a significance level of < 0.01.

patients. All who underwent the cooling test developed muscle weakness which disappeared after rewarming. Taking all reports together, cold environment should be now considered as a typical HyperPP trigger even though more intensive cooling than in PC may be required to elicit weakness. The milder Na^+ accumulation and depolarization of HyperPP fibers can explain the much shorter period of weakness of HyperPP muscle compared to PC. In agreement with the literature, only one of the PAM patients who carried a mutation at G1306 close to the PC mutation T1313M showed a slight cold-induced weakness whereas PAM patients carrying V1589M were not-cold-sensitive.[21,22]

The absence of substantial weakness in PAM patients can be simply explained by the in vitro observation that, under provocation, the membrane only slightly depolarized to around −60 mV. At this potential range, the membrane always was able to generate and propagate action potentials. In contrast to PC fibers, several PAM and also some HyperPP fibers were able to repolarize to normal potentials. As high $[K^+]_e$ is known to stimulate the Na^+ pump whereas its enzymatic activity is reduced in the cold, the unequal electrogenic

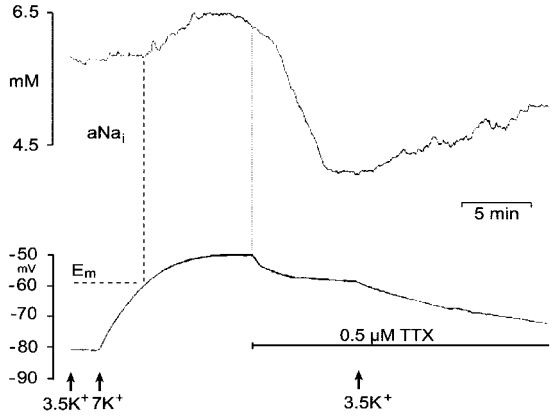

Figure 3. Recordings of intracellular Na^+ activity and resting potential. A fiber from a HyperPP patient carrying the T704M mutation was measured. Elevation of $[K^+]_e$ to 7 mM caused a membrane depolarization. As soon as the activation threshold (dashed horizontal line) was exceeded, aNa_i increased (beginning at the vertical dashed line). Although the steady-state was not reached at the time the bath solution was changed, the figure was taken because it shows in addition that TTX reduced the inward Na^+ current and repolarized the membrane to the reversal potential. Reduction of extracellular K^+ to 3.5 mM finally resulted in usual aNa_i and membrane polarization.

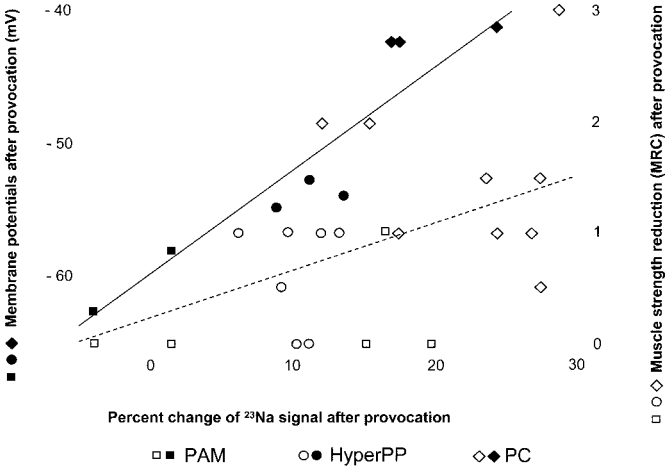

Figure 4. Analysis of the correlation between ^{23}Na signal increase, membrane potential, and muscle strength reduction. The filled symbols represent the mean resting membrane potentials of the muscle fibers taken from the eight patients (three PC, three HyperPP, two PAM) vs the muscular ^{23}Na signal increase of these patients. The open symbols show the decrease in muscle strength of all patients who underwent a provocation test (nine PC, seven HyperPP, five PAM) vs the muscular ^{23}Na signal increase of these patients. The degree of the membrane depolarization correlates with the percent change of the muscular ^{23}Na signal after provocation according to the function $y = 0.825x - 60.3$ (continuous line) and yields a correlation coefficient of $r = 0.92$ after Pearson. The muscle strength reduction is much less correlated with the muscular ^{23}Na signal ($r = 0.48$; $y = 0.043\ x + 0.24$, dashed line for plantarflexion).

Na^+ pump contributions at high $[K^+]_e$ and in the cold can explain the different polarization patterns in PAM and PC and the larger Na^+ accumulation in PC. Vice versa, the finding that $[Na^+]_i$ and ^{23}Na prior to provocation were significantly lower in PC than in normal controls shows that PC muscle is able to cope with an increased intracellular Na^+ accumulation in the warmth.

Usually, a sodium current through voltage-gated sodium channels is terminated by fast channel inactivation. If the fast inactivation is incomplete, the current decays by slow channel inactivation.[35] Functional expression of HyperPP mutations shows a persistent current attributed to the incomplete slow inactivation which is found in some but not all HyperPP mutations.[36] In contrast PC and PAM mutations slow fast inactivation but do not affect slow inactivation.[5-7,37] Therefore, slow inactivation should normally prevent a persistent current that is associated with a sustained membrane depolarization. However, here we show that sustained membrane depolarization is more pronounced in PC (−83 to −42 mV) than in HyperPP (−84 to −54 mV) and in PAM (−81 to −61 mV). In accordance with the large sustained membrane depolarization, PC and HyperPP fibers are paralyzed whereas PAM fibers are still excitable (see above).

In vivo ^1H MRI detected an increase in signal intensity, which reflects an edema as the consequence of an osmotically relevant Na^+ accumulation. An alternative explanation, on increase in $[H^+]_i$, has been excluded by ^{31}P NMR spectroscopy.[42] As earlier exercise studies indicate,[38] water passes the interstitial space and moves into muscle fibers faster than the simultaneous trans-capillary flow, suggesting that intracellular osmolarity provides the driving pressure in swelling. This seems to be the case for the depolarized PC and HyperPP muscle fibers in which $[Na^+]_i$ increases. The accumulation might become osmotically relevant and draw water in the provoked muscle fibers as visualized by a signal intensity increase on T2-weighted ^1H MR images. This shift of water might result in an elevated serum ion concentration as reported for Quarter horses during hyperkalemic paralytic attacks.[39,40] The elucidation of channelopathies of other tissues[41] might also take advantage from the presented in vivo techniques.

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