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⁺,1.4 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⁺], 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...
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 \(^{23}\text{Na}\) NMR signal is 22,000 times smaller than that of \(^1\text{H}\) and the extremely short T2 relaxation times of \(^{23}\text{Na}\) 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 \(^{23}\text{Na}\) MRI. Only with clinical MR units with broadband capability \(^{23}\text{Na}\) 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 \(^{23}\text{Na}\) MRI before, during, and after an episode of weakness or stiffness we implemented a three-dimensional radial \(^{23}\text{Na}\) 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 \(^{23}\text{Na}\). For this purpose, we examined 23 patients with PC, HyperPP, and PAM in whom diagnosis was genetically confirmed by \(^{23}\text{Na}\) and conventional \(^1\text{H}\) MRI. The \(^{23}\text{Na}\) 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 \(^1\text{H}\) MRI findings; median age, 27 years). The 23 patients and 10 volunteers were all examined by \(^{23}\text{Na}\) MRI. Eight of the patients underwent a muscle biopsy in addition to MRI.

Patient examination protocol. \(^{23}\text{Na}\) 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 \(^{23}\text{Na}\) 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 \(^{23}\text{Na}\) 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 dorsiflex 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

![Image](https://example.com/image.png)
Table 1 Analysis of muscular 23Na MRI signals

<table>
<thead>
<tr>
<th></th>
<th>Provoked lower leg</th>
<th>Reference lower leg</th>
<th>Percent change after cooling</th>
<th>Provoked lower leg</th>
<th>Reference lower leg</th>
<th>Percent change after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>0.80 ± 0.15</td>
<td>0.80 ± 0.16</td>
<td>22 ± 6*</td>
<td>-1 ± 5</td>
<td>-</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td>HyperPP</td>
<td>1.05 ± 0.22</td>
<td>1.03 ± 0.23</td>
<td>11 ± 2*</td>
<td>-2 ± 1</td>
<td>0 ± 7</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td>PAM</td>
<td>0.88 ± 0.11</td>
<td>0.88 ± 0.11</td>
<td>17 ± 2*</td>
<td>-1 ± 2</td>
<td>0 ± 4</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>Volunteers</td>
<td>0.99 ± 0.12</td>
<td>1.00 ± 0.11</td>
<td>-1 ± 2</td>
<td>-2 ± 3</td>
<td>-3 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

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.

Table 1 - Analysis of muscular 23Na MRI signals

In all but one HyperPP patient, frequent mutations were found: 4x M1592V, 2x T704M, 1x unidentified Na1.4 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, and 4x G1306A.

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 dorsiflexors 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).

23Na MR images. Since the muscular 23Na 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 23Na 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 23Na 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 23Na 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 23Na signal intensity in the warmed leg was even higher than immediately after cooling in both patients. This result may serve as further evidence that the increased 23Na signal is not a perfusion-dependent artifact. On the next day, the 23Na 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 23Na signal intensity (table 1). In contrast, this type of exercise did not cause a significant increase in the muscular 23Na signal intensity.
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$^+$ depolarized 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
coefficient of $r = 0.92$ after Pearson, suggesting that the $^{23}\text{Na}$ signal intensity increase reflects an intracellular Na$^+$ accumulation. In addition, the increase in the $^{23}\text{Na}$ 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 $^{23}\text{Na}$ 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 $^{23}\text{Na}$ content of skeletal muscle. More than half of the signal is due to intracellular $^{23}\text{Na}$ if the partial volume of the extracellular compartment is not greater than 7%. Since the extracellular space of skeletal muscle is less than 8%, this prerequisite is fulfilled for normal muscle. Vacuoles, the results of a substantial proliferation of the transverse tubular system, 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 $^{23}\text{Na}$ signal is composed of the weighted average of extra- and intracellular $^{23}\text{Na}$. $[\text{Na}^+]_e$ is about 10-fold higher than $[\text{Na}^+]_i$ both in brain and muscle according to our study. $[\text{Na}^+]_i$ 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, $[\text{Na}^+]_i$ might be increased. In contrast, $[\text{Na}^+]_e$ 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 $^{23}\text{Na}$ 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 $^{23}\text{Na}$ signal was almost the same. Therefore, we believe that $[\text{Na}^+]_e$ is virtually constant and any changes of the total $^{23}\text{Na}$ signal of skeletal muscle reflect alterations of the intracellular $^{23}\text{Na}$ 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. Thus, a decreased cMAP amplitude with cooling has been considered to indicate PC and not HyperPP. 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
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\(^+\)/H\(^+\) 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\(^+\)/H\(^+\) pump whereas its enzymatic activity is reduced in the cold, the unequal electrogenic

<table>
<thead>
<tr>
<th>Type of value</th>
<th>PC, n = 3</th>
<th>HyperPP, n = 3</th>
<th>PAM, n = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before provocation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_m) (mV)</td>
<td>(-83.3 \pm 4.8) (35)</td>
<td>(-82.5 \pm 5.3) (58)</td>
<td>(-84.4 \pm 3.9) (28)</td>
</tr>
<tr>
<td>aNa(_i) (mM)</td>
<td>(7.7 \pm 0.9) (14)</td>
<td>(5.8 \pm 0.8) (3)</td>
<td>(6.4 \pm 0.6) (9)</td>
</tr>
<tr>
<td>After provocation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_m) (mV) at 27 °C</td>
<td>(-75.7 \pm 3.6) (27)</td>
<td>(-41.9 \pm 6.5) (50)*</td>
<td>—</td>
</tr>
<tr>
<td>(E_m) (mV) at 7 K(^+)</td>
<td>(-70.1 \pm 3.9) (29)</td>
<td>—</td>
<td>(-53.8 \pm 8.9) (96)*</td>
</tr>
<tr>
<td>aNa(_i) at 27 °C</td>
<td>(10.2 \pm 1.4) (10)</td>
<td>(14.5 \pm 1.9) (5)</td>
<td>—</td>
</tr>
<tr>
<td>aNa(_i) at 7 K(^+)</td>
<td>(7.4 \pm 1.2) (10)</td>
<td>—</td>
<td>(10.2 \pm 0.8) (8)</td>
</tr>
</tbody>
</table>

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 \(< 0.01\).

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 \((r = 0.48\); \(y = 0.043x + 0.24\), 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 \(23Na\) 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.\(^5\) 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.\(^6\) In contrast PC and PAM mutations slow fast inactivation but do not affect slow inactivation.\(^5,7,37\) Therefore, slow inactivation should be able to cope with an increased intracellular Na\(^+\) than in normal controls shows that PC muscle is lower in PC than in normal controls shows that PC muscle is able to cope with an increased intracellular Na\(^+\) accumulation in the warmth.

In vivo \(^1\)H 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 \(^1\)H 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.

Acknowledgment

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References


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