Na\textsubscript{v}1.4 and Na\textsubscript{v}1.5 are modulated differently during muscle immobilization and contractile phenotype conversion

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Submitted 27 September 2010; accepted in final form 11 May 2011

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Muscle immobilization induces alteration in both muscle mass and contractile properties (4, 6–9, 18, 25, 31). Experimental immobilization has previously been produced by plaster cast (31) or joint fixation (18), mainly in shortened or lengthened position. These procedures immobilized all muscles around the joint, leading to the disuse of this articulation and decrease in neuromuscular activity (12, 18, 20). These changes in neural influences, which can be the result of reduction in afferent inputs, can modulate the contractile phenotype of muscle (30). Additionally, the fixation in shortened or lengthened position alters the functional length of muscle and promotes the evolution toward a fast or a slow contractile phenotype (18, 31).

Thus joint immobilization in shortened or lengthened position modifies the contractile phenotype of muscle through alteration in either or both neural inputs, functional length, and muscle shortening prevention. To specifically investigate the effect of this last parameter on muscle phenotype conversion during muscle immobilization, an alternative model involves the fixation of the distal tendon of one muscle to the adjacent bone (22, 32). It has been shown that single-muscle immobilization at neutral length preserves motor programs, leading to conservation in the neuromuscular activity of muscle (20, 22). Furthermore, muscle immobilization at neutral length, close to optimal length (L\textsubscript{O}), allows an optimal overlap of the myosin cross bridges and actin filaments from the first day of immobilization. Single-muscle immobilization at neutral length leads to isometric contraction (9, 20), similar to the activity of a postural muscle, and a conversion toward a slow phenotype might be assumed. Unexpectedly, previous studies using single-muscle immobilization, as well as other immobilization protocols, report a conversion of muscle toward a fast phenotype (18, 23).

The slow or fast contractile properties of skeletal muscle depend on its fiber-type distribution and electrophysiological properties (39). The density of voltage-gated sodium channels (Na\textsubscript{v}) contribute to muscle fiber excitability (11). Adult skeletal muscle expresses two isoforms of Na\textsubscript{v} (Na\textsubscript{v}1.4 and Na\textsubscript{v}1.5), exhibiting different electrophysiological properties (46). These channels display different inactivation curves, leading to differences in muscle fiber excitability (16). Modifications in the Na\textsubscript{v}1.4 expression during alteration of mechanical constraints were only reported by Desaphy et al. (11) in a model of hindlimb suspension. The authors reported evolution of soleus toward a rapid phenotype in parallel with increase in skm1 expression (11). However, this model is a model of hypogravity, and there is a lack of data concerning the evolution of sodium channel types and properties during muscle immobilization. We hypothesized that single-muscle immobilization modifies the expression and the ratio of Na\textsubscript{v}1.4/Na\textsubscript{v}1.5 and thus influences muscle excitability. We chose to study the effects of immobilization on peroneus longus (PL), which is a mixed-type muscle. PL contains the same proportion of the three motor unit (MU) types (13) and displays intermediate contractile properties between soleus and extensor digitorum longus (33). ATPase staining studies have shown that PL presents the different fiber types (2, 22). Contractile properties of whole PL and Na\textsubscript{v} properties in isolated fibers were determined after 4- and 8-wk immobilization in a neutral position and compared with control animals. We observed a...
transitory shift toward a faster contractile phenotype involving sodium current modifications, corresponding to an increase in Na$_{1.4}$ channel expression.

**MATERIAL AND METHODS**

**Muscle Fixation**

All procedures were performed according to our ethical committee recommendations. The experiments were authorized by department agreement no. 29 019–3 and performed according to the recommendation of the European Community directive no. 86/609. Experiments were performed on female Wistar rats weighing 260–280 g (n = 55; Centre d’élevage Dépré, Saint-Doulchard, France). Each animal was anesthetized by an intraperitoneal injection of pentobarbital sodium (40 mg/kg, CEVA). Surgery was performed under aseptic conditions. The left PL was set to a neutral length corresponding to a 90° flexion of ankle joint. The distal tendon of left PL was cut and attached with a nylon thread (Prolène 6.0) to a hole in the adjacent bone. Analgesics were administrated after the surgical procedure (100 mg/kg paracetamol per ora) during 4 days. After surgical procedure, the animals were randomly assigned to undergo 4 or 8 wk of PL immobilization (4-wk and 8-wk groups). All of the animals were housed at 23°C, with a 12-h day light and fed ad libitum with standard rat pellets and had free access to water. Force measurement, patch-clamp recordings, Western blot, or PCR analysis were conducted after the values obtained from the left peroneus of control animals of same age and weight (Table 1). Immobilized and control PL were weighed before dissociation, RT-PCR, Western blot analysis, or after force measurement.

To further evaluate the effects of the surgery on the contractile properties of muscle, the left peroneal shae was opened, and a hole was made in the adjacent bone (n = 5 animals). In “immobilized” animals, the shae and the skin were sutured by using a double-layer procedure, and paracetamol was given. Four or eight weeks after this surgery, force measurements in left PL were performed in these animals (sham 4 and 8 wk, respectively).

**Measurement of Isometric Contractile Properties**

Muscle and nerve preparations. The rats were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg). The PL and its tendon were isolated from the surrounding tissue without impairing the blood supply. The nerve of PL was freed, and all of the other hindlimb muscles were denervated. The hindlimb was abducted to the horizontal plane, and the body of the animal was maintained at 37°C by a monitored heating element. To prevent movements during contractions, the knee and ankle joints were held onto the supporting device. The distal tendon of PL was cut and attached to a force transducer (Kulite strain gauge, model BG 1000). The force transducer was mounted on a micrometer screw so that the muscle length could be accurately adjusted. The nerve of PL was carefully placed onto a platinum hook electrode acting as a cathode for electrical stimulation. The second electrode was placed in contact with the preparation at ~10 mm from the first one.

**Twitch and tetanic forces measurement.** PL was adjusted to its L0, at which maximal active force is elicited. A single twitch was elicited by an electrical stimulus of 0.05 ms duration at supramaximal intensity. Twitch force and time to peak were measured. Force/frequency relationship was determined by incremental stimulation frequencies (10, 20, 30, 40, 50, 80, 100, 120, 150 Hz) each for a duration burst of 0.7 s. Tetanic force corresponds to the maximal force developed by muscle during tetanic stimulation. Twitch and tetanic forces were normalized to muscle weight. The ratio of tetanic force to twitch force gives the tetanus-to-twitch ratio.

**Fatigue test.** The fatigability of PL was evaluated by using Burke’s protocol (5). This entailed a 40-Hz delivery burst for 330 ms every 1 s for 2 min. The fatigue index (%) was obtained from the following relation: (peak force of the 120th tetanus/peak force of the first tetanus) × 100.

**Active and passive length-force curves.** After a recovery period of 10 min, the active and passive length-force curves were determined by recording resting and total twitch forces at different muscle lengths. Active force was calculated by subtracting the resting force from the total force (14). The slope of the passive length-force relationship was calculated on the linear part of the curve.

At the end of the experiment, the rats were killed with a lethal dose of pentobarbital.

**Patch-Clamp Recordings**

Muscle isolation and enzymatic dissociation of muscle fibers. After anesthesia with pentobarbital (40 mg/kg ip) and cervical dislocation, PL muscles were rapidly excised from the hindlimb [control (n = 5), 4 wk (n = 4), 8 wk (n = 5)]. Muscles were placed in a dissociation medium [2-hydroxyethylpiperazine-N2-ethanesulfonic acid (HEPES)-buffered saline supplemented with 3.0 mg/ml collagenase (type II, Gibco-BRL, Gaithersburg, MD)] for 2 h at 37°C. After this incubation period, muscles were dissociated by using a gentle agitation, and isolated fibers were sampled in 35-mm Petri dish for patch-clamp recordings containing standard saline solution. Only fibers showing good appearance at visual inspection (no break, no bleb, no granular aspect, good contrast, visible radial striation) were used for patch clamp.

Electrophysiological studies. Fast sodium currents were recorded in cell-attached configuration (11, 24) with the macropatch clamp technique at room temperature (22 ± 2°C). A GeneClamp 500B amplifier, equipped with a CV-5-1GU headstage, allowing the clamping of currents up to 100 nA (Axon Instruments, Foster City, CA), was used. Micropipettes were pulled and polished from GC150TF-10 borosilicate glass (Harvard Apparatus, Natick, MA) using a horizontal pipette puller (Zeitz Instruments). Pipettes had resistances averaging 2 MΩ when filled with the standard saline solution (150 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 30 mM HEPES; pH 7.4). Voltage-clamp protocols and data acquisition were performed with WinWCP V3.8.5 (Whole Cell program, University of Strathclyde, Glasgow, UK) through a 12-bit analog-to-digital/digital-to-analog interface (CED 1401, Cambridge Electronic Design, Cambridge, UK). Currents were low-pass filtered at 5 kHz and digitized at 48 kHz.

Because sodium channel density is 5- to 10-fold higher on the end-plate border than away from the end plate (36), sodium currents were recorded on the extrajunctional membrane at a site >200 μm from the end plate. This could be visualized with phase contrast under an inverted microscope (Olympus IX 70) and with a progressive-scan digital camera (XCR500CE, DONISHA, Sony, Japan). Fibers were placed in a bath recording solution containing Cs$^+$ ions as the main cation (145 mM CsCl, 5 mM EGTA, 1 mM MgCl$_2$, 10 mM HEPES, pH 7.3) to inhibit potassium currents and to depolarize the membrane. Patch-clamp recordings were carried out after 10-min incubation in the recording solution. An amplifier compensation circuit canceled capacitance currents. To eliminate transient residual capacitance and leak currents, we used the P/4 subtraction procedure (1). Briefly, four hyperpolarizing pulses with an amplitude one-fourth of the pulse test amplitude were applied to the patch before the test pulse, thus allowing further determination and then subtraction of the residual
leak current. The holding potential was set to −100 mV, a value close to physiological values in intact skeletal muscle fibers and at which most of the channels are in a closed activatable state. At this holding potential, direct transitions from closed to inactivated state can occur, leading to few nonconductive sodium channels (3).

**Sodium current measurement and activation determination.** Maximal inward sodium current (I_{max}) was measured by applying to the patch membrane a cycle of 20-ms test pulses from the holding potential of −100 mV to increasing potentials (from −60 to +130 mV by 10-mV increments). This protocol was repeated three times for each patch to ensure sodium current stability. The patches with no reliable peak current amplitude were discarded. The slope of the quasi-linear part of the current-voltage relationship gives the maximal sodium conductance (g_{Na,max}). The activation curve was obtained by plotting g_{Na}/g_{Na,max} as a function of each imposed potential from −60 to +30 mV. Calculated values of g_{Na}/g_{Na,max} were fitted with the following Boltzmann equation:

\[
\frac{g_{Na}}{g_{Na,max}} = \frac{1}{1 + e^{\left(\frac{V - V_{1/2}}{h}\right)}}
\]

where \( V \) is imposed membrane potential; \( V_{1/2} \) is potential at which one-half of the channels is activated; and \( h \) is activation slope factor.

**Time constant determination.** The activation and inactivation time constants of the sodium current (\( \tau_a \) and \( \tau_h \), respectively) were calculated at −20 mV (11) by fitting the current (I) with the following Hodgkin-Huxley relation:

\[
I(t) = A \left[ 1 - e^{-(-t)} \right] p \left[ h_{in} - (h_{in} - 1) \cdot e^{-(-t)} \right]
\]

where \( A \) is voltage-depending part of the current; \( p \) is exponent of \( m \) (−3); \( h_{in} \) is equilibrium value reached by \( h \), according to the potential; and \( t \) is time in ms.

**Fast inactivation determination.** Steady-state fast inactivation was measured by applying 50-ms conditioning prepulses to various holding potentials from −120 to +10 mV, followed by a 20-ms test pulse up to −20 mV to activate Na\(^+\) current. To calculate the fast inactivation slope factor \( k_a \) and half-inactivation voltage \( V_{1/2} \), the steady-state fast inactivation relationships were fitted with the following Boltzmann equation:

\[
\frac{I_{Na}}{I_{Na,max}} = \frac{1}{1 + e^{\left(\frac{V - V_{1/2}}{k_a}\right)}}
\]

where \( I_{Na} \) is sodium current, and \( I_{Na,max} \) is maximal sodium current.

**Slow inactivation determination.** Slow inactivation was studied by applying a double-pulse protocol separated by 50 ms at −100 mV (11). The first pulse duration was 2,000 ms with various holding potentials from −140 to 0 mV. The second pulse duration was 20 ms with a potential at −10 mV to activate sodium current. The steady-state slow inactivation relationships were fitted with the following Boltzmann equation:

\[
I_{Na} = I_{Na,min} + \frac{I_{Na,max} - I_{Na,min}}{1 + e^{\left(\frac{V - V_{1/2}}{k_s}\right)}}
\]

where \( I_{Na,min} \) is nonzero residual current; \( V_{1/2} \) is half-inactivation voltage; and \( k_s \) is slow inactivation slope factor.

**Tetrodotoxin recordings.** Tetrodotoxin (TTX) (Alomone, Jerusalem, Israel) was used to distinguish between Na\(^+\)1.4 and Na\(^+\)1.5 currents and properties. After a first sequence of recordings in control condition, the pipette was removed, and TTX (300 nM) was added to the bath (16). A new pipette filled with the same solution containing TTX than the bath was sealed at the same site on the single fiber, to further compare sodium currents with or without TTX.

### Protein Content and Immunoblotting of Sodium Channel

The 4-wk \((n = 3), 8\)-wk \((n = 3), \) and control \((n = 3)\) PL were homogenized in a homogenization buffer (100 mM choline chloride, 50 mM phosphate potassium, 0.5 mM calcium chloride, 0.5 mM magnesium chloride, 1 mg/ml pepstatin, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml benzamidine, 8 μg/ml calpain I and II, and 0.2 mM PMSF, pH 7.4). Cell debris and nuclei were eliminated by centrifugation at 500 g for 10 min, and the clear supernatant was removed and centrifuged for 1 h at 100,000 g to pellet the membranes (in a Beckman Instruments with a Ti502 rotor, Fullerton, CA), which were subsequently resuspended in the same homogenization buffer. The protein content was measured with the Bradford method. Proteins were diluted 1:1 with the Laemmli sample buffer (S3401, Sigma), boiled, and subjected to separation by one-dimensional sodium dodecyl sulfate-polyacrylamide 10% gel electrophoresis (Laemmli). The separated proteins were transferred to a nitrocellulose membrane (0.45 μm, Millipore). The blot was blocked for 1 h at room temperature with 5% milk and Tween 20 in PBS and incubated with mouse or rabbit primary antibodies against Nav1.4 (3 μg/ml anti-SK1, monoclonal mouse IgG clone L/D3, S9568, Sigma) and Nav1.5 (4 μg/ml polyclonal rabbit IgG, S0819, Sigma), respectively. Revelation was performed by goat anti-mouse (1/1,250, A2429, Sigma) or goat anti-rabbit (1/1,250, A3687, Sigma) secondary antibodies, conjugated to alkaline phosphatase, followed by detection using nitro blue tetrazolium (N6876, Sigma)/BCIP (B6777, Sigma) substrate. d-Glyceraldehyde-3-phosphate dehydrogenase was used as internal marker with revelation by antibody (Santacruz). The membranes were photographed, and densitometric tracing was obtained with densitometry software (Mesurim Pro, e-mail: jean-francois.madre@ac-amiens.fr).

### RT-PCR

RNA was isolated using TRIZOL reagent from immobilized [4-wk \((n = 2), 8\)-wk \((n = 2)\)] and control PL \((n = 2)\). To minimize DNA contamination, eluted RNA solutions were treated with a DNase. Reverse transcription reactions contained 1 μg total RNA. For real-time PCR, all oligonucleotide primers were designed from published sequences (Table 2). d-Glyceraldehyde 3-phosphate dehydrogenase and β2-microglobulin were selected as controls. The specificity of forward and reverse primers was verified with the Infobiogen website (www.infobiogen.fr). All real-time PCR assays were carried out by using ABI Prism 7000 SDS (Applied Biosystem), software: 7000 System software version 1.2 (Applied Biosystem) using designed primers. For each sample, 2 μl of cDNA were combined with 12.5 μl of Power SYBR Green PCR master mix (Applied Biosystem), 10 pmol of each primer, and sterile water for 25-μl reaction containing 1× PCR master mix. The PCR assay was carried out in duplicate for each sample. The reactions were carried out with standard conditions as follows: 50°C for 2 min, 95°C for 10 min, then 40 cycles consisting of 95°C for 15 s and 60°C for 1 min. The cycling reaction was monitored by a dissociation curve to verify the amplification of a single product. To further compare the relative gene expression in immobilized PL, the value of 100 was given to control Nav1.4, Nav1.5, and β1-subunit mRNA expressions.

### Data Analysis and Statistics

All values are given in means ± SE. Statistical differences were determined by performing Student’s t-test, after checking the normality of distribution. Mann-Whitney’s test was used to compare mRNA expressions between control and immobilized PL. Kruskal-Wallis test was used to compare the force parameters in 4- and 8-wk “sham-operated” animals to control group. Differences were considered significant for \( p < 0.05. \)
Table 2. Primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Amplicon Size, bp</th>
</tr>
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<tbody>
<tr>
<td>α Na, 1.4</td>
<td>5′-GCTTGGGCTCTGACTGGTGA-3′</td>
<td>5′-ACAACGGTGAGCAGCCACTG-3′</td>
<td>51</td>
</tr>
<tr>
<td>α Na, 1.5</td>
<td>5′-GGGCCCCTGAAATACTATCGG-3′</td>
<td>5′-GCCATCCCAAAGACGAACATT-3′</td>
<td>85</td>
</tr>
<tr>
<td>β-actin</td>
<td>5′-GCTTGCCGCCATGGAAGATGCA-3′</td>
<td>5′-GAGATCCGGCTTGGAGAAG-3′</td>
<td>71</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-TCGCTGACGGCTGGAATGGGA-3′</td>
<td>5′-GGAGATCCGGCTTGGAGAAG-3′</td>
<td>71</td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>5′-TGCCATTCAGAAAAACTCC-3′</td>
<td>5′-GGAAGTTGGGCTTCCATTC-3′</td>
<td>71</td>
</tr>
</tbody>
</table>

Sequences of forward and reverse primers used to amplify voltage-gated sodium channel α- and β2-subunits, and the size expected for each PCR amplified product. GAPDH and β2-microglobulin were selected as controls. Na+, voltage-gated sodium channels.

RESULTS

Animal and Muscle Weights

During the immobilization period, animal weight showed a nonsignificant increase. In the 4-wk group, the animal weight was 273.4 ± 6.5 g at day 0 and 285.2 ± 8.1 g after 4 wk of immobilization (P = 0.23). In the 8-wk group, the body weight tended to increase during the immobilization period (269.1 ± 7.3 g at day 0 and 291.3 ± 9.4 g after 4 wk of immobilization, P = 0.12). The weight of control animals (279.1 ± 7.6 g) was not significantly different from the weight of 4-wk and 8-wk immobilized animals. In the sham 4-wk group, the animal weight was 276.9 ± 8.6 g at day 0 and 289.2 ± 9.3 g 4 wk after the surgery (P = 0.14, Mann-Whitney). In the sham 8-wk group, the animal weight was 274.5 ± 15.5 g at day 0 and 292.5 ± 13.5 g 8 wk after the surgery (P = 0.22, Mann-Whitney).

In control animals, the average muscle weight was 119.0 ± 3.9 mg, and the muscle-to-body wt ratio was 0.43 ± 0.01 mg/g. Immobilized PL showed significant atrophy (88.4 ± 5.4 mg) after 4 wk of immobilization compared with control (P < 0.001). The muscle-to-body weight ratio in the 4-wk group was 0.31 ± 0.02 mg/g. The muscle weight in the 8-wk group was 66.5 ± 7.2 mg, corresponding to a 0.23 ± 0.02 mg/g muscle-to-body weight ratio, which is significantly lower compared with the 4-wk group (P = 0.004) and the control group (P < 0.001). Thus, compared with control, the average reduction of muscle weight after 4 and 8 wk of immobilization was 25.7 ± 3 and 44.1 ± 3%, respectively. The muscle-to-body weight ratios were similar in the “sham-operated” animals (0.44 ± 0.02 and 0.41 ± 0.01 mg/g in 4- and 8-wk sham, respectively) and the control group.

Contractile Measurements

The contractile properties of the left PL were evaluated in seven animals in each group (control, 4 wk, and 8 wk). Force measurements were conducted in isometric condition at the L_0 (Figs. 1 and 2). Single-muscle immobilization leads to significant alteration in the contractile properties of PL, as shown in Table 3. However, when normalized to muscle weight, peak twitch and maximal tetanic force were not decreased in immobilized muscle. The time to peak force was lower in the 4-wk PL compared with control muscle (P = 0.004). After 8 wk of immobilization, the time to peak force showed a nonsignificant increase compared with that in the 4-wk group (P = 0.46). The tetanus-to-twitch ratio was significantly lower in the 4-wk group compared with control PL (P = 0.0064), and higher in the 8-wk group. Four weeks of immobilization increased PL fatigability during repetitive submaximal 2-min tetanic contractions. The fatigability index was lower in 4-wk compared with control PL (P = 0.00016). In the 8-wk group, the index of fatigability was significantly higher compared with that in the 4-wk group (P = 0.0082). As shown in Fig. 3, the force/frequency relationship displayed a significant rightward shift in 4-wk immobilized PL compared with control PL. In the 8-wk group, the force-frequency curve partially recovered the control value. The decreases in the tetanus-to-twitch ratio and in the index of fatigability, in addition to the “right-shift” in the force-frequency curve, were consistent with a more rapid phenotype in the 4-wk immobilized PL compared with control muscle. On the contrary, a fast-to-slow phenotype shift was observed between 4 and 8 wk of immobilization.

The effect of muscle length on active and passive forces was determined after setting L_0 to maximal active force (Fig. 4). When normalized to muscle length, the active force was similar in 4-wk and control PL (Fig. 4B) and increased in 8-wk muscle. As illustrated in Fig. 4D, there was a decrease in the slope of the passive length-force curve in the 4-wk group. The slope of the passive normalized length-force curve in control, 4-wk, and 8-wk groups were 4.5 ± 0.2, 3.8 ± 0.3, and 6.8 ± 0.6 N·g⁻¹·mm⁻¹, respectively. This suggests a transient de-
crease in the passive force of PL, after 4 wk of immobilization. To evaluate the involvement of the surgical intervention on such modifications of the contractile characteristics in PL, an additional series of force recordings were conducted. As summarized in Table 4, the twitch and the tetanic contractile properties were similar in the “sham-operated” and the control groups. The force-frequency relationship and the length-dependence of the force were unaffected by the surgical procedure (Figs. 5 and 6, respectively).

Maximal Sodium Currents

The effects of immobilization on inward sodium currents in control condition or in the presence of 300 nM of TTX are illustrated in Fig. 7. The $I_{\text{max}}$ was measured in control ($n = 23$), 4-wk ($n = 11$), and 8-wk ($n = 14$) immobilized fibers (Table 5). $I_{\text{max}}$ increased significantly after 4 wk of immobilization ($11.5 \pm 1.2$ in 4-wk vs. $7.8 \pm 0.8$ nA in control fibers, $P = 0.0076$). After 8 wk of immobilization, $I_{\text{max}}$ was lower compared with the 4-wk fibers ($P = 0.019$). In the presence of TTX, $I_{\text{max}}$ significantly decreased during the immobilization period ($P = 0.024$ between control and 4-wk immobilized fibers, and $P = 0.020$ between 4- and 8-wk immobilized fibers). In control fibers, $I_{\text{max}}$ in the presence of TTX represented $24.4 \pm 2.6\%$ of the $I_{\text{max}}$ in control condition, whereas the corresponding values in 4- and 8-wk immobilized fibers were $11 \pm 0.9$ and $12 \pm 1.6\%$, respectively.

Sodium Channel Properties

Time constants. To further compare the effect of immobilization and TTX on sodium channel time constants, $\tau_m$ and $\tau_h$ were calculated for one given membrane potential ($-20$ mV). Only a decrease in $\tau_h$ was observed in 8-wk immobilized fibers compared

Table 3. Effects of immobilization on the contractile properties of rat peroneus longus

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>4 wk</th>
<th>8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Twitch force, N</td>
<td>0.39 ± 0.02</td>
<td>0.29 ± 0.03*</td>
<td>0.30 ± 0.04*</td>
</tr>
<tr>
<td>Twitch force/muscle weight, N/g</td>
<td>3.5 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>4.1 ± 0.4†</td>
</tr>
<tr>
<td>Time to peak force, ms</td>
<td>52.6 ± 1.4</td>
<td>43.3 ± 2.6*</td>
<td>47.4 ± 1.9*</td>
</tr>
<tr>
<td>Tetanic force, N</td>
<td>1.99 ± 0.18</td>
<td>1.23 ± 0.20*</td>
<td>1.78 ± 0.23†</td>
</tr>
<tr>
<td>Tetanic force/muscle weight, N/g</td>
<td>17.9 ± 1.6</td>
<td>14.0 ± 2.4</td>
<td>25.1 ± 3.2†</td>
</tr>
<tr>
<td>Tetanus-to-twitch ratio</td>
<td>5.11 ± 0.17</td>
<td>4.20 ± 0.21*</td>
<td>6.04 ± 0.23†</td>
</tr>
<tr>
<td>Fatigability index, %</td>
<td>65.6 ± 2.4</td>
<td>29.4 ± 6.3*</td>
<td>51.6 ± 3.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of rats. The contractile properties of left peroneus longus (PL) were evaluated after 4 or 8 wk of immobilization and in control animals, in isometric condition at optimal length ($L_o$). Twitch and tetanic forces were normalized to muscle weight. *Significantly different from control ($P < 0.03$). †Significantly different from 4 wk ($P < 0.04$).
with control and 4-wk fibers ($P = 0.029$ and $P = 0.014$, respectively).

The effects of muscle immobilization on activation and fast and slow inactivation of Na, were evaluated in the absence and in the presence of 300 nM TTX. Activation and inactivation parameters are summarized in Table 5.

**Activation.** Figure 8 displays current-voltage relationship and activation curve of sodium currents. As shown in Fig. 8B, the activation curves were not significantly different between controls and 4- and 8-wk immobilized fibers. TTX shifted activation curves toward hyperpolarized potentials in both immobilized and control fibers. Control and 4- and 8-wk immobilized fibers exhibited similar $V_{a1/2}$ in the presence of TTX ($-26.2 \pm 2.6$, $-29.0 \pm 3.5$, and $-25.1 \pm 2.7$ mV, respectively, nonsignificant).

**Fast inactivation.** To obtain the steady-state fast inactivation relationship, the ratio of the peak current amplitude at a given prepulse potential ($120$ to $10$ mV) to the maximal peak current amplitude was plotted against the prepulse potential (Fig. 9A). There were no differences in the voltage dependence of inactivation between control ($n = 17$), 4-wk ($n = 10$), and 8-wk immobilized fibers ($n = 12$), when compared in control

### Table 4. Effects of the surgical procedure on the contractile properties of rat peroneus longus

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Sham 4 wk</th>
<th>Sham 8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Twitch force, N</td>
<td>$0.39 \pm 0.02$</td>
<td>$0.44 \pm 0.02$</td>
<td>$0.37 \pm 0.04$</td>
</tr>
<tr>
<td>Twitch force/muscle weight, N/g</td>
<td>$3.5 \pm 0.2$</td>
<td>$3.3 \pm 0.2$</td>
<td>$3.6 \pm 0.4$</td>
</tr>
<tr>
<td>Time to peak force, ms</td>
<td>$52.6 \pm 1.4$</td>
<td>$50.2 \pm 3.2$</td>
<td>$48.5 \pm 2.8$</td>
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<tr>
<td>Tetanic force, N</td>
<td>$1.99 \pm 0.18$</td>
<td>$2.16 \pm 0.37$</td>
<td>$2.06 \pm 0.29$</td>
</tr>
<tr>
<td>Tetanic force/muscle weight, N/g</td>
<td>$17.9 \pm 1.6$</td>
<td>$17.7 \pm 2.3$</td>
<td>$18.0 \pm 3.3$</td>
</tr>
<tr>
<td>Tetanus-to-twitch ratio</td>
<td>$5.11 \pm 0.17$</td>
<td>$5.29 \pm 0.95$</td>
<td>$5.75 \pm 1.57$</td>
</tr>
<tr>
<td>Fatigability index, %</td>
<td>$65.6 \pm 2.4$</td>
<td>$67.2 \pm 4.9$</td>
<td>$64.4 \pm 4.8$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$, no. of rats. The contractile properties of left PL were evaluated 4 or 8 wk after surgery and compared with that of control animals, in isometric condition at $L_o$ (Kruskal-Wallis test). Twitch and tetanic forces were normalized to muscle weight.
condition or in the presence of 300 nM TTX (Table 5). It should be noticed that TTX shifted the inactivation curves toward hyperpolarized potentials in both control and immobilized fibers.

**Slow inactivation.** The development of slow inactivation was studied in control \((n = 13)\), 4-wk \((n = 9)\), and 8-wk \((n = 10)\) immobilized fibers (Fig. 9B). The normalized nonzero residual current \(I_{\text{min}}/I_{\text{max}}\) was higher in the 4-wk compared with control fibers \((P = 0.024)\). In the 8-wk fibers, \(I_{\text{min}}/I_{\text{max}}\) was lower compared with that in the 4-wk fibers \((P = 0.038)\). Figure 9B shows that, in the presence of TTX, the slow inactivation curves of control and immobilized fibers were shifted toward hyperpolarized potentials. Subsequently, \(I_{\text{min}}/I_{\text{max}}\) in control and immobilized fibers was significantly decreased by TTX compared with control condition (Table 5).

**Sodium Channels and mRNA Expression**

\(\text{Na}_1.4\) and \(\text{Na}_1.5\) expressions were evaluated by Western blot analysis. \(\text{Na}_1.4\) expression increased in 4-wk PL and recovered to control level after 8 wk of immobilization, as shown in Fig. 10A. On the contrary, the amount of \(\text{Na}_1.5\) decreased throughout immobilization (Fig. 10B). RT-PCR analysis was used to quantify mRNA. \(\text{Na}_1.4\) showed a similar amount between control and 4- and 8-wk PL (Fig. 11), whereas \(\text{Na}_1.5\) was significantly lower in 4- and 8-wk muscles compared with control PL \((P = 0.025)\). \(\beta_1\)-subunit mRNA expression was decreased by immobilization, leading to a significant difference between 8-wk muscles and control \((P = 0.025)\).

**DISCUSSION**

Our aim was to investigate the effects of 4 and 8 wk of immobilization on the contractile properties and the \(\text{Na}_1\) of rat PL. An interesting result of this study is that the contractile properties of immobilized muscle display a transient conversion toward a faster phenotype. In parallel, we observed modifications in electrophysiological properties related to \(\text{Na}_1.4\)-to-\(\text{Na}_1.5\) ratio.

*Fig. 6. Effects of surgery on the length dependence of active (A) and passive forces (B). The active and the passive length-force relationships were evaluated 4 and 8 wk after the surgical procedure ("sham-operated" animals, see MATERIALS AND METHODS) and compared with control. Values are means ± SE.*

*Fig. 7. Effects of muscleimmobilization on the voltage-gated sodium channel currents. The currents were elicited with 20-ms pulses varying from −60 to +130 mV. The holding potential was −100 mV. The currents were recorded in control condition (A, C, E) and in the presence of 300 nM tetrodotoxin (TTX; B, D, F), in the same area of the muscle fiber membrane. A and B: control muscle fibers. C and D: 4-wk immobilized muscle fibers. E and F: 8-wk immobilized muscle fibers.*
in absolute value and relative to the membrane surface under the pipette. The activation and inactivation time constants of the sodium current (\( I_{Na} \)) are determined at -20 mV. Activation and inactivation parameters are derived from fits with the Boltzmann equation. 

Table 5. Sodium current parameters in fibers from immobilized and control rat peroneus longus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control TTX</th>
<th>4-wk TTX</th>
<th>4-wk TTX</th>
<th>8-wk TTX</th>
<th>8-wk TTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{Na} ), nA</td>
<td>7.8 ± 0.8</td>
<td>1.9 ± 0.2a</td>
<td>11.5 ± 1.2a</td>
<td>1.3 ± 0.2bc</td>
<td>8.6 ± 0.7c</td>
<td>0.9 ± 0.1bcde</td>
</tr>
<tr>
<td>( I_{Na} ), A/mm²</td>
<td>15.9 ± 1.6</td>
<td>3.9 ± 0.4a</td>
<td>23.4 ± 2.4a</td>
<td>2.6 ± 0.3bc</td>
<td>17.5 ± 1.4a</td>
<td>1.8 ± 0.2bcde</td>
</tr>
<tr>
<td>( g_{Na_{max}} ), μS</td>
<td>132.0 ± 14.7</td>
<td>26.7 ± 4.2a</td>
<td>171.5 ± 13.6a</td>
<td>16.1 ± 1.7bc</td>
<td>134.1 ± 11.9c</td>
<td>12.0 ± 1.1bcde</td>
</tr>
<tr>
<td>( g_{Na_{max}} ), S/mm²</td>
<td>268.8 ± 29.9</td>
<td>54.4 ± 8.6a</td>
<td>349.3 ± 27.7a</td>
<td>32.8 ± 3.3bc</td>
<td>273.1 ± 24.2c</td>
<td>24.4 ± 2.2bcde</td>
</tr>
<tr>
<td>( \tau_{a}, ) ms</td>
<td>0.19 ± 0.01</td>
<td>0.14 ± 0.02a</td>
<td>0.17 ± 0.02</td>
<td>0.12 ± 0.02e</td>
<td>0.20 ± 0.02</td>
<td>0.11 ± 0.02e</td>
</tr>
<tr>
<td>( \tau_{h}, ) ms</td>
<td>0.26 ± 0.01</td>
<td>0.20 ± 0.01a</td>
<td>0.29 ± 0.03</td>
<td>0.23 ± 0.02e</td>
<td>0.21 ± 0.02e</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Activation</td>
<td>23</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>( V_{1/2a}, ) mV</td>
<td>-13.2 ± 3.5</td>
<td>-26.2 ± 2.6a</td>
<td>-17.0 ± 4.4</td>
<td>-29.0 ± 3.5c</td>
<td>-14.6 ± 3.1c</td>
<td>-25.1 ± 2.7c</td>
</tr>
<tr>
<td>( K_a, ) mV</td>
<td>11.4 ± 0.6</td>
<td>9.9 ± 0.6</td>
<td>11.5 ± 1.5</td>
<td>9.6 ± 1.3</td>
<td>10.5 ± 0.6</td>
<td>8.6 ± 2.6</td>
</tr>
<tr>
<td>Fast inactivation</td>
<td>17</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>( V_{1/2b}, ) mV</td>
<td>-49.2 ± 3.6</td>
<td>-63.8 ± 13.6a</td>
<td>-46.5 ± 5.1</td>
<td>-62.7 ± 6.3c</td>
<td>-47.3 ± 3.4</td>
<td>-66.3 ± 2.3c</td>
</tr>
<tr>
<td>( K_b, ) mV</td>
<td>11.6 ± 0.7</td>
<td>11.8 ± 0.8</td>
<td>13.9 ± 1.9</td>
<td>14.0 ± 1.2</td>
<td>12.2 ± 1.2</td>
<td>-12.0 ± 0.9</td>
</tr>
<tr>
<td>Slow inactivation</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>( V_{1/2c, ) mV</td>
<td>-73.1 ± 6.2</td>
<td>-88.8 ± 4.3a</td>
<td>-57.7 ± 7.8</td>
<td>-93.2 ± 5.0a</td>
<td>-69.8 ± 5.8</td>
<td>-90.1 ± 5.0a</td>
</tr>
<tr>
<td>( K_c, ) mV</td>
<td>17.1 ± 1.6</td>
<td>13.5 ± 1.1a</td>
<td>19.1 ± 2.7</td>
<td>14.1 ± 1.9a</td>
<td>-17.6 ± 1.9</td>
<td>-15.3 ± 1.7</td>
</tr>
<tr>
<td>( I_{max}/I_{Na} )</td>
<td>0.48 ± 0.03</td>
<td>0.29 ± 0.02a</td>
<td>0.69 ± 0.05a</td>
<td>0.25 ± 0.03e</td>
<td>0.58 ± 0.02c</td>
<td>0.30 ± 0.03c</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Sodium currents were recorded in single fibers from left PL after 4 wk or 8 wk of immobilization in neutral position and compared with PL fibers from control animals. Patch-clamp recordings were collected in control condition and in the presence of 300 nM tetrodotoxin (TTX), at the same site on each muscle fiber. \( I_{Na} \) and \( g_{Na_{max}} \) are the maximal inward sodium current and the maximal sodium conductance, respectively, expressed in absolute value and relative to the membrane surface under the pipette. The activation and inactivation time constants of the sodium current (\( \tau_{a}, \) and \( \tau_{h}, \) respectively) were determined at -20 mV. Activation and inactivation parameters are derived from fits with the Boltzmann equation. \( V_{1/2a}, V_{1/2b}, \) and \( V_{1/2c} \) are the half-maximal activation and fast and slow inactivation potentials, respectively. \( K_a, K_b, \) and \( K_c, \) are the slope factors for activation and fast and slow inactivation processes, respectively. \( I_{max}/I_{Na} \) is the ratio of non-zero residual current to maximal current. Statistical analysis was performed between each group (control, 4 wk, and 8 wk) with distinction between control and TTX conditions. Significantly different from acontrol (\( P < 0.05), ^{b} \) control TTX (\( P < 0.03), ^{c} \) 4 wk (\( P < 0.02), ^{d} \) 4-wk TTX (\( P < 0.02 \)), and ^{e} \) 8 wk (\( P < 0.03 \)).

**Contractile Properties**

During the initial 4 wk of immobilization, the contractile properties of PL shift toward a more rapid phenotype, as shown by the lessening in the tetanus-to-twitch ratio (18) and the index of fatigability (5) associated with a “right-shift” in the force-frequency curve (21). Between 4 and 8 wk of immobilization, the opposite shift was observed, leading the immobilized muscle to partially recover the contractile properties of control PL phenotype. Earlier studies reported a fast conversion in immobilized muscle (18, 20, 45). However, these authors did not show any partial recovery, as they used only one immobilization duration period. As discussed before, in these studies, joint fixation was used for muscle immobilization, which can alter the motor programs (12) and thus the contractile phenotype of muscle (30). Muscle immobilization at neutral length leads to isometric contraction (20). This corresponds to the activity of a postural muscle, and conversion toward a slow phenotype might be expected. Nevertheless, a slow-to-fast conversion was observed during the early stage of immobilization in our study. As illustrated in Fig. 4D, the slope of the passive length-tension curve exhibits a decrease at 4 wk of immobilization compared with control and 8-wk immobilized muscle, whereas contraction time increases. Therefore, when fibers contract in 4-wk immobilized muscle, the passive resistance is less important and leads to a quicker force development, compared with control and 8-wk immobilized muscle. This decrease of muscle stiffness in 4-wk immobilized muscle could contribute to a quicker twitch contraction time and thus to a faster contractile phenotype. Single PL immobilization in a neutral position leads to muscle atrophy. This result is consistent with previous studies when muscle was immobilized in shortened or neutral positions, irrespective of the method of immobilization (20, 22, 45). However, when normalized to muscle weight, the contractile properties were not decreased, indicating that atrophy does not mainly affect the contractile proteins. In single-muscle immobilization at neutral length, motor programs and neural inputs are conserved (20, 22). Additionally, the immobilization of muscle at neutral length, close to \( L_0, \) allows maximal interdigitation between the myosin cross bridges and actin filaments since the first day of immobilization. Despite the preservation of these two factors, namely, neural influences and optimal functional length (30), contractile phenotype conversion and atrophy were observed during the immobilization period. Thus our present results provide evidence that the opportunity for muscle to shorten or lengthen is essential to maintain muscle trophicity and contractile properties. These data are consistent with the failure of electrostimulation to avoid the loss of mass in disused muscles (19). Countermeasure programs may also involve muscle stretching to prevent atrophy during immobilization (15, 27, 35).

**Sodium Currents and Muscle Phenotype**

The changes in the contractile phenotype of immobilized PL heighten our interest to focus on the evolution of \( Na^+ \) during muscle immobilization. In fact, previous studies have attempted to link the properties of sodium channels to a muscle contractile phenotype or a single-muscle fiber type (11, 36). After 4 wk of immobilization, PL exhibits a faster contractile
phenotype, as the maximal sodium current increases in dissociated fibers. Conversely, 8-wk immobilized PL displays a slower phenotype compared with 4-wk PL, whereas maximal sodium current decreases. It has been shown that peak inward sodium current, related to sodium channel density, is higher in “fast” compared with “slow” muscle fibers (11, 38). Therefore, the modifications in maximal sodium currents we observed, in parallel with changes in muscle contractile phenotype during immobilization, are consistent with previous studies in control muscle fibers. In fast fibers, the higher currents increase the excitability and contribute to a more rapid depolarization of muscle fiber (11). The type of sodium channels expressed in muscle fiber can also modulate its excitability (16), since Nav1.4 and Nav1.5 display different electrophysiological properties. Na\textsubscript{v}1.5 is resistant to nanomolar concentration of TTX, unlike Na\textsubscript{v}1.4 (10). Regarding the concentration we used, 97% of Na\textsubscript{v}1.4 channels were inhibited by TTX (16), thus allowing specific study of Na\textsubscript{v}1.5 properties. The comparison of our results obtained in the absence and in the presence of TTX indicates a transient enhancement of Na\textsubscript{v}1.4 current after 4 wk of immobilization, whereas Na\textsubscript{v}1.5 current decreases continuously throughout immobilization. In the same way, at the protein level, Na\textsubscript{v}1.4 expression is upregulated and Na\textsubscript{v}1.5 expression is downregulated during the early stages of immobilization and fast-phenotype conversion. Our results are in keeping with Desaphy et al. (11), who observed an increase in

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**Fig. 8.** Effects of muscle immobilization on the voltage dependence of sodium channel activation. Curves were determined in control condition and in the presence of 300 nM TTX. The holding potential was −100 mV. A: current-voltage relationship. Currents were normalized to membrane patch area. B: activation curves, corresponding to sodium conductance (g\textsubscript{Na \text{max}}) for each tested potential between −60 and +30 mV, were obtained by fitting data with the Boltzmann equation. Values are means ± SE.

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**Fig. 9.** Effects of muscle immobilization on the fast and slow inactivation processes. Curves were determined in control condition and in the presence of 300 nM TTX, by fitting data with the Boltzmann equation. The holding potential was −100 mV. A: steady-state fast inactivation. Inactivation prepulses of 50 ms to potentials ranging between −120 to +10 mV were applied, and peak sodium current (I\textsubscript{Na}) amplitudes were measured during a 20-ms test pulse whose amplitude was selected to give a response near the maximum. The peak values of inward currents were normalized to maximal amplitude and plotted against prepulse voltage. B: slow inactivation curve. A first pulse was applied for 2,000 ms, with various holding potentials from −140 mV to 0 mV. The second pulse duration was 20 ms, with a potential at −10 mV to activate I\textsubscript{Na \text{max}}, maximal sodium current. Values are means ± SE.
skm1 level in a slow-to-fast muscle conversion. Conversely, Zebedin et al. (47) found that Nav1.4 expression decreases in a fast to slow transition model using murine cell line C2C12. Modifications in Na₉,1.4 activation and inactivation time constants could also contribute in lessening the maximal sodium current after 8 wk of immobilization toward control values.

The τᵢᵣ displays a nonsignificant increase in 8-wk immobilized fibers, whereas τᵢ.onerror not found for sodium current. Likewise, no changes were observed in the time constants of sodium currents in the presence of TTX throughout immobilization. This indicates that immobilization does not affect Na₉,1.5 time constants. Thus, in 8-wk fibers, the reduction in the Na₉,1.4 τᵢᵣ contributes to reduction of the maximal sodium current, in addition to the decrease in channel protein expression.

**Sodium Channel Properties**

Activation and fast and slow inactivation curves were shifted toward hyperpolarized potentials in the presence of TTX. Filatov and Rich (16) described the same shift in the fast inactivation curves with TTX. Our results provide evidence that activation also occur at potentials more negative for Na₉,1.5 compared with Na₉,1.4. The voltage dependence of activation and fast inactivation are not significantly different between control and immobilized fibers, with and without TTX. These results indicate that immobilization does not affect the voltage dependence of activation or fast inactivation in Na₉,1.4 and Na₉,1.5.

Fig. 10. Western blot of voltage-gated sodium channels in control and 4-wk and 8-wk immobilized muscles. Expression of voltage-gated sodium channel (Na₉), Na₉,1.4 (A), Na₉,1.5 (B), and GAPDH (C) in the Western blot analysis and the correspondent densitometric analysis (absorbance) are shown. The value of 100 was given to the absorbance in the control group.

Fig. 11. Reverse transcription-real-time polymerase chain reaction in control and 4-wk and 8-wk immobilized muscles. The value of 100 was given to control Na₉,1.4, Na₉,1.5, and β₁-mRNA expressions. Values are means ± SE. *Different from control PL (P < 0.03). †Different from 4-wk immobilized PL (P < 0.03).
In contrast, the slow inactivation process in Na\textsubscript{a1.4} is significantly modulated during immobilization, with a transient rightward shift of the curve after 4 wk of immobilization. It should be emphasized that Na\textsubscript{a1.4} is more resistant to the slow inactivation process, compared with Na\textsubscript{a1.5}, according to the residual current. In the presence of TTX, the slow inactivation curves and the nonzero residual currents were not different between control and immobilized fibers. This suggests that Na\textsubscript{a1.5} slow inactivation is not altered by immobilization. Therefore, the modulation of slow inactivation curve observed without TTX during immobilization results from modification in the slow inactivation process of Na\textsubscript{a1.4}. Desaphy et al. (11) described an enhancement in the residual current concomitant with an increase in skm1 expression, which is consistent with our results in 4-wk immobilized fibers. This variation in the slow inactivation curve in Na\textsubscript{a1.4} raises the question of its physiological significance, because the contractile phenotype of muscle is also modified during immobilization. Simoncini and Stuhmer (42) proposed that the slow inactivation process regulates muscle membrane excitability via reduction in the number of excitable sodium channels, thus leading to a modulation in muscle fatigability. In the present study, Burke's index of fatigability was calculated. This provides a useful comparison between the fatigability of whole muscle and the slow inactivation process in single fibers, throughout immobilization. PL fatigability increases after 4 wk of immobilization and decreases after 8 wk, whereas the residual current increases and decreases, respectively. Therefore, our results conflict with usual conceptions concerning the slow inactivation process. Slow muscles are thought to be less affected by the slow inactivation process to preserve muscle excitability (42). Ruff et al. (37) showed that the slow inactivation curve in fibers from extensor digitorum longus appears at more negative potentials compared with fibers from soleus. This discrepancy possibly relates to different experimental protocols. It should be highlighted that the protocols used to elicit the slow inactivation process differ between studies, leading to difficulties in data comparison. As suggested by Desaphy et al. (11), these different protocols may correspond to different time-dependent processes. However, using our protocol, we observed a shift in 4-wk immobilized fibers, corresponding to a greater number of channels in an excitable state. Consequently, a higher frequency of recruitment is consistent with the faster phenotype that we observed in the fibers from 4-wk immobilized muscle.

Mechanical Regulation of Sodium Channels

The mechanism underlying the changes in channel types and density during immobilization remains to be defined. Modifications in Na\textsubscript{a1.4} and 1.5 expressions have been recently described in cultured muscle fibers (29). During this in vitro model of denervation, the Na\textsubscript{a1.5} and the β\textsubscript{1}-subunit expressions increased, whereas the levels of Na\textsubscript{a1.4} decreased. Despite differences in experiment duration (1 wk for fiber culture and 4 or 8 wk for muscle immobilization), this strongly argues for opposite effects of immobilization and denervation on muscle Na\textsubscript{a1.4} and 1.5 expressions (28).

It has been shown that β\textsubscript{1}-subunit enhances the membrane insertion of Na\textsubscript{a} (26). Nonetheless, we observed a decrease in the β\textsubscript{1}-subunit mRNA levels throughout immobilization, suggesting another mechanism that acts in our model. Given that the force parameters were unaffected by the surgical intervention in the “sham-operated” animals, muscle immobilization per se may explain such Na\textsubscript{a} and contractile modifications in immobilized PL. Because motor programs and thus neural inputs are preserved throughout immobilization (20, 22), an intrinsic factor acting in muscle (“myogenic” factor) could be forwarded as an explanation (18, 40). Active and passive components of muscle may be altered during immobilization (14, 43, 44).

In cat PL, Filippi and Troiani (17) have shown that the L\textsubscript{O} of the different types of MUs can differ from whole muscle L\textsubscript{O}. The L\textsubscript{O} of the fast-fatigable MU, and thus fast-twitch fibers, is near muscle L\textsubscript{O}, unlike slow and fatigue-resistant MUs. Thus the present model of whole PL immobilization at neutral length could promote the selective atrophy of the slowest muscle fibers during the initial 4 wk of immobilization (32, 43). In this model, transient alteration in CSA of type I fibers has been reported (32). This could explain the enhancement of the maximal sodium current after 4 wk of immobilization, given that fast-twitch fibers exhibit greater sodium currents compared with slow fibers (34, 36).

Modification in the resting tension of muscle could also modulate its contractile properties, as proposed by Goldspink et al. (23). The immobilization of muscle in a lengthened or a shortened position results in a slow or fast conversion, respectively (9, 18, 23, 31). It has been suggested that, in the lengthened position, the resting passive tension increases, whereas a decrease occurs in the shortened position (31). In this study, a decrease in the passive length-force curve was observed after 4 wk of immobilization. An attractive hypothesis is that the muscle membrane tension influences the distribution of the sodium channel types and, consequently, modulates the contractile phenotype of muscle. It has been suggested that the cytoskeleton interacts with muscle sodium channel (41). Four-week immobilized fibers exhibit more frequently a “coiled” aspect, suggesting that the cytoskeleton is altered. It could be hypothesized that muscle membrane tension controls Na\textsubscript{a1.4} insertion and/or function via the cytoskeleton. The chronic reduction in passive tension, as observed in 4-wk immobilized muscle, can be related to a modification in the cytoskeleton of muscle fiber and can thus enhance Na\textsubscript{a1.4} insertion. It should be noticed that Na\textsubscript{a1.4} channel density is increased, whereas the corresponding mRNA is only slightly modified, suggesting a reduction in the membrane turnover. Desaphy et al. (11) observed an increase in the sodium current density of soleus, in a model of rat hindlimb unloading. Hindlimb suspension leads to decrease of the gravity influence on whole hindlimb and provides data consistent with our concept. This experimental model is different from single-muscle immobilization, but the common effect might be a chronic reduction in the resting tension, leading to increase of the sodium channel density.

Therefore, immobilization induces adaptation of active and passive structure of muscle, leading to modulate Na\textsubscript{a} expression by different mechanisms. Such adjustments of muscle active and passive components may develop according to a different time scale during immobilization, resulting in transient modification in Na\textsubscript{a} expression and contractile properties.
Conclusion

Despite the preservation of neural influences and a functional length close to $L_O$, the contractile properties of immobilized muscle display a transitional fast phenotype conversion after 4 wk of immobilization. Between 4 and 8 wk of immobilization, the muscle tends to recover the initial contractile phenotype. During these muscle phenotype conversions, the Na$_{1.4}$ current first increases and then decreases, whereas Na$_{1.5}$ expression is downregulated throughout immobilization. This suggests that the opportunity for muscle to shorten or lengthen is necessary to preserve muscle electrophysiological and mechanical properties.

GRANTS

Public funding from the French Ministry of Research supported this work.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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