Early changes of type 2B fibers after denervation of rat EDL skeletal muscle

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The main goal of the study was to investigate whether and how sarcoplasmic reticulum properties of type 2B fibers from the fast-twitch EDL muscle are altered by denervation. We also studied, in denervated single fiber, the Ca$^{2+}$ sensitivity of myofibrillar proteins. To evaluate any possible relationships with the properties of denervated muscle, we extended our study at the whole muscle level to examine mechanical and electrophysiological properties.

Our results indicate that denervation causes significant modifications of sarcoplasmic reticulum and myofibrillar properties of type 2B fibers even during the first week of denervation. Moreover, most of the alterations observed at 2 days are reverted at 7 days after denervation. Therefore, these data support the idea of the dependency of type 2B phenotype on nerve activity and/or trophic factors.

**METHODS**

The study was approved by the Ethics Committee of the Medical Faculty of the University of Padova. The experiments were carried out on Wistar adult rats (3–6 mo old). The sciatic nerve was cut unilaterally at the level of the trochanter, while the animals were under ether anesthesia, and −1.0 cm of the peripheral nerve stump was removed to avoid reinnervation. Two or seven days later, the animals were killed, always under ether anesthesia, and the EDL muscle of both limbs was then excised and utilized for the experimental analyses.

**Single-Fiber Preparation**

Muscle fibers were chemically skinned as previously described (33, 40). Briefly, muscles were tied to a wooden stick and quickly immersed in an ice-cold skinning solution containing (mM) 170 potassium propionate, 2.5 magnesium propionate, 2.5 Na$_2$K$_2$ATP, 5 K$_2$EGTA, and 10 imidazole buffer, pH 7.0. The skinned fibers were stored at −20°C for no more than 4 wk in a skinning solution supplemented with 50% (vol/vol) glycerol, and, to minimize differences in mechanical and biochemical fiber properties as a result of unpredictable effects of the experimental procedure, attention was paid to store control and denervated preparations for the same period.

**Single-Fiber Functional Studies**

**Ca$^{2+}$ sensitivity of tension development.** pCa-tension curves (pCa is −log Ca$^{2+}$ concentration) were obtained by exposing the fiber sequentially to solutions with different free Ca$^{2+}$ concentration (33), calculated by using apparent association constants described by Orentlicher et al. (34). Single-fiber segments (~1.5 mm length) were inserted between two clamps, one fixed and the other connected to a tension transducer (model 801, AK Sensonor, Horten, Norway). Fibers were immersed in a relaxing solution, at room temperature (22–24°C), with the following composition (mM): 170 potassium propionate, 2.5 magnesium propionate, 5 Na$_2$K$_2$ATP, 5 K$_2$EGTA, and 10 imidazole buffer, pH 7.0. Fibers were stretched manually to a sarcomere length of 3 μm as measured by light diffraction with a helium-neon laser lamp (33). The tension generated in each pCa solution was continuously recorded, and the baseline tension was established as the steady-state voltage output recorded with the fiber in the relaxing solution. The minimum tension accepted to identify pCa threshold level was 5% of the maximum tension (pCa 5).

Specific tension for each single fiber was calculated by normalizing the maximum tension measured at pCa 5 to the fiber cross-sectional area, as calculated by three different diameter determinations along the fiber length, considering the fiber immersed in solution as a cylinder.

**Sarcoplasmic reticulum caffeine response.** The caffeine threshold for sarcoplasmic reticulum Ca$^{2+}$ release was determined as previously described (10, 33). The minimum tension accepted to identify caffeine threshold was 5% of the maximum tension measured with 20 mM caffeine. Fibers were first incubated for 30 s in a Ca$^{2+}$ loading solution (pCa 7.0 solution). Then, the fibers in a relaxing solution deprived of EGTA were challenged in a stepwise fashion with increasing concentrations of caffeine until tension was recorded, thus identifying the caffeine concentration threshold (10, 33, 41).

**Sarcoplasmic reticulum Ca$^{2+}$ accumulation properties.** Ca$^{2+}$ uptake by the fiber sarcoplasmic reticulum was measured at room temperature (22–24°C) either by a light-scattering method (39, 41), as previously reported (33), or by a caffeine contracture method (51).

With the first method, fibers were mounted in a chamber containing the relaxing solution and stretched to 180% of slack length to avoid interference in light-scattering measurements caused by actin-myosin interactions. Fibers were then incubated in a Ca$^{2+}$ loading solution (pCa 6.4). Ca$^{2+}$-loading activity by the sarcoplasmic reticulum was measured after the increase of the fiber light scattering after the addition of 5 mM oxalate (45). The plateau level of light scattering represents the maximum capacity for Ca$^{2+}$ uptake of the sarcoplasmic reticulum (40, 45). The calibration procedure for converting the light-scattering signal to fiber Ca$^{2+}$ concentration was performed by using $^{54}$Ca$^{2+}$ as described in detail (40). The relative increase in light scattering was proportional to the Ca$^{2+}$ concentration inside the fiber. The proportionality constants for control, 2-day-denervated, and 7-day-denervated fibers were 0.199 ± 0.035 (n = 6), 0.159 ± 0.032 (n = 5), and 0.210 ± 0.031 (n = 6) nM $^{54}$Ca$^{2+}$/light-scattering unit/g fiber protein$^{-1}$, respectively. Accordingly, these values were used in the analysis of data obtained under the relative experimental condition.

The caffeine contracture method is an indirect technique that allows estimating the amount of Ca$^{2+}$ stored in the sarcoplasmic reticulum after various periods of Ca$^{2+}$ loading (51). The fibers were first exposed to the relaxing solution containing 20 mM caffeine to deplete the sarcoplasmic reticulum of Ca$^{2+}$. After being rinsed in the relaxing solution, the fiber was incubated in pCa 7.0 loading solution for 15, 30, 60, 120, and 180 s. The amount of Ca$^{2+}$ sequestered by the sarcoplasmic reticulum was then determined by exposing the fiber to 20 mM caffeine in a relaxing solution deprived of EGTA. For each fiber, the contracture force was plotted against loading duration and data were fit, via nonlinear regression ($r^2 = 0.93–0.99$), to an equation of the form $F = 100(1 - e^{-K_{Ca}t})$, where F is the normalized contracture force, $K_{Ca}$, is the rate constant for Ca$^{2+}$ uptake, and t is the loading duration.

**SDS-PAGE of Single Fibers and Whole Muscle**

All of the fibers investigated by the above-described procedures were subsequently analyzed by 7% PAGE and identified by MHC isofoms expressed (11, 13). Briefly, after each analysis, single-fiber segments were placed into a microcapillary and dissolved with 20 μl of SDS-PAGE solubilization buffer (12). Five microliters of each sample (20 samples for each run) were electrophoresed, and proteins were revealed.
No more than one action potential could be generally recorded from a stimulated single fiber without signs of membrane damage as revealed by a decrease of resting membrane potential. The maximum rate of rise and the maximum rate of fall of the action potential were determined by computing the first derivative of the digitized spike. The conduction velocity of the action potential along the fiber was calculated on the computer-restored cathodic ray oscilloscope tracings by relating the measured distance between the recording and stimulating electrodes, by using a micrometer lens mounted on a stereomicroscope, with the time interval between the stimulus artifact and the action potential onset. The latter was established to begin at the point of intersection of the extrapolated steepest part of the rising phase of action potential with the baseline (7).

Statistical Analysis

Means ± SE were calculated from individual values by standard procedures. Results were analyzed by one-way analysis of variance performing multiple comparisons against the control group (SigmaStat, Jandel Scientific). The 0.05 level of probability was established for statistical significance. pCa-tension data were fitted by a least squares method using the Table Curve fitting program (Jandel Scientific), according to the following equation: $y = \text{max} \times n_{H}/(\text{max} + k_{H})$, where max is the maximal value of pCa-tension curve, which was normalized to 1; $k_{H}$ is the pCa at 50% of maximum tension (i.e., $P_{0.5}$); $x$ and $y$ refer to coordinates shown in Fig. 2; and $n_{H}$ is the Hill coefficient.

RESULTS

Myofibrillar Protein Ca\(^{2+}\) Sensitivity of Single Denervated Fibers

The second day after denervation, type 2B fibers showed a left shift of the pCa-tension curves, which then reversed in direction by displaying a moderate right shift after 7 days (Fig. 2). The pCa threshold for tension development of 2-day-denervated type 2B fibers was almost identical to that of control fibers.
have affected the myofilament (or phosphorylatable) myosin light chains may respectively, in control, 2-day denervated, and 7-day denervated, and 7-day-denervated fibers, respectively. The Hill coefficient, an estimate of cooperative interactions between myofilaments (11), showed a significant reduction (P < 0.05) after 2 days [2.28 ± 0.17 (n = 18)] compared with control [2.93 ± 0.14, (n = 35)] and an increase after 7 days [3.32 ± 0.20, (n = 21); P < 0.05 vs. the 2-day-denervated fibers].

The specific tension of single skinned fibers, calculated by normalizing the maximum tension to the fiber cross-sectional area, was not modified at 2 days [13.8 ± 1.4 N/cm² (n = 19)] and was slightly reduced at 7 days [10.9 ± 0.7 N/cm², (n = 16)], compared with control [12.6 ± 1.4 N/cm² (n = 17)].

The rate of tension development of denervated type 2B fibers was transitory and significantly reduced 2 days after denervation with respect to the control, showing significant differences between 2-day- and 7-day-denervated fibers (P < 0.01) [0.28 ± 0.04 (n = 17), 0.17 ± 0.02 (n = 11), and 0.29 ± 0.03 mN/s (n = 9) in control, 2-day denervated, and 7-day denervated, respectively].

### Two-Dimensional Analysis of Myosin Light Chains

To evaluate whether phosphorylation of the regulatory (or phosphorylatable) myosin light chains may have affected the myofilamentary Ca²⁺ sensitivity of denervated EDL fibers (46), we performed two-dimensional analyses of myosin light chains. Figure 3 shows that phosphorylation of the regulatory light chain (2F-P) was not changed after 2 days, whereas it was greatly reduced after 7 days of denervation. Densitometric analyses confirm indeed that the percentage of phosphorylated 2F myosin light chain was 42.7 ± 3.2 (n = 9), 42.8 ± 4.6 (n = 6), and 27.6 ± 5.4 (n = 8) in control, 2 days after denervation, and 7 days after denervation, respectively, with the difference between control and 7-day-denervated muscle being significant (P < 0.01). In addition, surprisingly, the light chain 3F-to-light chain 2F protein band ratio was significantly reduced (P < 0.01) 2 days after denervation and returned to control values at 7 days [control, 0.25 ± 0.01 (n = 9), 2-day denervated, 0.17 ± 0.02 (n = 6); 7-day denervated 0.22 ± 0.02 (n = 8)].

Last, the expression level of parvalbumin progressively increased during the first week of EDL muscle denervation. In fact, the parvalbumin-to-myosin light chain ratio was 0.65 ± 0.06 (n = 9), 0.78 ± 0.08 (n = 6), and 0.83 ± 0.05 (n = 8) in control, 2-day-denervated, and 7-day-denervated muscles, respectively, with the increase at 7 days being significant (P < 0.05) compared with control.

#### Sarcoplasmic Reticulum Ca²⁺ Uptake and Release

Light-scattering analysis of sarcoplasmic reticulum properties of type 2B fibers shows that denervation caused a significant decrease in the sarcoplasmic reticulum Ca²⁺ accumulation capacity at 2 days after denervation. Importantly, this trend reversed at 7 days after denervation to produce a significant increase in the Ca²⁺ accumulation capacity (Table 1). Significant changes, i.e., first a decrease and then an increase, were also observed in the rate of Ca²⁺ uptake. Accordingly, K⁰Ca, calculated by the caffeine method (51), was 1.66 ± 0.11 (n = 12), 1.39 ± 0.13 (n = 9) and 2.18 ± 0.18 (n = 12) in control, 2-day-denervated and 7-day-denervated fibers, respectively, with a significant difference (P < 0.05) between the denervated fibers (Fig. 4).

Similarly, sarcoplasmic reticulum Ca²⁺ release properties of type 2B fibers were also modified during the first week of denervation. After 2 days of denervation, uptake. According to the caffeine method (51), was 1.66 ± 0.11 (n = 12), 1.39 ± 0.13 (n = 9) and 2.18 ± 0.18 (n = 12) in control, 2-day-denervated and 7-day-denervated fibers, respectively, with a significant difference (P < 0.05) between the denervated fibers (Fig. 4).

Similarly, sarcoplasmic reticulum Ca²⁺ release properties of type 2B fibers were also modified during the first week of denervation. After 2 days of denervation,

![Figure 3: Two-dimensional analysis of myosin light chain isoforms composition in control, 2-day-denervated, and 7-day-denervated fibers. Only the myosin light chain region is shown. Data shown are representative of experiments. 1F, 2F, and 3F, myosin light chain fast isoforms; 2F-P, phosphorylated myosin light chain 2; PA, parvalbumin.](image-url)

#### Table 1. Ca²⁺ transport activities of the sarcoplasmic reticulum of type 2B fibers from denervated rat EDL muscle

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2-day Denervated</th>
<th>7-day Denervated</th>
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<tbody>
<tr>
<td>Ca²⁺ uptake capacity, μmol/mg fiber protein</td>
<td>0.54 ± 0.06(15, 3)</td>
<td>0.28 ± 0.03*(9, 3)</td>
<td>0.70 ± 0.06‡(12, 3)</td>
</tr>
<tr>
<td>Rate of Ca²⁺ uptake, nmol min⁻¹·mg fiber protein⁻¹</td>
<td>43.6 ± 4.8(15, 3)</td>
<td>24.3 ± 2.4*(9, 3)</td>
<td>62.6 ± 8.4‡(12, 3)</td>
</tr>
</tbody>
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Values are means ± SE; nos. in parentheses are no. of fibers and of animals, respectively. EDL, extensor digitorum longus. *P < 0.05; ‡P < 0.01 vs. controls. ‡P < 0.01 vs. 2-day-denervated fibers.
type 2B fibers showed marked increase of the sensitivity to caffeine of sarcoplasmic reticulum Ca\(^{2+}\) release (Fig. 5). Specifically, the mean caffeine concentration able to induce Ca\(^{2+}\) release was significantly reduced in 2-day-denervated fibers from 8.95 ± 0.50 to 2.36 ± 0.32 mM (Fig. 5). The higher sensitivity to caffeine exhibited by denervated fibers was confirmed by the observation that the ratio between the tension development and the caffeine concentration was significantly increased in 2-day-denervated fibers vs. control.

As expected by the very short denervation time, densitometric analysis of MHC isoform composition from SDS-PAGE gels demonstrated that MHC expression level was not significantly modified in the first week of denervation. The MHC of the EDL control contained 5.6 ± 0.9% type 1, 30.8 ± 3.2% type 2A, 25.8 ± 2.2% type 2X, 61.0 ± 2.9% type 2B, and 8.1 ± 0.9% type 1 (n = 6); the 2-day-denervated MHC contained 6.4 ± 0.7% type 1; and 7-day-denervated MHC contained 5.6 ± 0.9% type 2A, 51.7 ± 6.6% type 2B and 11.9 ± 2.9% type 1 (n = 3). Also in agreement with previous reports (2, 27), we observed that denervation induced substantial alterations of electrophysiological properties of EDL (Table 3). The resting membrane potentials were significantly reduced after 2 days, which persisted by 7 days. Major changes of action potentials were observed 7 days after

![Graph](image)

**Fig. 4.** Caffeine contracture force recorded after Ca\(^{2+}\) loading for various time periods. Values are means ± SE of data obtained from control (12 fibers, 3 muscles), 2-day-denervated (9 fibers, 3 muscles), and 7-day-denervated (12 fibers, 3 muscles) type 2B fibers. *P < 0.02, 7-day-denervated fibers vs. control.

**Fig. 5.** Percentage distribution of caffeine threshold for sarcoplasmic reticulum Ca\(^{2+}\) release of control, 2-day-denervated, and 7-day-denervated extensor digitorum longus single type 2B muscle fibers. Total number of type 2B fibers examined was 43, 22, and 27, respectively, from 4 animals in each group. *P < 0.05 vs. control.

**Table 2.** Contractile properties of denervated rat EDL muscle

<table>
<thead>
<tr>
<th></th>
<th>Control (10)</th>
<th>2-day Denervated (4)</th>
<th>7-day Denervated (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraction time, ms</td>
<td>40.0 ± 1.1</td>
<td>41.3 ± 2.7</td>
<td>60.3 ± 3.7†‡</td>
</tr>
<tr>
<td>Half-relaxation time, ms</td>
<td>30.3 ± 1.9</td>
<td>26.6 ± 5.6</td>
<td>56.2 ± 5.7†‡</td>
</tr>
<tr>
<td>Twitch specific tension, mN/mm²</td>
<td>73.1 ± 5.5</td>
<td>60.9 ± 4.2</td>
<td>82.6 ± 6.1‡</td>
</tr>
<tr>
<td>Tetanic specific tension, mN/mm²</td>
<td>220.9 ± 14.7</td>
<td>176.2 ± 15.4</td>
<td>128.2 ± 6.1†‡</td>
</tr>
<tr>
<td>Maximum rate of rise of tetanus, mN/ms</td>
<td>8.90 ± 0.47</td>
<td>8.06 ± 0.22</td>
<td>4.75 ± 0.53†</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses are no. of experiments. *P < 0.05 vs. control. †P < 0.001 vs. control. ‡P < 0.05 vs. 2-day-denervated muscles.
denervation. The maximum rates of rise and fall of action potential were both reduced at 7 days by 69% and 68%, respectively, whereas the reductions at 2 days were smaller (18% and 27%). The amplitudes of action potential were both reduced at 7 days by 69 and 53%.

Values are means ± SE; nos. in parentheses are no. of fibers and of animals, respectively. RMP, resting membrane potential; MRR, maximum rate of rise; MRF, maximum rate of fall; AP, amplitude of action potential. *P < 0.05 vs. control. †P < 0.05 vs. 2-day-denervated muscles.

Table 3. Electrophysiological properties of denervated rat EDL muscle

<table>
<thead>
<tr>
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<th>Control (22, 3)</th>
<th>2-day Denervated (17, 3)</th>
<th>7-day Denervated (15, 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP, mV</td>
<td>−72.6 ± 1.2</td>
<td>−65.4 ± 1.3</td>
<td>−61.5 ± 1.0</td>
</tr>
<tr>
<td>MRR, V/s</td>
<td>146.9 ± 10.3</td>
<td>120.8 ± 13.2</td>
<td>46.0 ± 5.0</td>
</tr>
<tr>
<td>MRF, V/s</td>
<td>63.6 ± 2.9</td>
<td>46.4 ± 4.4</td>
<td>20.5 ± 2.6</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>84.7 ± 1.9</td>
<td>64.7 ± 2.4</td>
<td>53.3 ± 2.7</td>
</tr>
<tr>
<td>Conduction velocity, m/s</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.2*</td>
<td>1.0 ± 0.1*</td>
</tr>
</tbody>
</table>

DISCUSSION

The nerve-dependence of type 2B muscle fibers is a debated question, at least as far as the expression of MHC isoform is concerned. In fact, because type 2B fibers develop in the absence of both mechanical load and neural stimulation (8, 37, 42, 47), according to some authors, type 2B fibers should be considered a default phenotype. However, some recent reports show a definite role of innervation for the expression of type 2B MHC (15, 29) and for the postnatal differentiation of type 2B fibers (1).

The present work shows that, during early phases of muscle denervation, when changes in MHC expression are not evident yet, there are substantial modifications of myofibrillar and sarcoplasmic reticulum properties in type 2B fibers from fast-twitch muscle. These effects appear to be even more marked than those observed in denervated type 1 fibers from soleus muscle (33), and they contradict the hypothesis that type 2B fibers are insensitive to the action of the nerve. Because these changes of type 2B fiber properties occurred before any modifications in the MHC isoform expression, our results corroborate the opinion that early changes of muscle contractile properties are primarily due to alterations of the muscle activation process (24, 27, 31, 33, 43, 44, 53). Consistently, changes of the excitation-contraction coupling process were noted in the denervated rat EDL muscle (16).

Denervated type 2B fibers showed a higher myofibrillar Ca$^{2+}$ sensitivity at 2 days and a lower sensitivity at 7 days. Ca$^{2+}$ sensitivity of myofibrillar proteins of the different fiber types is distinctive according to the expression of specific regulatory proteins, in particular of troponin and tropomyosin isoforms (10, 35, 42). For example, threshold for tension development is mainly attributed to the specific fast or slow isoform of troponin C (35). Even though 1-wk denervation of hind-limb muscles is not enough to change the expression level of myofibrillar proteins of fast muscles (23, 26), Ca$^{2+}$ sensitivity could be influenced by several other factors and mechanisms that may operate in denervated muscle, like posttransductional modifications of involved proteins that include oxidation of sulfhydryl groups (3, 52), glycation (22), deamination (4), and phosphorylation of myofibrillar proteins. It is indeed known that phosphorylation of myosin light chain 2, by a specific Ca$^{2+}$/calmodulin-dependent protein kinase, causes a leftward shift of the pCa-tension relationship in fast-twitch fibers (28, 46). Our data show that the level of myosin light chain 2F phosphorylation significantly decreased 7 days after denervation, providing a possible explanation for the rightward shift of pCa-tension curves. The lower phosphorylation level of myosin light chain 2F we noted is consistent with the reported broad decrease of phosphorylation of fast-phenotype markers during denervation (50). It is interesting to note that 2 days of denervation seem enough to alter myosin light chain 3F-to-myosin light chain 2F stoichiometric ratio. Because a relationship between the maximal shortening velocity and the amount of myosin light chain 3F (6, 18) has been demonstrated, the lower expression level of myosin light chain 3F could explain, at least in part, the reduced rate of tension developed by the type 2B fibers 2 days after denervation.

After 2 days of denervation, we observed a significant decrease of sarcoplasmic reticulum Ca$^{2+}$ uptake capacity and rate of type 2B fibers, followed by a marked increase at 7 days. Apparently, the alterations on Ca$^{2+}$ uptake rate cannot be attributed to modifications in Ca$^{2+}$-ATPase content, because no changes in the expression of both sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase Ca$^{2+}$-pump isoforms (SERCA-1 and SERCA-2) occur during the first week of denervation (43). On the other hand, the rise of Ca$^{2+}$ uptake capacity in the 7-day-denervated type 2B fibers is consistent with the increase of calsequestrin-to-Ca$^{2+}$-pump protein ratio (38) and the proliferation of junctional sarcoplasmic reticulum membranes reported in 15-day-denervated rabbit fast muscle (53).

Changes of sarcoplasmic reticulum Ca$^{2+}$ release properties in 2-day-denervated fibers is likely associated to the substantial elevation of sensitivity to opening of the Ca$^{2+}$ release channel, as indicated by the higher caffeine sensitivity of Ca$^{2+}$ release. Because, in leakage tests performed according to Trachez et al. (49), the spontaneous Ca$^{2+}$ release was smaller in fibers from 2-day-denervated muscles than in controls, it is unlikely that the changes are related to membrane leakiness (unpublished observations).

Therefore, our results indicate that the SR Ca$^{2+}$ uptake and release, key properties in the excitation-coupling process, are under nervous control in type 2B fibers. Fiber Ca$^{2+}$ sensitivity also appears to be affected by innervation. The observed changes in type 2B fibers after denervation were more marked than in type 1 fibers from soleus muscle (33).
account the very low level of neuromotor activity discharged on type 2B with respect to type 1 fibers (21), it is possible that 2B fibers are particularly sensitive to the lack of nerve trophic factors, as hypothesized by others (14, 19, 20).

Moreover, because in type 2B fibers, as early as 2 days after denervation, alterations take place that partly disappear (sensitivity to caffeine of sarcoplasmic reticulum Ca\(^{2+}\) release, myosin light chain 3F expression level) and partly reverse (Ca\(^{2+}\) sensitivity of myofibrillar proteins and sarcoplasmic reticulum Ca\(^{2+}\) accumulation properties) 7 days after denervation, one can hypothesize that the initial dramatic effects of inactivity caused by nerve deprivation are attenuated or even reverted by muscle fibillation, a cell membrane electric activity that mimics a chronic low-level stimulation and that is known to occurs at 2–3 days after denervation (30).

Last, our results confirm that denervation causes substantial early changes of contractile and membrane properties of the whole rat fast-twitch EDL muscle (17). Differently from soleus muscle (33), and despite of the observed modifications in sarcoplasmic reticulum and myofibrillar properties, changes of contractile properties were not evident at 2 days of denervation. It is possible that the alterations of the membrane and of myofibrillar and sarcoplasmic reticulum properties, detected in single type 2B fibers abolish each other’s effects, resulting in the lack of significant alterations in the contractile properties of the whole muscle. On the other hand, in the 7-day-denervated EDL muscle, we observed a general slowing of contractile properties with a significant increase of contraction and relaxation times, as well as of the maximum rate of rise of tension. The observed increase of whole muscle parvalbumin level in the 7-day-denervated EDL muscle, expected to contribute to shorten the relaxation time (25), probably only attenuates the slowing of contractile properties. Moreover, the resting membrane potential, the amplitude of the action potential and the conduction velocity were progressively reduced during the first week of denervation. Because MHC isoform composition does not change in the first week of denervation (our results; Ref. 23), the reduction of maximal rate of rise of tension, a property normally ascribed to actin-myosin cycling rate (35, 42), could be attributed to posttranslational modifications, such as, for example, the reduction of myosin light chain 2F phosphorylation. It is also possible that the reduction of impulse conduction velocity contributed to the reduction of the rate of rise of tension, as has been proposed for the denervated fast muscle of the cat (27).

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