Adaptations of motoneuron properties to chronic compensatory muscle overload

P. Krutki, A. Hałuszka, W. Mrówczyński, P. F. Gardiner, and J. Celichowski

1Department of Neurobiology, University School of Physical Education, Poznań, Poland; and 2Spinal Cord Research Center and Faculty of Kinesiology & Recreation Management, University of Manitoba, Winnipeg, Canada

Submitted 2 December 2014; accepted in final form 16 February 2015

Krutki P, Hałuszka A, Mrówczyński W, Gardiner PF, Celichowski J. Adaptations of motoneuron properties to chronic compensatory muscle overload. J Neurophysiol 113: 2769–2777, 2015. First published February 18, 2015; doi:10.1152/jn.00968.2014.—The aim of the study was to determine whether chronic muscle overload has measurable effect on electrophysiological properties of motoneurons (MNs), and whether duration of this overload influences intensity of adaptations. The compensatory overload was induced in the rat medial gastrocnemius (MG) by bilateral tenotomy of its synergists (lateral gastrocnemius, soleus, and plantaris); as a result, only the MG was able to evoke the foot plantar flexion. To assure regular activation of the MG muscle, rats were placed in wheel-equipped cages and subjected to a low-level treadmill exercise. The intracellular recordings from MG motoneurons were made after 5 or 12 wk of the overload, and in a control group of intact rats. Some of the passive and threshold membrane properties as well as rhythmic firing properties were considerably modified in fast-type MNs, while remaining unaltered in slow-type MNs. The significant changes included a shortening of the spike duration and the spike rise time, an increase of the afterhyperpolarization amplitude, an increase of the input resistance, a decrease of the rheobase, and a decrease of the minimum current necessary to evoke steady-state firing. The data suggest higher excitability of fast-type MNs innervating the overloaded muscle, and a shift towards electrophysiological properties of slow-type MNs. All of the adaptations could be observed after 5 wk of the compensatory overload with no further changes occurring after 12 wk. This indicates that the response to an increased level of chronic activation of MNs is relatively quick and stable.

MUSCLE OVERLOAD CAN BE ACHIEVED by workload training (Fry 2004; McDonagh and Davies 1984), ablation or tenotomy of synergists (Gardiner et al. 1991; Noble and Pettigrew 1989; Olha et al. 1988), peripheral nerve damage, or denervation of synergistic muscles (Degens et al. 1995). Overload induces measurable adaptive effects in the whole muscle, its motor units (MUs), and individual muscle fibers. The most prominent response is muscle hypertrophy, reflected in the increase of muscle mass, cross-section area, and the increased strength (Allbrook 1981; Seynnes et al. 2006). Some biochemical adaptations related to this hypertrophy have been reported in muscle fibers (Baldwin et al. 1982; Dearth et al. 2013; Ianuzzo and Chen 1979). Several studies have reported changes in contractile properties of MUs in response to chronic muscle overload. They point on increased tetanic forces of all MU types, longer contraction times of fast resistant and slow MUs, decreased fatigue resistance of fast fatigable units, and altered proportions of MUs within an overloaded muscle (Noble and Pettigrew 1989; Olha et al. 1988).

It is well documented that motoneurons are highly susceptible to altered levels of physical activity (Gardiner et al. 2005). Hyperpolarized membrane potentials and voltage thresholds, lower spike rise times, and higher cell capacitances of fast-type motoneurons have been established in rat in response to exercise training (Beaumont and Gardiner 2003). On the other hand, elevated rheobase currents, lower spike and afterhyperpolarization (AHP) amplitudes, lower cell capacitances, and rightward shift of frequency-current (f–I) relationships have been found in response to decreasing the activity of MUs, by muscle unweighting (Cormery et al. 2005). However, so far there have been no studies investigating influence of compensatory muscle overload on properties of motoneurons.

Therefore, the aim of our study was to determine whether a chronic neuromuscular overload has measurable effects on the electrophysiological properties of motoneurons innervating the overloaded muscle. The study was conducted using Wistar rats, and the medial gastrocnemius (MG) muscle was subjected to a compensatory overload induced by tenotomy of its synergists (lateral gastrocnemius, soleus, and plantaris); as a result, only the MG was able to evoke the foot plantar flexion. This muscle is a heterogeneous structure, composed of three MU types, making investigation of adaptive changes in membrane and firing properties of either fast or slow types of the MG motoneurons possible. This way we could verify whether observations concerning overload-induced changes in contractile properties of fast and slow MUs are reflected in adaptations of the properties of their innervating motoneurons. In this study the electrophysiological properties of motoneurons were recorded, using intracellular recording techniques, 5 or 12 wk after the operation, and the results were compared with a control group. Thus, we also intended to determine the rate and stability of likely adaptations of motoneuron properties due to the overload.

MATERIALS AND METHODS

The study was conducted using 24 adult male Wistar rats (body mass of 423 ± 33 g, at the age of 7 mo on a day of the final acute experiment) that were randomly assigned to the following three groups: two groups of rats subjected to compensatory overload of the MG muscle 5 wk (5wkOL, n = 8) or 12 wk (12wkOL, n = 8) before the final experiment, and the control group (n = 8) of age-matched, not operated, and untrained animals.

The animals were housed in standard cages (2 per each) in a room with humidity and temperature maintained constant, with a 12:12-h
dark-light daily cycle and unrestricted access to food and water. All experimental procedures were approved by the Local Ethics Committee. The maintenance and care of animals followed the European Union guidelines and the Polish Law on the Protection of Animals.

Muscle overload. The compensatory overload of the MG muscle was induced by tenotomy of the lateral gastrocnemius, soleus, and plantaris muscles (Gardiner et al. 1991; Kryciak et al. in press). The operation was performed under general anesthesia (60 mg/kg ip pentobarbital sodium). The MG muscle was exposed, and its two heads (medial and lateral) were separated. Next, the lateral head was cut from the Achilles tendon and tied proximally into the fascia surrounding the muscles. Similarly, plantaris and soleus muscles were separated, and their distal tendons were tied proximally into the fascia, to prevent their participation in force production during movements; then the overlying muscles were sutured, and skin was closed with steel clips. As a result of the operation, the foot plantar flexion during daily locomotor activity could be evoked exclusively by the MG muscle.

After the surgery, for 1 wk of convalescence animals were housed individually while antibiotics and painkillers were administered. After this period rats were placed in wheel-equipped cages and additionally performed treadmill exercises (for 1 h daily 5 days/wk at the speed of 27 cm/s). According to observations of Silva et al. (1997) and Cavalcanti et al. (2009) such intensity of physical exercise of rats is considered low. The aim of wheel and treadmill locomotor exercises of the operated rats was on one hand to prevent the excessive stretch of the MG muscle by antagonists (Antonio and Goneya 1993) and on the other hand to induce regular activation of the MG muscle to ensure its actual compensatory overload.

Electrophysiological experiments. The final acute experiments were carried out on deeply anesthetized animals (initial dose of 60 mg/kg ip pentobarbital sodium and additional doses of ~10 mg·kg⁻¹·h⁻¹ throughout the whole experiment). The depth of anesthesia was controlled by lack of pinna and withdrawal reflexes during preparation and the continuous electrocardiogram monitoring of the heart rate (300–360 beats/min) during the recording session. The experiments were terminated by a lethal dose of pentobarbital sodium (180 mg/kg).

The femoral vein was catheterized for drug administration, and then tracheotomy was made to insert a tracheal tube. Rats were artificially ventilated (volume 4–5 ml, stroke rate 100–120/min; Jelfa) was administered at regular intervals (the first dose of 0.4 mg/kg and additional doses of ~1 mg/kg). In the experiments the doublet threshold (DT) was also determined as a criterion of membrane potential stabilization, antidromic action potentials (APs) from single MG motoneurons were recorded in a bridge mode. They were recognized from a constant and short latency of a spike and its all or nothing appearance. Only stable recordings with the resting membrane potentials of at least −50 mV and the amplitudes of APs over 50 mV were taken into consideration (Baćzyk et al. 2013; Gardiner and Kernell 1990). Ten antidromic spikes were averaged, and the following basic membrane properties were calculated: the resting membrane potential, the AP amplitude, the AP duration, the AP rise time, the AHP amplitude, the AHP time-to-peak, and the AHP half-decay time (AHP-HDT). In the electrophysiological study on the rat gastrocnemius MUs, Gardiner (1993) demonstrated that the latter parameter is sufficient to separate slow- and fast-type motoneurons. When the AHP-HDT was ≤20 ms a motoneuron was recognized as a fast one, whereas at >20 ms it was considered slow (Beaumont and Gardiner 2002, 2003). Cell input resistance was measured from an average of 40 short pulses (100 ms) of I-na hyperpolarizing current, recorded in a DCC mode (4–8 kHz). Rheobase current was determined as the amplitude of a 50-ms current pulse required to elicit a single spike.

Subsequently, depolarizing current injections of 500-ms square-wave pulses (at amplitudes increasing or decreasing in steps of 0.1–2 nA) were used in a DCC mode to determine the maximum and minimum steady-state firing (SSF) frequencies. Stimulations were separated by 5-s intervals. The SSF frequencies were calculated from the last three interspike intervals during the 500-ms square pulse. Current injected at those frequencies was used to determine the f-I relationship. The f-I slope was calculated for each motoneuron. From these recordings the doublet threshold (DT) was also determined as a minimum current to evoke the initial doublet of APs (with the first interspike interval <10 ms), and the DT-to-rheobase ratio was calculated (Mrówczyński et al. 2010).

At the end of each experiment, the overloaded MG muscle was carefully dissected, removed, and immediately weighed.

Statistical analysis. Comparisons between the control, 5wkOL, and 12wkOL groups with respect to the body and muscle mass as well as motoneuron membrane and rhythmic firing properties were made with the ANOVA Kruskal-Wallis test, and subsequent post hoc comparisons were made. The differences were considered significant at P < 0.05 or highly significant at P < 0.01. For the relationship between rheobase and input conductance the Pearson correlation coefficient was calculated, and the linear regression was derived.

RESULTS

Body and muscle mass. No significant differences in the mean body mass were observed before the electrophysiological experiments (after the operation, convalescence, and training periods) between the control, 5wkOL, and 12wkOL groups. The respective mean values amounted to 407 ± 28, 436 ± 44, and 435 ± 24 g. The evidence of the actual compensatory overload was provided by hypertrophy of the MG muscle in either 5wkOL or 12wkOL groups compared with control. The mean MG muscle mass in the control group amounted to 1,041 ± 77 mg, and was considerably higher in the 5wkOL group (1,283 ± 128 mg, P < 0.05) and in the 12wkOL group (1,355 ± 221 mg, P < 0.05). This hypertrophy was also reflected in the significantly higher muscle-to-body mass ratio in both overloaded groups compared with control. The mean values of this ratio were 0.26 ± 0.02% in the control group, 0.29 ± 0.01% (P < 0.05) in the 5wkOL group, and 0.31 ± 0.04% (P < 0.01) in the 12wkOL group. The observed results are in line with several previous studies on overload effects on
Passive and threshold properties of motoneurons were altered as a result of the chronic overload in fast-type motoneurons. Either after 5 or 12 wk of overload, adaptive changes were reflected in a significant shortening of the AP rise time (by 9 and 8%, respectively) and an increase of the AHP amplitude (by 45 and 33%, respectively). The input resistance was significantly increased in fast

Table 1. Passive and threshold properties of motoneurons

<table>
<thead>
<tr>
<th></th>
<th>Slow</th>
<th>Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5wkOL</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>56.12 ± 6.74</td>
<td>56.25 ± 4.35</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>57.76 ± 5.91</td>
<td>58.21 ± 4.32</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>1.98 ± 0.34</td>
<td>1.99 ± 0.25</td>
</tr>
<tr>
<td>AP rise time, ms</td>
<td>1.02 ± 0.16</td>
<td>1.06 ± 0.14</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>5.56 ± 2.62</td>
<td>4.86 ± 1.40</td>
</tr>
<tr>
<td>AHP time-to-peak, ms</td>
<td>10.80 ± 4.50</td>
<td>9.18 ± 0.92</td>
</tr>
<tr>
<td>AHP-HDT, ms</td>
<td>25.85 ± 5.08</td>
<td>26.81 ± 4.48</td>
</tr>
<tr>
<td>IR, MΩ</td>
<td>3.48 ± 1.46</td>
<td>3.06 ± 1.26</td>
</tr>
<tr>
<td>Rheobase, nA</td>
<td>2.68 ± 1.32</td>
<td>3.48 ± 1.58</td>
</tr>
<tr>
<td>DT, nA</td>
<td>8.82 ± 3.40</td>
<td>10.67 ± 3.22</td>
</tr>
<tr>
<td>DT/rheobase</td>
<td>3.40 ± 1.31</td>
<td>3.34 ± 1.02</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD; n, no. of rats. 5wkOL, 5 wk overload; 12wkOL, 12 wk overload; RMP, resting membrane potential; AP, action potential; AHP, afterhyperpolarization; HDT, half-decay time; IR, input resistance; DT, doublet threshold. Comparisons between control, 5wkOL, and 12wkOL groups were made with the ANOVA Kruskal-Wallis test: *P < 0.05 and **P < 0.01.
motoneurons on average by 29% in the 5wkOL group and by 25% in the 12wkOL group, whereas rheobase was significantly decreased by 28 and 26%, respectively. Distributions of the above parameters in the whole sample of slow and fast motoneurons are reflected by plots in Fig. 1.

The significant correlation was observed between rheobase and input conductance in all groups of motoneurons. For slow motoneurons the correlation coefficients were 0.73, 0.78, and 0.76 ($P < 0.001$), whereas for fast motoneurons they were 0.76, 0.79, and 0.63 ($P < 0.001$) in the control, 5wkOL, and 12wkOL groups, respectively. For either slow or fast motoneurons, the regression lines of 5wkOL and 12wkOL groups fell into the 95% confidence band of the control motoneurons (Fig. 2). Therefore, one may conclude that the overload did not significantly affect the relationship between rheobase and input conductance seen for control motoneurons.

In line with changes in rheobase, a tendency for the lower DT was observed in fast motoneurons in both overloaded groups compared with control ($P = 0.054$, a decrease of the mean values by 10 and 11% for 5wkOL and 12wkOL groups, respectively; Table 1). The relative threshold for doublet generation (measured as a ratio of current necessary to evoke doublet to the rheobase) exceeded two times rheobase, and there were no statistical differences between groups. It should be mentioned that doublets were observed frequently with increased intensity of current injection, but not in all the cases, which confirms earlier observations (Bawa and Calancie 1983; Kirkwood and Munson 1996; Mrówczyński et al. 2010). Doublets could be determined in 11 out of 14 slow and 29 out of 35 fast motoneurons in the control group, in 9 out of 12 slow and 27 out of 31 fast motoneurons in the 5wkOL group, and 11 out of 13 slow and 24 out of 29 fast motoneurons in the 12wkOL group.

**Rhythmic firing properties.** Examples of the SSF at the minimum and maximum SSF frequencies and respective $f$-$I$ plots for slow and fast motoneurons from all groups investigated are shown in Fig. 3. No differences between the SSF parameters were determined for slow motoneurons (Fig. 4). However, a significant decrease of the minimum SSF current was observed in fast motoneurons for the 5wkOL (by 29%) and 12wkOL (by 26%) groups compared with control (Fig. 4A). The maximum SSF current as well as the minimum and maximum frequencies during SSF were not different between motoneurons of the control and overloaded groups (Fig. 4, B–D). Moreover, no significant differences were observed in the slopes of the $f$-$I$ relationships determined for slow or fast motoneurons of the control, 5wkOL, and 12wkOL groups. The respective mean values of the $f$-$I$ slopes were $4.03 \pm 1.43$, $3.66 \pm 1.19$, and $3.73 \pm 0.83$ Hz/nA for slow and $3.12 \pm 1.17$, $2.86 \pm 1.08$, and $3.09 \pm 0.82$ Hz/nA for fast motoneurons.

The above alterations in rhythmic firing properties of fast motoneurons influenced $f$-$I$ relationships. Figure 5 shows the $f$-$I$ relationships for the whole sample of motoneurons under the study. The $f$-$I$ relationships for fast motoneurons of both overload groups were shifted leftward compared with the control group, with no changes in the slopes of these relationships. Again, no differences in the $f$-$I$ relationship were found between the 5wkOL, 12wkOL, and control groups of slow motoneurons, with only a minor tendency to a rightward shift of the $f$-$I$ relationships of the overloaded groups.

**DISCUSSION**

The study indicates that a compensatory muscle overload induces type-specific adaptations in electrophysiological properties of rat spinal motoneurons and that these adaptations are relatively quick and stable, since they can be observed after 5 weeks of overload.
wk with no further development after 12 wk. These results confirm previous observations that motoneurons are highly susceptible to altered motor behavior, and adaptive changes in motoneuron properties due to an increased or decreased level of physical activity can appear in just a few weeks from its onset (Beaumont et al. 2004; Cormery et al. 2000, 2005). On the other hand, one might expect more pronounced adaptations after longer periods of muscle overload, which did not happen. This may suggest that, since overload produces muscle hypertrophy, relative overload of the motoneurons decreases, and thus adaptations stabilize.

Diverse effects of muscle overload on fast and slow motoneurons. Changes in excitability and firing characteristics occurred only in motoneurons of the fast type, whereas membrane properties of slow motoneurons remained unaltered. There are several possible explanations of this diversity. First of all, after tenotomy of the lateral gastrocnemius, soleus, and plantaris muscles, the MG becomes the only muscle active during plantar flexion. The rat MG muscle contains only 10–12 slow MUs, which are innervated by the most excitable motoneurons, what amounts to 12–15% of the total number and produce about 5% of the total MG muscle force (Celichowski and Drzymała-Celichowska 2007; Krutki et al. 2006). Therefore, after the operation, even for relatively low-force movements, recruitment of fast MUs innervated by less excitable motoneurons would be necessary, whereas for high-force movements one might expect activation of a majority of MUs in the muscle. This obviously leads to more frequent recruitment of fast MUs than under normal conditions in intact rats. Moreover, an additional activity time of all MUs in the over-
loaded muscle would have a relatively low effect on highly resistant to fatigue slow MUs. Hennig and Lømo (1985) suggested that in normal rat hind limb muscles, slow MUs are active for \( \frac{1}{2} - 0.35 \% \) of the total daily time (i.e., 5.3–8.4 h). On the other hand, a total daily activity time of fast resistant to fatigue MUs is 1.6–5% (23–72 min) and for fast fatigable MUs just 0.04–0.22% (0.5–3 min). Thus, changes in the recruitment process due to overload would be expected to have considerably more impact on fast vs. slow MUs.

Adaptations in threshold and rhythmic properties of motoneurons. Several basic membrane properties and rhythmic firing properties of fast motoneurons were altered in response to the muscle overload, indicating most of all an increased excitability of these cells compared with motoneurons of intact rats. An increase in the cell excitability (expressed principally by a reduction in the rheobase and the minimum current necessary to evoke SSF) is generally linked to an increase in a cell’s input resistance and the AHP amplitude (Bakels and Kernell 1993, Gardiner 2006, Gustafsson and Pinter 1984). Indeed, we have indicated a 26–28% decrease of the rheobase, a 26–29% decrease of the minimum SSF current, which coincided with a 25–29% increase of the cell input resistance, and a 33–45% increase of the AHP amplitude of the AP. Contrary to the previous study of Gollvik et al. (1986) who reported a 20–30% increase in AHP duration in all motoneuron types during postnatal development of the overloaded MG muscle in the cat, no changes in the AHP duration were observed in our study.

These data suggest a shift from fast-type to slow-type motoneurons and a change toward smaller size. One cannot totally exclude a possibility that the observed changes in input resistance and rheobase might be due to alterations in membrane conductances and not a smaller size of a motoneuron. However, the latter suggestion is supported by the linear relationship between rheobase and input conductance (see Fig. 2) that has not been significantly affected by overload, either after 5 or 12 wk. Therefore, fast-type motoneurons in the overloaded groups are activated at lower current thresholds than control, and this is consistent with their smaller size, as estimated by input conductance (Kalmar et al. 2009). Previous studies evaluating motoneuron size in response to compensatory overload have provided contradictory results. Finkelstein et al. (1991) indicated decreased motoneuronal soma size following muscle hypertrophy in plantaris motoneurons, but in research of Roy et al. (1999) the mean motoneuron soma area was not affected after functional overload of the same muscle.

On the other hand, some data in our study indicate that adaptations evoked by overload cannot be explained simply by a shift from one type of motoneuron to another, and other biophysical changes in the membrane should be considered. A significant shortening of the mean rise time of a spike (by 0.08–0.09 ms) was found in fast motoneurons for both over-
The overload-induced changes should also be compared with control, and the overall spike duration was similarly shortened by 0.09–0.10 ms (change not significant) while these values in slow motoneurons are considerably longer (see Table 1). Such a change in the overloaded groups suggests increased sodium activation by modulation of ion channels responsible for initiation of APs (Hodgkin and Huxley 1952; Siegelbaum and Koester 2013) and unchanged voltage-dependent calcium-independent potassium conductance primarily involved in the repolarization phase of a spike (Viana et al. 1993). Unaltered processes of activation of calcium-dependent potassium channels cannot be unequivocally excluded, since they also play a role in the generation of the AHP period (Barrett and Barret 1976), and its amplitude has been modified in motoneurons following overload. Moreover, minimum and maximum frequencies of the SSF in 5wkOL and 12wkOL groups are not different from the control group and are still considerably higher than those determined for slow motoneurons. This suggests that overload does not cause a shift from one motoneuron type to another but instead causes diverse changes in the biophysical properties of motoneurons.

It is worth noting that many of the overload-induced changes in motoneuron properties are opposite to those previously observed in experiments based on the opposite paradigm, after muscle inactivation by unloading of the hind limb (Cormery et al. 2005). The authors have reported significantly elevated rheobase currents, lower AHP amplitudes, or rightward shifts of f-I relationships for both fast and slow motoneurons after elimination of weight bearing for 2 wk.

The overload-induced changes should also be compared with effects of endurance training on a treadmill. Beaumont and Gardiner (2003) have shown that motoneurons of rats running on a treadmill (2 h/day for 16 wk at a speed of 30 m/min at 10% grade) have significantly lower values of spike rise time compared with control, which is similar to that seen in overloaded rats. However, contrary to the current study, intense endurance training resulted in larger mean cell capacitance in fast motoneurons, suggesting their larger size and indexes of excitability (rheobase and cell input resistance) were not modified. Thus, we may assume that adaptations in motoneuron properties documented in our study were due to the compensatory overload and not due to the exercise alone, which in this study was much milder in intensity and duration compared with the Beaumont and Gardiner study. Overload might be seen as more prolonged and less dynamic than the running exercise involving 2 h/day of intense contractile muscle activity.

The motoneuron threshold and firing properties are determined not only by basic membrane properties, but also inhibitory and excitatory postsynaptic potentials contribute to motoneuron excitability (Nunez-Abades et al. 2000) and influence discharge rates (Powers and Binder 2000). Tenotomy of several muscles thus leaving only one muscle operating on the ankle joint may affect synaptic input to motoneurons from either peripheral sources (e.g., from muscle receptors of un-loaded synergists) or supraspinal pathways (due to changes in recruitment pattern). Moreover, if the knee flexion moment remains intact, the synaptic drive to lumbar motoneurons should be modified to stabilize at the knee. Therefore, changes in activity of the spinal neuronal network due to altered motor behavior might also play a role in the observed increase in motoneuronal excitability as a result of overload. These factors, however, still await detailed explanation. Moreover, one should consider the fact that pentobarbital sodium anesthesia used in this study substantially affects synaptic influences. Different synaptic actions might influence motoneuron excitability during their normal activity than under experimental conditions.

Functional considerations. Overload-induced adaptations in motoneuron properties have implications for the force output of fast MUs and for the recruitment process. According to the size principle, excitability of a motoneuron highly correlates with the force generated by an active MU (De Luca et al. 1982; Heckman and Binder 1991). However, in the present study fast MUs became more excitable (see Table 1), whereas the parallel study has indicated greater forces produced by fast MUs due to 3 mo overload (Krysciak et al. In press). The latter observation is in accordance with several previous reports on overload effects on properties of muscle fibers or MUs (Walsh et al. 1978; Olha et al. 1988; Noble and Pettigrew 1989). Krysciak et al. (in press) have also shown a decrease of the twitch-to-tetanus ratios, which indicates more efficient force development during tetanic contractions. Therefore, we may conclude that greater forces of fast MUs, together with their higher excitability, allow the overloaded muscle to achieve at the same synaptic input to the motor nucleus larger forces while engaging less MUs, which could also be recruited earlier. One should note, however, that overload-induced alterations in contractile parameters were reported also in slow MUs (Krysciak et al. In press; Olha et al. 1988), which is not the case in our study on motoneurons.

Lower values of rheobase current and a leftward shift of the f-I relationship observed for fast motoneurons, either after the 5 or 12 wk of the compensatory overload, indicate their ability to become recruited earlier (and possibly more frequently) and to achieve higher firing rates at the same stimulus intensities than respective motoneurons of the intact rats. Therefore, it is likely that a weaker synaptic input would be necessary for motoneuron activation, but, at the same level of membrane...
depolarization, the discharge frequencies in the overloaded group of fast motoneurons would be higher than in the control group. This is especially important with respect to earlier reference to the necessity of recruitment of more MUs (especially fast) to make possible effective plantar flexion with only one muscle active at the ankle joint after the operation. Moreover, we have indicated that the minimum or maximum SSF frequencies have not been altered in fast motoneurons of the overloaded groups, so they still have ability to generate high frequencies of discharges that are necessary to evoke high levels of MU force. We have reported in our recent study on MUs (Krysciak et al. In press) that force-frequency curves for all types of MUs after 3 mo of the compensatory overload are shifted rightward compared with control toward a faster profile, so the MUs develop the same relative levels of tetanic forces at higher stimulation frequencies than MUs of the control group. In line with hypertrophy of muscle fibers, this would contribute to develop higher forces during activity of the overloaded muscle. However, it is worth noting that this rightward shift concerned the steep parts of the curves (at around 50–60% of the maximum force), corresponding to a limited range of frequencies, and not for the values corresponding to the maximum SSF frequencies of motoneurons.

Overload-induced adaptive changes in properties of motoneurons and MUs are important from the practical point of view because effects of chronic overload can be observed following intensive long-term physical work or overtraining in sportsmen (Lehmann et al. 1997), so results of this research may be helpful in setting optimal loads in sports training. Knowledge about specific adaptations in excitability and firing properties of motoneurons controlling muscles substituting paralyzed or tenotomized muscles due to neurodegenerative diseases (Finkel 2013) or tendon transfers (Henry and Chambers 1997) may be useful in choosing appropriate rehabilitation techniques and in optimizing levels of physical exercise in patients.

ACKNOWLEDGMENTS
Segments of this study concerning effects of the 5-wk muscle overload constitute part of the doctoral thesis submitted by A. Hałuszka.

GRANTS
This study was funded by Polish National Science Center Grant No. 2012/04/M/NZ4/00190.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


