Fast-to-Slow Conversion Following Chronic Low-Frequency Activation of Medial Gastrocnemius Muscle in Cats. II. Motoneuron Properties

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INTRODUCTION

The motor unit, consisting of the motoneuron and the muscle fibers that it innervates, is a highly integrated structure. Individual motor units making up a given muscle can range widely in terms of their muscle and motoneuron properties. However, within an individual motor unit the muscle fibers all have similar mechanical and biochemical properties (Burke 1981; Nemeth 1990), and these are coordinated with the properties of the motoneuron (reviewed in Mendell et al. 1994). Thus motoneurons innervating slow (type S) motor units have cell bodies with low rheobase (Rh), large input resistance (\(R_N\)), and long afterhyperpolarization (AHP) half-decay time, and have axons that conduct slowly. Motoneurons innervating fast (type F) motor units have cell bodies that are less excitable (higher Rh) and larger (lower \(R_N\)) and generate AHPs of briefer duration; their axons conduct more rapidly. Differences in Rh and \(R_N\) exist also between motoneurons that supply fast contracting fatigable (type FF) versus fatigue-resistant (type FR) motor units (Zengel et al. 1985).

In addition to differences in the intrinsic properties of motoneurons innervating these motor units, the properties of the synaptic connections on them also exhibit substantial heterogeneity (Burke 1981). One would anticipate that motoneurons with large values of \(R_N\) would produce larger excitatory postsynaptic potentials (EPSPs) than motoneurons with small values of \(R_N\). However, the differences between the connections on type S and type F motoneurons go beyond this to involve the manner in which synaptic transmission from group Ia fibers to motoneurons is altered during trains of high-frequency stimulation (167 Hz). Type S motoneurons uniformly exhibit a progressive decrease in EPSP amplitude (negative modulation), whereas type F motoneurons are variable, tending to show less amplitude decrease (type FR) or even amplitude increase (positive modulation: type FF) during the train (Mendell et al. 1995). Although the loci of the differences accounting for this type specific behavior are not known with certainty, it appears that presynaptic mechanisms are at least in part responsible (Mendell et al. 1994).

The existence of these coordinated properties in muscle fibers, motoneurons, and their synapses raises the question as to their determination. It seems most unlikely that these diverse properties are regulated completely independently, and so it becomes important to ascertain whether there is an organizing principle. The literature (reviewed in Gordon and Pattullo 1993) suggests two general explanations for these correlations. The first explanation invokes an orthograde mechanism: muscle units differentiate, for example, as a result of differential activity imposed on them by intrinsically different activity levels in the motoneurons or because of trophic factors secreted by different motoneuron types (Buller et al. 1960). The second explanation invokes a retrograde mechanism: muscle fibers, once differentiated into slow oxidative (type SO), fast oxidative glycolytic (type FOG), or fast glycolytic (type FG), may modify properties of motoneurons via retrograde transport of some substance(s). Specific properties of the motoneurons and synapses might be modified either directly as a result of activity or secondarily to differentiation of the muscle, via a retrograde mechanism.
Experimental evidence exists supporting each of these hypotheses. For example, Foehring and colleagues (Foehring and Munson 1990; Foehring et al. 1987a,b; see also Dum et al. 1985a,b) cross-regenerated the muscle nerve of the heterogeneous (∼75% type F, 25% type S) medial gastrocnemius (MG) into the homogeneous all-type-S soleus muscle of cats. As evidence for an orthograde effect, those researchers found that 31% of the soleus muscle units were now fast twitch [time to peak (TTP) <40 ms], a condition never seen in normal soleus. As evidence for a retrograde effect, the proportion of type S motoneurons (52% with AHP >35 ms) was much greater than in normal MG (∼25%). Conversion was not complete in all cases: there were some hybrid motor units with long contraction time but short AHP.

In the companion paper (Gordon et al. 1997), the MG muscle of cats was made soleus-like (all type SO muscle fibers, all slow twitch) as a result of chronic electrical stimulation of the MG nerve by indwelling electrodes. In the present paper we investigate 1) whether the associated MG motoneurons and the Ia synapses on them also developed properties typical of type S motoneurons, and 2) whether the correspondence among muscle, motoneuron, and synaptic properties seen in the normal adult cat was maintained after chronic electrical stimulation. Although we found clear evidence for changes in motoneuron and synaptic properties as well as muscle properties, the resultant matches were not complete. Specifically, we found that the muscle underwent a more complete conversion than did the motoneurons innervating it. On the basis of the present results as well as previous publications (Czech et al. 1978; Foehring et al. 1987b) (see DISCUSSION), we suggest that the clearest example of retrograde determination of motoneuronal properties is that seen from natural type SO muscle when MG motoneurons are crossed into soleus muscle (MG → SOL motoneurons): MG fast muscle fibers made slow by chronic stimulation appear to exert relatively less effect on their innervating motoneurons.

Some data are from previous experiments (Foehring and Munson 1990; Foehring et al. 1986a,b, 1987a,b; Mendell et al. 1995) or the companion paper (Gordon et al. 1997), and the work has been presented in abstract form (Gordon et al. 1993, 1994; Munson et al. 1996). Most literature cited in those previous papers is not referred to again in the present work.

**METHODS**

Experiments were performed on random-source mature female cats. Animals were deeply anesthetized during all surgical and experimental procedures and were killed by anesthetic overdose after acute experiments. Data for normal and axotomized MG, for normal soleus, and for MG → SOL motoneurons are from experiments previously published (Foehring and Munson 1990; Foehring et al. 1986a,b, 1987a,b), as are data for unstimulated MG motoneurons crossed into the sural nerve (MG → SUR motoneurons: Mendell et al. 1995).

**Preparatory procedures**

Cuff electrodes were implanted on the MG nerve in the popliteal fossa in six otherwise unoperated cats, permitting chronic stimulation of the MG muscle nerve and monitoring of muscle properties (Gordon et al. 1997). Stimulation (2.5-s trains at 20 Hz, 1 per 5 s, 24 h/d) commenced ∼1 mo after electrode implantation and continued for 8 wk (2 cats) or 11–12 wk (4 cats). Strength, speed, and fatigability of the MG muscle were assessed at intervals of ∼1 wk (Gordon et al. 1997).

In two other cats the MG nerve was first cross-anastomosed with the caudal cutaneous sural nerve and the MG muscle was excised (MG → SUR cats). Eighteen or 24 mo later (allowing time for restoration of normal motoneuron properties) (Nishimura et al. 1991) a cuff electrode was implanted around both the MG and lateral gastrocnemius (LG) nerves. This permitted monitoring of contraction of the LG and soleus muscles in response to the chronic electrical stimulation (which commenced 6 mo later), thus assuring the adequacy of stimulation, which lasted 12 or 13 wk.

**Experimental procedure**

Motoneuron electrical and muscle unit mechanical properties were determined with the use of standard intracellular recording and stimulation procedures as described previously (e.g., Foehring and Munson 1990). Synaptic potentials in response to low-frequency stimulation and to trains of stimuli were obtained as described in Mendell et al. (1995). The right and left triceps surae muscles were analyzed for whole muscle contractile properties, for speed- and fatigue-related enzymes, and for fiber size (Gordon et al. 1997).

Numerical data are expressed as means ± SE (Marks 1982).

**RESULTS**

**Conversion of muscle by chronic stimulation**

The chronically stimulated MG muscles became slowly and weakly contracting (Table 1). This is illustrated in Fig.

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**TABLE 1. Properties of whole muscle contractions**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MG Stim Time, days</th>
<th>Whole MG TTP, ms</th>
<th>Whole MG Twitch, mN</th>
<th>Whole LG TTP, ms</th>
<th>Whole LG Twitch, mN</th>
<th>Whole SOL TTP, ms</th>
<th>Whole SOL Twitch, mN</th>
</tr>
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<tbody>
<tr>
<td>ACY</td>
<td>75</td>
<td>68</td>
<td>4,704</td>
<td>36</td>
<td>6,850</td>
<td>66</td>
<td>4,253</td>
</tr>
<tr>
<td>ACZ</td>
<td>76</td>
<td>51</td>
<td>3,332</td>
<td>31</td>
<td>9,163</td>
<td>54</td>
<td>3,332</td>
</tr>
<tr>
<td>ADA</td>
<td>56</td>
<td>86</td>
<td>3,412</td>
<td>36</td>
<td>11,182</td>
<td>94</td>
<td>5,067</td>
</tr>
<tr>
<td>ADB</td>
<td>59</td>
<td>64</td>
<td>4,126</td>
<td>49</td>
<td>5,733</td>
<td>90</td>
<td>3,489</td>
</tr>
<tr>
<td>ADQ</td>
<td>87</td>
<td>106</td>
<td>6,213</td>
<td>40</td>
<td>13,641</td>
<td>71</td>
<td>4,824</td>
</tr>
<tr>
<td>ADR</td>
<td>87</td>
<td>85</td>
<td>5,027</td>
<td>41</td>
<td>10,008</td>
<td>84</td>
<td>2,959</td>
</tr>
<tr>
<td>Mean exp’t</td>
<td>73 ± 5 (6)</td>
<td>77 ± 8 (6)</td>
<td>4,656 ± 418 (6)</td>
<td>37 ± 2 (6)</td>
<td>9,430 ± 1,176 (6)</td>
<td>77 ± 6 (6)</td>
<td>3,987 ± 350 (6)</td>
</tr>
<tr>
<td>Mean normal</td>
<td>none</td>
<td>31 ± 1 (10)</td>
<td>12,593 ± 1,064 (10)</td>
<td>31 ± 2 (11)</td>
<td>12,338 ± 965 (11)</td>
<td>76 ± 7 (11)</td>
<td>4,439 ± 418 (11)</td>
</tr>
</tbody>
</table>

All ± values are means ± SE. Numbers in parentheses are sample size. Values for medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL) are from same legs. TTP, twitch time to peak; mean exp’t, data from present experiments; mean normal, data from normal, unoperated cats (Foehring et al. 1988).
SLOWED MUSCLE SLOWS MOTONEURONS

1, in which are shown whole muscle twitch contractions for the three triceps surae muscles of an experimental limb after 8 wk of stimulation (Table 1, experiment ADA). In normal cats the time course and force developed by twitches of the LG and MG muscles are identical (Foehring et al. 1988). In the six intact animals with chronically stimulated MG (Table 1), whole muscle twitch TTP ranged from 51 to 106 ms (mean 77 ms), whereas mean TTP of normal MG is 31 ms (Foehring et al. 1988). Mean whole muscle twitch tension decreased from a normal mean value of 12,593 to 4,656 mN. Stimulated MG muscles exhibited neither fatigue nor sag (Gordon et al. 1997).

The normally heterogeneous MG muscles were converted nearly completely to type SO by chronic stimulation: virtually all muscle fibers (>99%) (Gordon et al. 1997) exhibited enzymatic properties characteristic of type SO muscle (normal MG contains ~20% type SO muscle fibers) (Foehring et al. 1988). By the usual criteria, then, chronic activation of the MG muscle conferred on it contractile and histochemical properties similar to those of normal soleus (Fig. 1, Table 1).

We studied single MG muscle units activated by stimulation of the impaled motoneurons in three cats (Table 2). These muscle units were found to be slowly (mean TTP: 65 ± 3 ms, mean ± SE) and weakly (mean tetanic tension: 75 ± 7 mN) contracting, nonfatiguing, and nonsagging (force did not decline during unfused tetanic activation; cf. Gordon et al. 1997). These values are similar to those of both normal MG type S muscle units (58 ± 4 ms, 69 ± 10 mN) (Foehring et al. 1986a) and normal soleus muscle units (82 ± 4 ms, 78 ± 10 mN) (Foehring et al. 1987a). Thus the single muscle unit properties agree with the whole muscle contractile and histochemical properties in suggesting the conversion of MG muscle to soleus-like muscle by chronic stimulation.

Modification of motoneurons by chronic stimulation

With chronic stimulation (Table 2, Fig. 2), mean Rh averaged over all cells decreased (from 15 ± 1 nA to 9 ± 1 nA), mean R<sub>N</sub> increased (from 1.1 ± 0.1 MΩ to 1.5 ± 0.1 MΩ), mean AHP (measured as half-decay time) increased (from 28 ± 1 ms to 36 ± 8 ms), and mean conduction velocity decreased (from 91 ± 1 m/s to 86 ± 1 m/s). The significance of these changes was evaluated with the use of a nested analysis of variance procedure to distinguish variability between treatments (control vs. stimulated) from variability between animals. Changes in Rh were significant (P < 0.01), as were changes in R<sub>N</sub> and AHP (P < 0.05). The directions of these changes were toward values characteristic of motoneurons supplying fatigue-resistant motor units (Zengel et al. 1985) and were thus consistent with the changes in the muscle properties.

Although these changes are consistent with conversion of the motoneurons toward all fatigue-resistant, they are in part consistent also with effects of nerve injury (e.g., axotomy) (Foehring et al. 1986b). To test whether these changes might be the result of nerve injury, we compared the AHP distributions of normal, axotomized, and stimulated MG motoneurons. Whereas the range of AHP values is reduced by axotomy (Foehring et al. 1986b), it was increased and expanded to higher values by chronic stimulation (Fig. 3). We conclude that the stimulation-induced alterations of motoneuron properties are not injury effects.

Particularly noteworthy in Fig. 3 is the profound effect of chronic stimulation on the AHP values >30 ms, i.e., those

FIG. 1. Whole muscle contractions of triceps surae muscles (Table 1, experiment ADA). A: all records on same scale. B: records rescaled so that maximum amplitudes are identical. In unstimulated triceps surae, time to peak tension and magnitude of medial gastrocnemius (MG) and lateral gastrocnemius (LG) twitches are identical, and both are different from values for soleus (SOL; Table 1). After 8 wk of stimulation, MG and normal soleus have similar contractile properties. Calibration: 20 ms, 1,650 mN.
TABLE 2. Properties of motor units

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MG Nerve</th>
<th>Stim Time, days</th>
<th>MU TTP, ms</th>
<th>MU Tetanic, mN</th>
<th>Rheobase, nA</th>
<th>$R_w$, nM</th>
<th>AHP, ms</th>
<th>CV, m/s</th>
<th>EPSP MOD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACY</td>
<td>Intact</td>
<td>75</td>
<td>45 ± 5 (3)</td>
<td>44 ± 5 (4)</td>
<td>8 ± 1 (4)</td>
<td>1.7 ± 0.3 (4)</td>
<td>33 ± 4 (4)</td>
<td>84 ± 3 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>ACZ</td>
<td>Intact</td>
<td>76</td>
<td>58 ± 2 (18)</td>
<td>88 ± 10 (18)</td>
<td>8 ± 5 (17)</td>
<td>1.5 ± 0.11 (17)</td>
<td>47 ± 6 (17)</td>
<td>81 ± 1 (18)</td>
<td>NA</td>
</tr>
<tr>
<td>ADA</td>
<td>Intact</td>
<td>56</td>
<td>82 ± 2 (12)</td>
<td>65 ± 10 (12)</td>
<td>9 ± 8 (12)</td>
<td>1.6 ± 0.31 (12)</td>
<td>43 ± 7 (12)</td>
<td>81 ± 2 (12)</td>
<td>−41 ± 6 (12)</td>
</tr>
<tr>
<td>ADB</td>
<td>Intact</td>
<td>59</td>
<td>NA</td>
<td>NA</td>
<td>9 ± 3 (5)</td>
<td>1.4 ± 0.5 (5)</td>
<td>32 ± 5 (5)</td>
<td>85 ± 2 (5)</td>
<td>−32 ± 6 (4)</td>
</tr>
<tr>
<td>ADQ</td>
<td>Intact</td>
<td>87</td>
<td>NA</td>
<td>NA</td>
<td>10 ± 5 (19)</td>
<td>1.3 ± 0.11 (17)</td>
<td>34 ± 4 (19)</td>
<td>85 ± 1 (19)</td>
<td>−34 ± 4 (19)</td>
</tr>
<tr>
<td>ADR</td>
<td>Intact</td>
<td>87</td>
<td>NA</td>
<td>NA</td>
<td>10 ± 5 (14)</td>
<td>1.5 ± 0.2 (14)</td>
<td>24 ± 3 (14)</td>
<td>100 ± 2 (14)</td>
<td>−18 ± 9 (14)</td>
</tr>
<tr>
<td>Mean stim’d</td>
<td>MG</td>
<td>73 ± 5 (6)</td>
<td>65 ± 3 (33)</td>
<td>75 ± 7 (34)</td>
<td>9 ± 1 (71)</td>
<td>1.5 ± 0.11 (69)</td>
<td>36 ± 8 (71)</td>
<td>86 ± 1 (72)</td>
<td>−31 ± 10 (49)</td>
</tr>
<tr>
<td>Mean normal</td>
<td>MG</td>
<td>35 ± 2 (80)</td>
<td>343 ± 20 (78)</td>
<td>15 ± 1 (91)</td>
<td>1.1 ± 0.11 (73)</td>
<td>28 ± 1 (91)</td>
<td>91 ± 1 (79)</td>
<td>−6 ± 32 (55)</td>
<td></td>
</tr>
<tr>
<td>Mean axotMG†</td>
<td>Axotomized</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>6 ± 3 (28)</td>
<td>1.7 ± 1.01 (29)</td>
<td>29 ± 6 (28)</td>
<td>61 ± 7 (29)</td>
<td>−53 ± 12 (27)</td>
</tr>
<tr>
<td>Mean normal</td>
<td>soleus‡</td>
<td>82 ± 4 (43)</td>
<td>78 ± 7 (44)</td>
<td>5 ± 0 (42)</td>
<td>2.1 ± 0.1 (41)</td>
<td>57 ± 2 (42)</td>
<td>74 ± 1 (39)</td>
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<td></td>
</tr>
<tr>
<td>ADO</td>
<td>MG → SUR</td>
<td>85</td>
<td>6 ± 1 (8)</td>
<td>1.5 ± 0.2 (9)</td>
<td>29 ± 2 (8)</td>
<td>73 ± 2 (9)</td>
<td>NA</td>
<td></td>
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</tr>
<tr>
<td>ADP</td>
<td>MG → SUR</td>
<td>94</td>
<td>6 ± 1 (12)</td>
<td>1.7 ± 0.3 (11)</td>
<td>36 ± 4 (15)</td>
<td>76 ± 2 (18)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean stim’d</td>
<td>MG</td>
<td>90 ± 3 (2)</td>
<td>6 ± 1 (20)</td>
<td>1.6 ± 0.2 (20)</td>
<td>33 ± 3 (23)</td>
<td>75 ± 2 (27)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean unstim’d§</td>
<td>MG</td>
<td>None</td>
<td>13 ± 1 (36)</td>
<td>1.2 ± 0.1 (32)</td>
<td>21 ± 1 (33)</td>
<td>82 ± 2 (37)</td>
<td>−27 ± 3 (32)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All ± values are means ± SE. Numbers in parentheses are sample size. MU, motor unit; NA, not available; MG → SUR, MG nerve crossed to sural nerve; $R_w$, input resistance; AHP, afterhyperpolarization half-decay time; CV, conduction velocity; EPSP MOD, modulation of EPSP amplitude. For other abbreviations, see Table 1. *Means from normal, unoperated cats (Foehring et al. 1986a, 1987b; Mendell et al. 1995). †Means from cats with MG nerve axotomized 5–15 wk (Mendell et al. 1995). ‡Means from normal, unoperated cats (Foehring et al. 1987a,b). §Means from unstimulated cross-innervated cats (Mendell et al. 1995).

presumably from type S motoneurons (Zengel et al. 1985). This suggests that natural type SO muscle fibers (i.e., those naturally of type S motor units, as opposed to former types FG and FOG muscle fibers made type SO by chronic stimulation) are especially competent in modulating the properties of their motoneurons (see DISCUSSION).

Correlation of muscle and motoneuron properties

A predictable relationship exists between AHP and TTP of normal MG and normal soleus motor units: type F motor units have brief AHP and brief TTP, and type S motor units have long AHP and long TTP (e.g., Fig. 6 of Zengel et al.)

![Graph](image-url)
FIG. 3. Cumulative sum histograms of AHPs of normal MG, axotomized MG, and chronically stimulated MG motoneurons. Note that the range of values is reduced from normal by axotomy, but is increased by chronic stimulation, confirming that stimulation-induced changes in motoneuron properties do not result from nerve injury. Note also that AHP values >30 ms for the stimulated population are especially prolonged, indicating a special competence for natural type I muscle fibers in altering motoneuron properties (details in text).

1985; Fig. 7 of Cope et al. 1986). These relationships are illustrated in Fig. 4A for normal MG and normal soleus motor units. As a population, soleus type S motor units have somewhat longer AHP and TTP than do MG type S motor units.

When the heterogeneous (75% type F, 25% type S) MG muscle nerve is made to innervate the normally all-type-S soleus muscle, considerable conversion of motor unit properties occurs (compare soleus portion of Fig. 4A with corresponding plot of MG → SOL: Fig. 4B). Some MG motoneurons retain their brief AHP and convert the formerly type S muscle units to short-TTP type F units, an example of an orthograde motoneuron-to-muscle effect. Other units have long AHP and long TTP, typical of type S units of normal soleus. Foehring et al. (1990) argued that the numbers of type S units in the cross-inervated soleus were greater than could be accounted for by the proportion of type S motoneurons in normal MG; thus there must have been also a retrograde muscle-to-motoneuron conversion.

Given this and other evidence of retrograde determination of motoneuron properties by soleus muscle (see discussion), we were interested in the extent to which endowing MG muscle with soleus-like contractile and histochemical properties would affect the innervating MG motoneurons. The plot of motoneuron AHP versus muscle unit TTP for the 32 units in three chronically stimulated preparations (Fig. 4C) reveals a general trend for large values of AHP to be associated with long contraction times, but with considerable variability, particularly at longer contraction times. The distribution of values appears to divide into two clusters: one (n = 22) with short AHPs (20–40 ms) but a wide range of TTPs (37–90 ms); the other (n = 10) with long AHPs (55–95 ms) and long TTPs (56–92 ms). On the basis of AHPs, the first cluster could be the former type F motoneurons, now innervating muscle units with the full range of contraction times. If so, both their AHPs (normal range: ~15–30 ms) and TTPs (normal range ~20–35 ms) are greater than normal. On the basis of both AHP and TTP, the second cluster could be the former type S population. If so, their AHPs (normal range: ~30–70 ms) and their contraction times (normal range: ~40–80 ms) are also greater than normal. The percentage of motor units in each group (S/F: 31%/69%) is similar to the percentages of such motor units in normal MG (S/F: 25%/75%).

Although we refer to types F and S in the experimental population, the type F muscle units in particular are clearly
different from those in normal MG. In contrast to normal type F muscle units, all or virtually all muscle fibers in the stimulated muscles were identified histologically as type SO, no muscle units exhibited sag, and none fatigued. All muscle units in the experimental population contracted more slowly than normal type F muscle units. These motor units differ also from type F units in the MG → SOL experiments, in which some innervating MG motoneurons conferred normal type F contractile (TTP, sag, fatigability) and type FG or FOG histochemical properties on soleus muscle units (Foehring et al. 1987b).

Normal MG type F motoneurons have Rh/RN ratios >7; normal type S motoneurons have Rh/RN < 7 (Zengel et al. 1985). Because chronic stimulation has decreased Rh and increased RN, the same criteria would not be valid for stimulated motoneurons. However, we can calculate an equivalent value for stimulated motoneurons on the basis of the mean changes in Rh and RN in these preparations compared with intact controls. From Table 2, mean Rh/RN for controls is 15/1.1 = 13.6; for chronically stimulated motoneurons mean Rh/RN is 9/1.5 = 6. Adjusting the value of Rh/RN = 7 (see above) proportionately, we obtain a dividing value between type F and type S of Rh/RN = 3.08. The values of Rh/RN for stimulated motoneurons are plotted in Fig. 5. Nine of 10 putative type S motoneurons (AHP >55 ms), but only 2 of 22 putative type F motoneurons (AHP <40 ms) have Rh/RN ratios <3, further suggesting the validity of our interpretation that most motoneurons with AHP <40 ms are former type F motoneurons.

In the companion paper (Gordon et al. 1997) it was shown that chronically activated MG muscle units retain a range of contractile properties. To test whether those differences might be remnants of the prestimulation properties of the motor units, we plotted two muscle unit properties (tetanic tension and TTP) along with motoneuron AHP (Fig. 6). If previous motoneuron-muscle unit relations remain, we would expect motoneurons with long AHP to be associated with the weaker, slower muscle units, and the opposite for motoneurons with short AHP. Figure 6 shows this to be the case. We found tetanic tension for the long-AHP (>55 ms) motor units to be half that of the short AHP units (48 ± 8 mN vs. 88 ± 8 mN). As advanced in the companion paper, neuromuscular activity alone was unable to dictate adult muscle contractile properties.

**Was muscle necessary to produce the changes?**

**Stimulation of motor nerves cross-innervating skin**

We investigated the role of muscle in inducing these changes in the motoneurons. This was accomplished by cutting the MG nerve and allowing it to regenerate into skin via cross-anastomosis with the sural nerve (MG→SUR motoneurons) (Nishimura et al. 1991). Twenty-four or 30 mo later the MG nerve was stimulated for 85 or 94 days with the use of a cuff electrode. If muscle were necessary for the motoneuron alterations, one would anticipate no change in these MG→SUR motoneuron properties.

**FIG. 4.** Plots of AHP vs. time to peak tension of (A) normal control MG and soleus motor units, (B) motor units in which MG motoneurons cross-innervate soleus (MG→SOL units), and (C) chronically stimulated intact MG motor units. Note that the MG→SOL population contains many fast (type F) motor units [brief AHP, brief time to peak (TTP): orthograde effect] not seen in normal soleus, and excessive type S motor units (long AHP, long TTP: retrograde effect). Chronic stimulation prolongs minimum values of MG AHP and TTP, both suggesting conversion toward values of type S motor units. Details in text.
butions of motoneuron properties in unstimulated MG → SUR and stimulated MG → SUR motoneurons are shown in Fig. 7. The nested analysis of variance procedure revealed significant increases in AHP ($P < 0.05$) and decreases in $R_N$ ($P < 0.05$). The decrease in $R_h$, although similar to the changes observed after stimulation of intact motoneurons, was not significant. Thus changes in $R_h$, $R_N$, conduction velocity, and AHP were all at least qualitatively similar whether or not muscle was present, suggesting that stimulation altered motoneuron properties even in the absence of a target muscle.

**Alteration of synaptic mechanisms**

A measure of synaptic function that differs between type F and type S motoneurons is EPSP modulation, defined as the increase or decrease in EPSP amplitude during a high-frequency burst of impulses (Collins et al. 1984, 1988). In normal motoneurons there is a tendency for EPSP modulation to be positive in putative type FF motoneurons and to be negative in most putative type FR and all type S motoneurons (Mendell et al. 1995). In the present data we found EPSP modulation to be negative in 48 of 49 motoneurons (Fig. 8). These findings are consistent with the notion that the slowing of the muscle is associated with corresponding changes in the motoneuron and synaptic properties of these motor units. However, the mean amplitude of the EPSPs in these motoneurons measured in two of the four preparations (ADQ, ADR) was not elevated (1.95 ± 0.24 mV, $n = 31$; normally 2.0 ± 0.2 mV) (Mendell et al. 1995), as might be anticipated from the fact that the motor units developed type fatigueresistant properties.

**DISCUSSION**

In the present work we tested the hypothesis that chronic-stimulation-induced conversion of the normally heterogeneous MG muscle to a homogeneous all-type-S soleus-like composition would be accompanied by muscle-induced conversion of the innervating MG motoneurons to type S phenotype, maintaining the normal correspondence between properties of muscle units and of their innervating motoneurons. Precedent for such conversion comes from the work of Foehring et al. (1987b, 1990), in which cross-innervation into the all-type-S soleus muscle conferred type S properties on many former type F MG motoneurons. Conversion in the opposite direction occurs as well: Cope et al. (1986) and Munson et al. (1986) reported that motoneurons and muscle units of soleus and MG, respectively, of cats with chronically transected spinal cord changed properties such as to make the motor units more type F-like. Properties of motoneurons and muscle units remained coordinated; changes were likely due to reduced motoneuron and muscle activity, a manipulation opposite that in the present work.
Here the MG muscles subjected to chronic activation acquired characteristics typical of normal all-type-SO muscle (soleus) (see Gordon et al. 1997). This was true whether we examined the contractile properties of the whole muscle or of individual muscle units or the histochemical composition of the individual muscle fibers. The muscle became less fatigueable, the contraction time was longer, and the force developed was decreased. Stimulated muscle fibers characterized histochemically were almost all type SO (Gordon et al. 1997).

There was an alteration also of properties of motoneurons and their synapses toward values characteristic of fatigue-resistant units, the same direction in which the muscle had shifted as a result of the chronic stimulation. These shifts occurred in five measured variables: \( R_h, R_n, \) conduction velocity, AHP, and EPSP amplitude modulation (not all were significant). Interestingly, the shift was not the same for all these variables. With the use of normal and axotomized MG motoneurons as reference points, we found that \( R_h, R_n, \) axonal conduction velocity, and EPSP amplitude modulation acquired values intermediate between the two reference points, whereas AHP became longer, opposite to the changes observed after axotomy. EPSP amplitude, measured in two of these preparations, exhibited no change. Thus the alterations induced by long-term stimulation are not uniform, suggesting independent control mechanisms for these motoneuron and synaptic parameters.

Despite these alterations in properties, a population of putative former type F motor units was identified (RESULTS). Most motoneuron properties were still within normal limits; however, the muscle units were greatly changed. All were slowly contracting (up to 3 times the normal maximum for type F), none sagged (normally all sag), and histochemical composition was type SO and not type FG or FOG. We conclude that although changes in motoneuron properties had occurred as a consequence of stimulation, full conversion of type F motoneurons to type S had not occurred.

A population of putative former type S motor units was also identified. Their muscle unit contractions were also slowed from normal. The longest contraction times for stimulated type S muscle units were the same as those for stimulated type F units, suggesting a common ceiling effect. Motoneuron AHPs, which for normal type S motoneurons may be as brief as \( \approx 40 \) ms, were especially sensitive to stimulation: AHPs increased considerably in the stimulated population, all being \( > 55 \) ms. The type S motor units of stimulated MG resembled the normal (type S) motor units of soleus in the electrical properties of their motoneurons and the contractile and histochemical properties of their muscle units.

Similar experiments have been conducted by Donselaar et al. (1986): in four cats with dorsal rhizotomies the common peroneal nerve was stimulated chronically for 8 wk. In seeming contrast with the positive results of the present study, those activated motoneurons did not differ from normal controls with regard to soma size or staining for succinic dehydrogenase, an oxidative enzyme associated with small motoneurons.

**Consideration of mechanisms**

Our working hypothesis assumed in part that coordinated changes in motoneuron and muscle unit properties would originate with the muscle. Thus the effect of the activation would be primarily to slow the muscle and secondarily to alter the motoneurons through a retrograde mechanism. From the present data we cannot rule out the possibility of an interaction in the opposite direction, i.e., a primary action of the activity on the motoneuron and an orthograde action on the muscle. One compelling reason to adopt the retrograde mechanism as the major factor in the modification of motoneuron properties is that the muscle has clearly undergone a more complete change than have the motoneurons. All of the muscle units in the normally heterogeneous MG muscle became type SO after stimulation, whereas the motoneuron composition, although changed from normal, still had a substantial group of type F motoneurons. If one accepts the assumption that the properties of muscle and motoneuron are actively coordinated (see INTRODUCTION), it seems very unlikely that the muscle would have converted completely in response to only partial conversion of the motoneurons. The retrograde hypothesis would imply that the apparently completely converted muscle fibers were able to respecify the motoneurons only partly. However, our finding that alteration of motoneurons in response to stimulation of the motor nerve does not require the presence of the muscle (i.e., alteration can occur with the motor nerve cross-regenerated into skin with removal of the normal target muscle) indicates that activity of muscle is not a necessary factor in determining the change in the motoneuron.

In addition, we cannot rule out the possibility of parallel but independent effects of stimulation on muscle and motoneuron properties. However, we reject that possibility as unlikely because I there is no a priori reason to expect stimulation to directly alter motoneuron properties at all (Czeh et al. 1978), much less in a direction fully coordinated.
with changes in muscle properties; 2) there is evidence that activation of muscle alone without activation of motoneurons can modify motoneuron properties (Czeh et al. 1978); and 3) Foehring et al. (1987b, 1990) have shown the ability of soleus muscle to convert properties of some cross-regenerated MG motoneurons from type F to type S.

Kuno and colleagues reported that 7–13 days of stimulation of the soleus nerve and muscle of cats with transected spinal cord maintained the AHP duration of soleus motoneurons, which otherwise declined (Czeh et al. 1978). This effect was observed also if stimulation was applied distal to, but not proximal to, a TTX block of the nerve. This finding suggests the likelihood of a muscle-derived activity-dependent influence (perhaps a neurotrophin: see below) on motoneuron properties and makes unlikely a direct effect of stimulation on motoneurons.

Incompetence of converted muscle in altering type F motoneurons, or inability of type F motoneurons to be converted?

Former type F motoneurons retained AHPs near to normal values, despite conversion of their muscle units to the type S phenotype. This could signify that MG type F motoneurons are incapable of such change and/or that MG type II muscle made type I is incapable of effecting such change. Evidence has been presented (Foehring et al. 1987b, 1990; see also Mendell et al. 1994) that MG type F motoneurons that cross-innervate large (i.e., powerful) soleus muscle units then express type S motoneuron properties. In the present work, AHPs of native MG type S motoneurons (i.e., those innervating natural type I muscle fibers) were greatly prolonged by activation (Fig. 3), whereas those of former type F motoneurons (i.e., innervating former type II muscle fibers) were changed only marginally. Together, these results suggest that 1) properties of type F motoneurons are modifiable under proper conditions and 2) natural type I muscle fibers have competence that converted type II muscle fibers do not.

Putative role of neurotrophins

Candidate agents in the periphery for influencing motoneurons retrogradely include the trophic factors brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) that are known to act on tropomyosin related kinase-B (trkB) receptors present on motoneurons and their axons (reviewed in Friedman et al. 1995). There is sufficient overlap in the trophic factor composition of muscle and of prenatal skin to suggest that the same trophic factor(s) might be involved in both muscle-derived and skin-derived effects, particularly if the denervated tissues dedifferentiated to express fetal amounts of neurotrophins (reviewed in Mendell 1995). For example, NT-4/5 and BDNF are known to influence motoneuron properties (Friedman et al. 1995), and their mRNAs are present in both muscle and skin (Henderson et al. 1993; Schecterson and Bothwell 1992).

Recent experiments point specifically to the likely involvement of NT-4/5, which has been shown to be present in muscle in adult mammals and whose activity has been demonstrated to be highest in type I (type SO) muscle fibers (Funakoshi et al. 1995). Those investigators demonstrated that muscle NT-4/5 increases in a stimulation-dependent
manner in type I (but not type II) muscle fibers after only brief muscle stimulation (hours). Muscle NT-4/5 mRNA is reduced after sciatic nerve lesion or nerve block, suggesting that loss of muscle activity is responsible for decrease of this mRNA in muscle (Funakoshi et al. 1995; Griesbeck et al. 1995). This may also explain the findings of Cope et al. (1986) and Munson et al. (1986) that motor units become more type F-like after spinal cord transection, and thus reduced muscle activity (see also Kuno 1984). This raises the interesting possibility that in the present experiments, NT-4/5 upregulated in type SO (also types FG and FOG) muscle fibers by electrical stimulation was the trigger for modification of motoneuron properties (e.g., decreasing axonal conduction velocity) as well as the synapses on them (making them more type S). Such a mechanism is consistent with other observations that natural type SO muscle is especially and perhaps uniquely competent in modifying motoneuron properties (Czeh et al. 1978; Foehring et al. 1987b, 1990) (Fig. 3). The possibility that NT-4/5 is also upregulated in skin cross-innervated by motor nerves, promoting rescue of motoneurons innervating this target, requires elucidation.

This interpretation is complicated by the fact that putative actions of NT-4/5 on conduction velocity of motor axons differ according to context. Deprivation of NT-4/5, either by axotomy or by axotomy-mimicking treatment with trkB–immunoglobulin G (Munson et al. 1997), slows conduction velocity, and conduction velocity of motor axons slowed by axotomy is restored (increased) by provision of exogenous NT-4/5 (Munson et al. 1997). We speculate that in the present situation long-term stimulation induces upregulation of muscle NT-4/5 and that this in turn elicits type S-like properties in the innervating motoneurons, including slowing of conduction velocity toward type S values.

Conclusions

We have demonstrated that the conversion of the MG muscle to all type SO after chronic electrical activation is accompanied by a change in the properties of the MG motoneurons toward type S. Although the muscle underwent an apparent complete conversion, the motoneuron pool retained identifiable populations of former type S and former type F motoneurons, suggesting that the peripheral change is primary and the central change is secondary, i.e., a retrograde mechanism. For the type S population, the degree of change in the motoneurons appears to be proportional to the changes in mechanical properties. For the type F population, however, motoneuron properties were less altered, remaining generally within the limits of normal type F parameters despite the total conversion of their muscle units to type SO. The Ia synapses on the motoneurons also underwent changes consistent with those of the motoneurons, exhibiting negative modulation of EPSP amplitude during high-frequency stimulation, as occurs also in normal type S motoneurons.

Although we find evidence for the action of a retrograde
periphery-to-motoneuron mechanism, it is not dependent on the presence of muscle and, in the case of type F muscle made type S by chronic stimulation, it is not sufficient to convert type F motoneurons to type S, as can normal soleus type S muscle (Foehring et al. 1987b, 1990). Perhaps most attractive is the suggestion that both muscle and motoneuron properties can be determined by some third factor, such as a neurotrophin (i.e., the muscle is not necessary), which would have a greater effect on the muscle than on the motoneuron.

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REFERENCES


