Progress of Age-Related Changes in Properties of Motor Units in the Gastrocnemius Muscle of Rats

Miho Sugiura1,3 and Kenro Kanda2,3,4
1Epidemiology and Health Promotion Research Group, 2Motor and Autonomic Nervous System Integration Research Group, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015; 3Graduate School of Humanities and Sciences, Ochanomizu University, Bunkyo, Tokyo 112-8610; and 4Section for Human Neurophysiology, Research Center for Frontier Medical Engineering, Chiba University, Chiba 263-8522, Japan

Submitted 2 October 2003; accepted in final form 8 April 2004

Sugiura, Miho and Kenro Kanda. Progress of age-related changes in properties of motor units in the gastrocnemius muscle of rats. J Neurophysiol 92: 1357–1365, 2004. First published April 14, 2004; 10.1152/jn.00947.2003. The mechanical properties of individual motor units in the medial gastrocnemius muscle, as well as the whole muscle properties and innervating motor nucleus, were investigated in dietary-restricted, male Fischer 344/DuCrj rats at ages of 4, 7, 12, 21/22, 27, 31, and 36 mo. The tetanic tension of the type S units continuously increased until the age of 36 mo. Those of type FF and FR units declined from 21/22 to 27 mo of age but did not change further while the whole muscle tension decreased greatly. The atrophy of muscle fibers, the decline in motoneuron number and axonal conduction velocity, and the decrease in the posttetanic potentiation of twitch contraction of motor units seemed to start after 21/22 mo of age and were accelerated with advancing age. Prolongation of twitch contraction time was evident for only type S and FR units in 36-mo-old rats. The fatigue index was greatly increased for type FF units in 36-mo-old rats. These findings indicated that the progress of changes in various properties occurring in the senescent muscle was different in terms of their time course and degree and also dependent on the types of motor unit. The atrophy and decrease in specific tension of muscle fibers affected the decline in tension output of motor units. This was effectively compensated for by the capture of denervated muscle fibers over time.

INTRODUCTION

Motor units are the functional units for muscle activity and have a distinct anatomical structure comprising the motoneuron and innervated muscle fibers (Burke 1981). Therefore investigations are important to determine how these changes progress with age to elucidate the underlying mechanisms for sarcopenia and muscle weakness that occurs with age. Mechanical and morphological properties of motor units in the aged rat have been reported previously (Edström and Larsson 1987; Einsiedel and Luff 1992; Kadhiresan et al. 1996; Kanda and Hashizume 1989; Kanda et al. 1986; Larsson 1995; Larsson and Ansved 1988, 1995; Larsson and Edström 1986; Pettigrew and Noble 1991). These studies have demonstrated that changes in the properties of motor units with advancing age are affected by multiple factors, such as degenerative changes of motoneuron and muscle fiber, the denervation and reinervation process, transformation of muscle fibers, etc. Age-related changes have also suggested differences between slow and fast muscles or among different types of motor units within a muscle. However, some controversies are noted among findings on this matter. These include findings on the differential change in tetanic tension produced by each type of motor unit, on the increased number of type I muscle fibers, and on the change in the twitch contraction time. Some of these discrepancies may concern motor unit properties that were the result of rats being at different stages of the life span of the rat because in most studies, only two age groups were investigated. Furthermore, the mean survival times for the colony are not known in many cases, although the ages of the animals used are stated in the literature. To elucidate the underlying mechanisms for age-related changes in the neuromuscular system, the exact progress of each property and the differential alterations among different types of motor units with age must be clarified. In this respect, we examined various mechanical properties of individual motor units in the medial gastrocnemius muscle of dietary restricted (DR) rats ranging from 4 to 36 mo of age (7 groups) in the present study. Moreover, motoneuron number in the medial gastrocnemius (MG) motor nucleus, number and cross-sectional area of muscle fibers, and tension output of the whole MG muscle were also obtained in these rats.

METHODS

Experimental animals

The animals used in these studies were pathogen-free, male Fischer 344/DuCrj rats. After weaning, the rats could access food (ordinary commercial pellets) only 3 day/wk (Monday, Wednesday, and Friday; 3 × 24 h/week). They were housed three per cage and maintained on a 12:12 light-dark schedule (lights on at 0600) at 22°C. At the time of the experiments, the rats were 4 (n = 8), 7 (n = 10), 12 (n = 8), 21/22 (n = 8), 27 (n = 12), 31 (n = 8), or 36 (n = 11) months of age. The body weight of these DR rats was ~60% of that for ad-libitum-fed (AL) rats at the age of 24 mo. In rats maintained under these conditions, the incidence of diseases such as nephrosis and various cancers is lower, and the mean survival time of the rats in the same facility was 1,080 days, which is substantially longer compared with 860 days for AL rats (Dr. Kuramoto, personal communication). All rats used appeared healthy (judged from food intake and mobility) and had no visible subcutaneous tumors.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Surgical and experimental procedures

Two days before the final experiment, the rat was anesthetized with pentobarbital sodium (Nembutal, 35–45 mg/kg ip) or halothane in a mixture of nitrous oxide and oxygen (2:1), and the nerve innervating the MG muscle in the right leg was freed from the surrounding tissues. A fresh solution of horseradish peroxidase (HRP; 40%, 0.1–0.5 µl) was injected into the nerve to the MG muscle near the entry to the muscle using a glass micropipette and a pressure system. The wound was sutured, and the rat was returned to the cage. In the final experiments, the rat was anesthetized with pentobarbital sodium (35–50 mg/kg) administered intraperitoneally. The MG muscle of the left leg was dissected from the surrounding tissues but was not separated from the lateral gastrocnemius muscle to maintain good blood circulation. The nerve to the MG muscle was also freed of the surrounding tissues for recording action potentials of axons to the MG muscle. Except for the MG muscle, each of the hind-limb muscles was denervated by sectioning of the nerves. The lumbar sacral spinal cord was exposed by a laminectomy. The leg and lumbar sacral spine were immobilized in a metal frame by means of clamps. The distal tendon of the MG muscle was attached to an isometric strain gauge (BG-300 or BG-1000, Kulite) with a small steel hook. The exposed portions of the spinal cord and limb were covered with pools of mineral oil. Heating pads and radiant heat kept the body temperature and the oil pools at 36–38°C (for the rectal and spinal cord) or at 35–37°C (for the leg). Blood pressure and expired CO2 level were monitored throughout the experiment.

In some experiments, 4% Ficoll (Pharmacia Fine Chemicals) solution in lactated Ringer solution (Otsuka Pharmaceuticals) was infused to maintain the blood pressure. After registering the mechanical properties of the whole muscle, as well as of individual motor units, the muscle was quickly excised bilaterally, fixed at about the middle of the physiological length, and frozen in isopentane cooled in liquid N2. The muscle specimens were stored in a deep freezer. Finally, the rats were perfused transcardially with warmed physiological saline (300 ml) followed by a cooled fixative (700 ml of a mixture of 1.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer) after a supplemental dose of pentobarbital, if necessary. The lumbar spinal cord was removed and immersed in 30% sucrose solution overnight for histological analysis.

Mechanical properties of the muscle

Before registering mechanical properties of individual motor units, the muscle length was adjusted so as to produce the maximum whole muscle tetanic tension. Subsequently individual motor-unit tension was registered at this muscle length. Motor units were isolated by stimulating single MG axons in finely dissected ventral root filaments. The ventral roots of the lower lumbar segments (usually L4 and L5) were sectioned at a point near the entry to the spinal cord and limb were covered by pools of mineral oil. Heating pads and radiant heat kept the body temperature and the oil pools at 36–38°C (for the rectal and spinal cord) or at 35–37°C (for the leg). Blood pressure and expired CO2 level were monitored throughout the experiment.

In some experiments, 4% Ficoll (Pharmacia Fine Chemicals) solution in lactated Ringer solution (Otsuka Pharmaceuticals) was infused to maintain the blood pressure. After registering the mechanical properties of the whole muscle, as well as of individual motor units, the muscle was quickly excised bilaterally, fixed at about the middle of the physiological length, and frozen in isopentane cooled in liquid N2. The muscle specimens were stored in a deep freezer. Finally, the rats were perfused transcardially with warmed physiological saline (300 ml) followed by a cooled fixative (700 ml of a mixture of 1.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer) after a supplemental dose of pentobarbital, if necessary. The lumbar spinal cord was removed and immersed in 30% sucrose solution overnight for histological analysis.

Histochemical and morphological analysis of muscle fibers

After storage in a deep freezer (−75°C), the muscle was cut into cross-sectional blocks that were about 5 mm thick. Sections were cut from these blocks at a 10–15 µm thickness in a cryostat and stained for myofibrillar ATPase at pH 9.4 to distinguish the type I and II muscle fibers. Several serial sections were cut at consecutive 100 µm to 1-mm intervals and reconstructed to show the fiber composition in the mid-belly portion. This process was necessary to estimate the number of type I muscle fibers making up the muscle, because individual sections did not contain all of these fibers. In some muscles, the type II fibers were further subdivided into three groups, type IIA, IIX, and IIB muscle fibers. The type IIA fibers were stained darkly for ATPase following alkaline preincubation at pH 10.4 and lightly for ATPase following acid preincubation at pH 4.6; the type IIB were stained immediately for ATPase both at pH 10.4 and pH 4.6; and the type IIX were stained darkly for ATPase at pH 10.4 and immediately at pH 4.6. (K. Kanda and S. Asaki, unpublished data; see also De Ruiter et al. 1996; Larsson et al. 1993; Lind and Kernell 1991). Cross-sectional area of individual type I (>200 fibers/muscle) and type II muscle fibers (>500 fibers/muscle) were measured using an image analysis program (National Institutes of Health image, ver. 1.62). The type II muscle fibers were measured in two different regions of the muscle: the red (the mid-belly portion) and white (the caudo-medial portion) regions. The prevalence of different types of muscle fibers in the red region were 18.7% for the type I, 21.2% for the type IIA, 54.3% for the type IIX, and 5.8% for the type IIB fibers. Those in the white region were 25.5% for the type IIX, and 75.5% for the type IIB fibers. We observed some small and angulated fibers that were scattered in the muscle of aged rats. However, the number of those fibers and the number of rats in which those fibers were observed were limited. Furthermore, it was hard to distinguish whether those fibers were functioning. Therefore we included these fibers for measuring cross-sectional area. Three to four muscles (i.e., rats) of each age group
 were measured. No correction was made for the pinnation of the

The estimation of the total number of muscle fibers and the specific tension as follows. The mean muscle fiber length was measured in rats used in other experiments. Two rats in each age group were fixed with 10% formaline. The knee and ankle angles were maintained at about 120 and 90° during fixation, respectively. The MG muscle was removed and sectioned longitudinally and in parallel with muscle fibers at about a 2-mm thickness. These sections were teased so that individual muscle fibers might be identified from their origin to the end apponeurosis under an operating microscopy. The fiber length was measured at its proximal, medial, and distal portions and then averaged. The total fiber cross-sectional area (CSA) of each muscle was calculated using these values (the formula: muscle mass divided by the product of length and density of the muscle fibers). The density of the skeletal muscle was taken as 1,060 kg m⁻³ (Brooks and Faulkner 1988; Kadhiresan et al. 1996). The mean specific tension of the muscle fibers was determined by dividing tetanic tension by the total fiber CSA.

Histological analysis of the motor nucleus

The spinal segments were identified by the insertion of the dorsal roots and the cord was trimmed to include the L₄–S₁ segment. Serial sections (40 μm in thickness) were cut horizontally and processed by the TMB method. Sections containing labeled neurons were photographed and printed on transparent film. Individual HRP-labeled neurons were identified and counted under microscopic observations with the aid of photomontage maps. In parts of the experiments, this process was possible through a computer assisted image analyzer (i.e., NeuroLucida; MicroBrightField). Cross-sectional area of soma was also measured for each identified motoneuron. The number of α- and γ-motoneurons were estimated from the size distribution in each rat.

Data analyses

The data of the mechanical properties of muscle units were pooled in each age group. As for the cross-sectional area of muscle fibers, the mean value was calculated for each rat, and then the mean value for each age group was obtained. The effect of aging and the difference between the motor unit types were examined by a two-factor ANOVA, and group comparisons were made initially using ANOVA, and subsequent post hoc comparisons were made with the Bonferroni/Dunn procedure. The criterion for accepting statistical significance was \( P < 0.05 \).

**R E S U L T S**

**Body mass, muscle mass, and tetanic tension produced by the whole muscle**

The body mass, the wet weight of the MG muscle, and the maximum tetanic tension produced by indirect electrical stimulation of the muscle nerve for each age group are summarized in Table 1. The body weight increased up to the age of 21/22 mo and then tended to decrease thereafter, although the difference between the 36- and 21/22-mo-old rats was not statistically significant. The wet weight of the MG muscle also increased greatly from 4 to 7 mo of age, remained unchanged at 27 mo, and then declined at 36 mo of age. The maximum tension output increased up to the age of 21/22 mo in parallel with the body weight change. Thereafter, it decreased by 42.1% from 21/22 to 36 mo of age (Fig. 1A).

**Number and size of muscle fibers**

The estimated total number of muscle fibers in each age group shown in Table 1 are generally consistent with those of previous reports (Kadhiresan et al. 1996; Kanda and Hashizume 1989). The changes in the total number of muscle fibers with age seems to be rather small; the decrease was ~3.8% from 4 to 36 mo of age, and this difference was not statistically significant. The direct count of the total number of the type I fibers was determined by dividing tetanic tension by the total fiber CSA.

![FIG. 1](http://jn.physiology.org/doi/10.2210/jn.2004.06.23.5)

**TABLE 1. Body mass, muscle mass, and tetanic tension produced by indirect electrical stimulation of the muscle nerve**

<table>
<thead>
<tr>
<th>Age Groups, mo</th>
<th>Body weight, g</th>
<th>Wet weight, mg</th>
<th>MG P₀ N</th>
<th>Estimated Total No. of M. Fibers</th>
<th>Estimated SpT N/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 4 (6)</td>
<td>142.5 ± 20.2</td>
<td>310 ± 15</td>
<td>6.44 ± 0.59†</td>
<td>18,447 ± 91.0</td>
<td>25.9 ± 1.5</td>
</tr>
<tr>
<td>B: 7 (8)</td>
<td>219.0 ± 21.7</td>
<td>466 ± 36</td>
<td>8.07 ± 0.78</td>
<td>18,335 ± 141.7</td>
<td>23.1 ± 2.0</td>
</tr>
<tr>
<td>C: 12 (6)</td>
<td>252.7 ± 20.8</td>
<td>450 ± 27</td>
<td>8.49 ± 0.32</td>
<td>14,924 ± 88.7</td>
<td>25.2 ± 0.7</td>
</tr>
<tr>
<td>D: 21/22 (6)</td>
<td>270.2 ± 25.4</td>
<td>458 ± 30</td>
<td>8.67 ± 0.55</td>
<td>16,867 ± 111.2</td>
<td>24.9 ± 1.6</td>
</tr>
<tr>
<td>E: 27 (10)</td>
<td>268.2 ± 18.3</td>
<td>455 ± 40</td>
<td>7.58 ± 0.80§</td>
<td>17,733 ± 155.5</td>
<td>21.9 ± 1.6</td>
</tr>
<tr>
<td>F: 31 (9)</td>
<td>259.7 ± 21.7</td>
<td>390 ± 31</td>
<td>7.54 ± 0.84‡</td>
<td>17,451 ± 158.8</td>
<td>17.7 ± 3.6</td>
</tr>
<tr>
<td>G: 36 (7)</td>
<td>241.1 ± 27.0</td>
<td>345 ± 53</td>
<td>5.01 ± 0.62*</td>
<td>17,746 ± 274.9</td>
<td>18.2 ± 4.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Number in each group in parentheses. Wet Wt, wet weight of the medial gastrocnemius muscle; MG P₀, Maximum tetanic tension of the medial gastrocnemius muscle; Est. SpT, estimated specific tension. *Different from groups A–E; †Different from groups B–G; §Different from groups B–D; ‡Different from group G.
fibers showed that this remained constant throughout the ages examined (Fig. 2A).

The mean CSA of both the type I and II muscle fibers increased greatly from age 4 to age 7 mo (Fig. 2B). The type I muscle fibers remained unaltered ≤36 mo of age. A two-factor ANOVA (age × fiber type) for type II fibers in adult and aged groups indicated an age effect: $F(4,22) = 10.281$. The magnitude of atrophy tended to be greater for the type II fibers in the white region (the majority were IIb fibers) compared with the type II fibers in the red region (the majority were IIa and IIx fibers) because only type II fibers in the white region in 36-mo-old rats were smaller than those in 7-, 12-, or 21/22-mo-old rats (Fig. 2B). We also noticed an increased irregularity in the arrangement of muscle fibers in aged rats. The mean specific tension of muscle fibers declined with age by ~30% from 4 to 36 mo of age (Table 1).

Motoneuron number, size, and axonal conduction velocity

The number of retrogradely labeled motoneurons in each age group is shown in Fig. 3A. A two-factor ANOVA (age × motoneuron) indicated an age effect: $F(6,80) = 20.263$, and an interaction: $F(6,80) = 10.316$. The number of presumed $\alpha$-motoneurons in the young to middle age (4- to 21/22-mo-old) groups were not different from each other. It decreased ≥27 mo of age, and this trend seemed to be accelerated with advancing age. Presumed $\alpha$-motoneurons decreased preferentially without any change in the number of presumed $\gamma$-mo-

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** A: the number of type I muscle fibers in the gastrocnemius muscle. The number remains unaltered throughout the ages examined in the present experiments. The thick horizontal bars indicate the mean values. B: the cross-sectional area of muscle fibers. Note that the type I muscle fibers did not show atrophy until 36 mo of age, whereas the decrease in cross-sectional area of type II fibers tended to begin at 21/22 mo of age. Most of the type II fibers in the red region were type IIa and IIx, whereas most of those in white region were type IIb (see METHODS). The vertical bars indicate the SE; asterisk, different from groups B–D.

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** A: the total number of motoneurons (including both $\alpha$- and $\gamma$-motoneurons) and the number of presumed $\alpha$-motoneurons. The $\alpha$-motoneurons are segregated from $\gamma$-motoneurons by the size distribution in each rat. Note that the number of $\alpha$-motoneurons was similar until 21/22 mo of age, thereafter decreasing, whereas that of $\gamma$-motoneurons remained constant throughout the ages. *, different from 4-, 7-, 12-, and 21/22-mo-old rats. B: the axonal conduction velocities of each unit type. The decline with age was smaller for type S units than type F (FR and FF) units. |, the SD +*, different from values for type FR and FF units.
toneurons. The mean soma CSA increased greatly from 4 to 7 mo of age and tended to increase further in the 36-mo-old rats.

The mean axonal conduction velocity of α-motoneurons gradually increased ≤21/22 mo of age and thereafter declined irrespective of the motor-unit type (Fig. 3B). A two-factor ANOVA (age × unit type) indicated an age effect: \( F(6,794) = 97.549 \), a type effect: \( F(2,794) = 46.467 \), and an interaction: \( F(12,794) = 8.258 \). The decrease with advancing age was greater for FR and FF units compared with that for the type S units, and at the age of 36 mo, the conduction velocity of the type S units was faster than those of the type FF and FR units, whereas in younger (4- to 27-mo-old) rats values for the type FF and FR motor units were faster than that of the type S motor units. An analysis for only type FF and FR units showed no unit-type effect or interaction. The mean conduction velocity value of type FR units was consistently faster than type FF units in young and adult (4- to 21/22-mo-old) rats, whereas in old (≥27 mo) rats the relation was reversed.

Mechanical properties of individual motor units

The mechanical properties of a total of 822 motor units (4-mo: 113 units in 6 rats; 7-mo: 107 units in 6 rats; 12-mo: 131 units in 5 rats; 21/22-mo: 111 units in 6 rats; 27-mo: 189 units in 10 rats; 31-mo: 89 units in 6 rats; and 36-mo: 82 units in 7 rats) were investigated in this study. Motor units could be classified into three categories: type FF, FR and S units using the “sag” property and fatigability. Figure 4 shows the frequency distribution of the fatigue index of each age group. The units distributed bimodally with a clear trough. Fatigable and fatigue-resistant motor units were separated according to this distribution pattern. Thus the fatigue index value distinguishing between the type FR and FF was 0.5 for the 4- to 31-month-old groups, and it was 0.6 for the 36-mo-old group because the distribution was shifted toward the right. The latter might be justified by the findings that motor units with a fatigue index <0.1 were not found in 36-mo-old rats and that the mean fatigue index of units assigned to the type FR did not show a tendency to decline with age. The mean fatigue index for the type FF motor units from 4 to 31 mo of age was similar (0.108–0.171) but that in 36-mo-old rats was 0.394, which was greater than those in other age groups.

The percentage of type S units in the sample taken from young and middle-aged rats (4- to 22-mo old) was ~17.8%. Because the mean number of motor units (i.e., number of α-motoneurons) in the same age group was 96, the number of type S motor units could be considered 17. The number of type S units was also calculated from the values for the mean tetanic tension, the number of type I muscle fibers, the mean cross-sectional area of type I fibers, and the specific tension. The specific tension was considered the same as the average value for all fibers composing the muscle, that is, the value estimated from the whole muscle tension and the total cross-sectional area. The number of type S units obtained in this way were 16.9 for 4, 17.1 for 7, 16.9 for 12, 17.3 for 21/22, 17.0 for 27, 17.0 for 31, and 17.0 for 36 mo of age. Thus the number of type S units estimated were very consistent in the young groups. The number of type S units appeared to remain very much unaltered up until the age of 36 mo. This suggests the preferential loss of fast-twitch motor units because the total number of motor units decreased. The numbers of type FF and FR units were then estimated by the ratio of observed type FR to FF units. The decrease in the number of the type FR (43 in 4-mo-old rats to 28 in 36-mo-old rats) was greater than that of the type FF units (35 in 4-mo-old rats to 26 in 36-mo-old rats).

As for the initial twitch contraction (i.e., twitch contraction before PTP) of type FR and FF units, a two-factor ANOVA (age × unit type) revealed an age effect: \( F(6,609) = 12.649 \) and an interaction: \( F(6,609) = 2.838 \). A regression analysis indicated an increasing trend of twitch contraction time with age for type FR and S units but not for type FF units (Fig. 5A). The magnitude of PTP was the greatest for the type FR units

![Fatigue index](https://www.jn.org)

FIG. 4. The frequency distributions of the fatigue index of motor units in each age group. □, motor units with the “sag;” ■, units without the sag. A: 4 mo; B: 7 mo; C: 12 mo; D: 21/22 mo; E: 27 mo; F: 31 mo; G: 36 mo. Note that the distribution for the fatigable units in 36-mo-old rats is greatly shifted to the right.
(3.4–3.7), then for the type FF units (2.2–2.4), and smallest for the type S units (1.2–1.3) in young rats. The PTP was gradually weakened with age after 21/22 mo of age and was very little for all units at the age of 36 mo (1.2 ± 0.1 for type FR units, 1.2 ± 0.1 for type FF units, 0.9 ± 0.1 for type S units; Fig. 5B). A two-factor ANOVA (age × unit type) for type FF and FR units indicated an age effect: $F(6,605) = 31.974$, a type effect: $F(1,605) = 61.643$, and an interaction: $F(6,605) = 4.727$.

The mean tetanic tension produced by individual motor units is shown in Fig. 1B. A two-factor analysis (age × unit type) indicated an age effect: $F(6,797) = 4.184$ and a unit-type effect: $F(2,797) = 449.678$. They increased greatly from 4 to 12 mo of age. The mean tetanic tension for the type S units tended to continuously increase ≥36 mo old. The type FR and FF units tended to decrease in tension from 22 to 27 mo of age but thereafter remained unaltered. These findings were quite different from the trend of the whole muscle tension output, which was greatly decreased. The change in the tension produced by individual motor units was minimal compared with the great decline in the whole muscle tension of the aged rat. The CSA of type II muscle fibers seemed to start declining at early senescent age. The specific tension also decreased with age. These two changes may cause the decline in the tension output by the

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** A: the twitch contraction time. The time to peak of the twitch contraction before posttetanic potentiation of type S and FR units was prolonged during the period between 31 and 36 mo of age, but that of type FF remained unchanged. B: posttetanic potentiation of twitch contraction (PTP). The values are the ratio of twitch tension after PTP to twitch tension before PTP. Note that the decline of PTP is greater for type FR units than for type FF units. C: twitch-to-tetanus ratio. The twitch/tetanus ratio increased in 36-mo-old rats for type S units, decreased in 36-mo-old for type FR units, and did not change for type FF units. | SD.

**DISCUSSION**

In this study, we demonstrated differential progress of changes in various properties of motor units. Age differentially affected the different types of motor unit. The atrophy of muscle fibers, the decline in motoneuron number and axonal conduction velocity, and the decrease in the posttetanic potentiation of twitch contraction of motor units seemed to start after 21/22 mo of age and were accelerated with advancing age. Prolongation of twitch contraction time for S and FR units and the increased fatigue index for type FF units were evident only in 36-mo-old rats.

The change in the tension produced by individual motor units was minimal compared with the great decline in the whole muscle tension of the aged rat. The CSA of type II muscle fibers seemed to start declining at early senescent age. The specific tension also decreased with age. These two changes may cause the decline in the tension output by the
whole muscle as well as individual motor units. The maintenance of the motor-unit tension clearly indicates that some units acquired extra-muscle fibers that were probably once denervated due to death of innervating motoneuron. In some of these units, this process reduces or even exceeds a decrease in tension due to muscle fiber atrophy and a decrease in the specific tension. Recapturing of denervated muscle fibers and remodeling of motor units in the aged have been reported repeatedly in human subjects (Campbell et al. 1973; Stålberg and Thiele 1975) and in experimental animals (Einsiedel and Luff 1992; Kadhiresan et al. 1996; Kanda and Hashizume 1989; Larsson 1995). Using the glycogen depletion technique and the computer-assisted method, Ansved et al. (1991) found some characteristics in spatial reorganization of motor-unit fibers due to a denervation-reinnervation process. However, the extent of denervation-reinnervation process in the whole muscle has not been well documented. The present findings that the total number of muscle fibers did not change and that motor-unit tension in very old rats did not differ from that in young rats suggest that a large proportion of fibers are recaptured and survived. Enlargement of motor units was also supported by the estimated innervation ratios that were calculated from the values of mean tetanic tension produced by motor units, mean cross-sectional area of muscle fibers of corresponding types and the specific tension. Appearance of a decline in tension from 21/22 to 27 mo of age for type FF and FR units might indicate that the capture of extra-muscle fibers and the subsequent recovery of the contractile function of reinnervated muscle fibers take time to overwhelm the effects of atrophy and a decrease in specific tension. This remodeling of the motor units may be related to findings of Masakado et al. (1994), who reported that the variance in the relationship between the motor-unit tension and recruitment threshold was greater in the elderly subject compared with younger ones (but see also Spiegel et al. 1996). Erim et al. (1999) also observed a disturbance of the onionskin phenomenon in the relationship between firing rate and recruitment threshold in a human muscle.

The twitch contraction time of the type FR and S units tended to become longer with age although we did not find such a difference in previous experiments (Kanda and Hashizume 1989; Kanda et al. 1986; Pettigrew and Noble 1991; but see also Larsson and Ansved 1988; Larsson et al. 1991; Thompson and Brown 1999). Among many factors, the process of excitation-contraction coupling (E-C coupling) is considered to influence the time-to-peak twitch contraction (see for review Burke 1981). The aging effect on sarcoplasmic reticulum and the E-C coupling process has been reported previously (De Coster et al. 1981; De Luca and Camerino 1992; Gonzalez et al. 2000; Larsson and Salviati 1989; Margreth et al. 1999; Narayanan et al. 1996; Plant and Lynch 2003). Interestingly, Narayanan et al. (1996) reported that the rate of ATP-supported Ca\(^{2+}\) uptake by the sarcoplasmic reticulum was lower in the aged rat compared with the adult for the soleus muscle, but not for the gastrocnemius muscle. They also found that the time-to-peak tension was prolonged for the soleus in the aged but not for the gastrocnemius. Thus the present study might indicate that there is a similar difference in the aging effect between fast and slow fibers within the MG muscle. The difference might also reflect the differential effect of aging on twitch time and PTP between the type FR and FF motor units found in the present experiment.

An increased fatigue index for the type FF units in 36-mo-old rats was quite impressive. This trend was also noticed in 31-mo-old rats (Fig. 4F). Kadhiresan et al. (1996) reported a similar finding that the fast-intermediate (FI) motor units (i.e., motor units with fatigue indices between 0.5 and 0.75 in their definition) were sampled only in muscles of old rats. Possible explanations for this change are as follows. First, type FF units captured many muscle fibers that once belonged to type FR units (i.e., type Ia and IIX fibers). These re-captured fibers keep their fatigue-resistant properties and raise the fatigue index of type FF units. Second, muscle fibers transform from the type Iib to type IIX in old muscle (Larsson et al. 1991). Motor units consisted of type IIX fibers are more fatigue resistant than motor units of type Iib fibers in the young rats (De Ruiter et al. 1996; Larsson et al. 1991; Kanda, unpublished observation). Thus the type FF motor units in 36-mo-old rats become more fatigue resistant. Finally, the type Iib fibers become more fatigue resistant because of an increased duty cycle of the type FF units. Zajac and Faden (1985) suggested that the recruitment order of motor units in the cat was in the order of the magnitude of tetanic tension and/or motor unit types (i.e., in order of S, FR, and FF). According to this recruitment pattern and the little change in tension demand to the muscle, the high-threshold type FF units in the aged rats might be more frequently recruited and thereby increasing their activity. This may increase the fatigue resistance of the type FF motor units.

Overall, the least degenerative age-related changes were found among the type S units, and the strongest changes were found among the type FR units. The difference between the type S units and type F (including both type FF and FR units) is especially interesting because reactive oxygen radicals are considered to be a major cause of cell damage with age (Sohal and Weindruch 1996). Type S units are the most active among all motor units (Hennig and Lømo 1985). The type S motoneurons and type I muscle fibers, therefore might produce more reactive oxygen species compared with the other types. Nevertheless, it seems that the type S motor units survive well. This indicates that the type S motoneurons and type I muscle fibers might have a stronger antioxidant function compared with type FF and FR units.

In the present experiments, we used dietary-restricted (DR) rats (see METHODS) because the incidence of various diseases in those rats were lower and they lived longer compared with ad-libitum-fed (AF) rats. It was therefore relatively easy to produce aged rats. In the present experiment, the motor units in DR rats were not compared with those in AF rats in detail. However, we noted some differences between the present experiments and the previous experiments in which AF rats were used. There was an increase in the number of type I muscle fibers and the maximum tetanic tension produced by the type S motor units was less extensive in the aged, DR rats compared with that of the AF rats. This difference was consistent with the findings in our previous experiments in which rats were randomly assigned to DR or AF groups and raised under different feeding condition (Kanda 2002). The difference in the degree of motoneuronal loss (Kanda 2002), the amount of muscle activity (Goodrick et al. 1983; Yu et al. 1985), and the plasma level of neurotrophic factors such as IGF-I (Breese
et al. 1991; Tomita et al. 2001) and corticosteroid hormone (Everett et al. 1985; Sabatino et al. 1991) between DR and AF rats may lead to the distinctive changes observed in these two groups of rats (see Hall 1990; Ishii et al.1994; Scheff and DeKosky 1983; Streppel et al. 2002; Tam et al. 2001). However, further study is needed to explain the difference in aging between the AF and DR rats.

The MG muscle mass became much bigger from 4 to 7 mo of age, maintained its level from 12 to 22 mo, and thereafter declined. In parallel with this change, the muscle strength, the maximum tetanic tension produced by electrical stimulation of the muscle nerve, became altered. The number of innervating motoneurons remained constant up until 21/22 mo of age and thereafter decreased rapidly. Thus the senescence changes both in the muscle and innervating motoneurons seem to progress in parallel and may be accelerated with age. Studies on the human neuromuscular system have demonstrated that changes become evident at 50–60 yr of age (Campbell et al. 1973; Massa et al. 1992; Stålberg and Favcett 1982; Stålberg and Thiele 1975; Tomlinson and Irving 1977), and its progress should be considered exponential rather than linear with age after maturation. These findings indicate that age-related changes in the neuromuscular system of both rats and humans are generally similar when looking at the progress with relative age to their life span. Thus age-related changes in rat motor units deserve further study to understand the mechanisms for human sarcopenia and muscle weakness occurring in the aged.

Acknowledgments

The authors thank Dr. Kuramoto for supplying the dietary restricted rats and S. Asaki for excellent technical assistance.

References


