Caloric Restriction Does Not Offset Age-Associated Changes in the Biophysical Properties of Motoneurons

Jayne M. Kalmar, Duane C. Button, Kalan Gardiner, Farrell Cahill, and Phillip F. Gardiner
Spinal Cord Research Center, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada

Submitted 28 May 2008; accepted in final form 4 September 2008

Kalmar JM, Button DC, Gardiner K, Cahill F, Gardiner PF. Caloric restriction does not offset age-associated changes in the biophysical properties of motoneurons. J Neurophysiol 101: 548–557, 2009. First published September 10, 2008; doi:10.1152/jn.90617.2008. Age-associated changes in neuromuscular function may be due to a loss of motor neurons as well as changes in their biophysical properties. Neuronal damage imposed by reactive oxygen species may contribute to age-related deficits in CNS function. Thus we hypothesized that aging would alter the functional properties of motoneurons and that caloric-restriction would offset these changes. Intracellular recordings were made from lumbar motoneurons of old Fisher Brown Norway (FBN) fed ad libitum (oldAL, 30.8 ± 1.3 mo) or on a fortified calorie-restricted diet from 14 wk of age (oldCR, 31.0 ± 1.8 mo). Basic and rhythmic firing properties recorded from these aged motoneurons (MN)s were compared with properties recorded from young FBN controls (young, 8.4 ± 4.6 mo). Compared with young MNs, old MNs had a 104% greater (P < 0.001) afterhyperpolarization potential (AHP), a 21.1% longer AHP half-decay properties recorded from young FBN controls (young, 8.4 ± 4.6 mo). For high- and low-threshold MNs, was lower in the old group, we conclude that aging alters the biophysical properties of MNs in a fashion that cannot be simply attributed to a loss of high-threshold MNs. Surprisingly, caloric restriction, which is known to attenuate aging-associated changes in hindlimb muscles, had no effect on the progress of aging in the innervating MNs. 

INTRODUCTION

As early as the seventh decade (60–70 yr of age) of life, isometric tests of maximal voluntary knee extensor torque in men reveal a loss of muscle strength ranging from 25% (Larsson and Karlsson 1978) to 49% (Young et al. 1985). Similar losses have been reported in isokinetic protocols (Poulin et al. 1992; Vandervoort et al. 1990) and in other lower extremity (e.g., Cunningham et al. 1987) and upper extremity (e.g., Bassey and Harries 1993) muscles. Furthermore, aging is associated with changes in the rate of fatigue during prolonged skeletal muscle activity via both central (Yoon et al. 2008) and peripheral (Baudry et al. 2007) mechanisms. In light of the impact of neuromuscular deficits on mobility, morbidity, and loss of independence in old adults, it is critical to gain some understanding of the mechanisms that contribute to these age-associated changes in neuromuscular output.

Age-associated reductions in twitch amplitude, twitch contraction, and relaxation times (Vandervoort and McComas 1986), twitch potentiation (Klein et al. 2002; Vandervoort and McComas 1986), muscle fiber number (Lexell et al. 1983, 1988), and an increased proportion of type 1 muscle fibers in some (Jakobsson et al. 1988; Larsson 1983) but not all (Grimby et al. 1982; Lexell et al. 1988) studies indicate that impaired neuromuscular function is due in part to changes within the muscle itself. However, these changes may be preceded or accompanied by age-induced changes in the motoneurons that innervate muscle such that impaired neuromuscular function is secondary to, or at least exacerbated by, the effects of aging on the motoneuron. It has been suggested that aging results in a selective loss of the high-threshold motoneurons that innervate fast twitch (type II) muscle fibers, followed by reinnervation of these muscle fibers by low-threshold motoneurons and subsequent conversion of reinnervated muscle fibers from a fast (type II) to a slow (type I) phenotype (Kanda and Hashizume 1989). However, it is also possible that aging alters the basic and rhythmic firing properties of motoneurons thereby impairing force production via suboptimal neural activation.

In other regions of the CNS, neuronal function appears to be compromised by cumulative damage from reactive oxygen species (Butterfield et al. 2001; Martin and Grotewiel 2006). This is the basis of the “free radical theory” of aging (Beckman and Ames 1998; Colavitti and Finkel 2005). According to this theory, a lifelong calorie-restricted diet should limit cellular damage by reactive oxygen species, delay age-related functional deficits, and even prolong life. In the CNS, caloric-restriction reduces an age-associated loss of spinal motoneurons (Kanda 2002), increases dopaminergic transmission (Diao et al. 1997), and offsets age-related declines in hippocampal function, memory, and spatial learning (Adams et al. 2008; Eckles-Smith et al. 2000; Fontan-Lozano et al. 2007; Martin and Grotewiel 2006; Okada et al. 2003). Therefore the objective of this study was to characterize the effects of aging on the biophysical properties of aged rat lumbar motoneurons after a lifetime ad libitum or caloric-restricted diet. We hypothesized that aging would alter the functional properties of motoneurons and that caloric restriction would offset these changes.

METHODS

Animal care and dietary regimen

Thirty female Fischer 355/Brown Norway (FBNF1) rats were obtained from the National Institute on Aging (NIA) aging colony at an age of 24 wk of age (oldAL, 30.8 ± 1.3 mo) or on a fortified caloric-restricted diet from 14 wk of age (oldCR, 31.0 ± 1.8 mo). Basic and rhythmic firing properties recorded from young FBN controls (young, 8.4 ± 4.6 mo). For high- and low-threshold MNs, was lower in the old group, we conclude that aging alters the biophysical properties of MNs in a fashion that cannot be simply attributed to a loss of high-threshold MNs. Surprisingly, caloric restriction, which is known to attenuate aging-associated changes in hindlimb muscles, had no effect on the progress of aging in the innervating MNs.

INTRODUCTION

As early as the seventh decade (60–70 yr of age) of life, isometric tests of maximal voluntary knee extensor torque in men reveal a loss of muscle strength ranging from 25% (Larsson and Karlsson 1978) to 49% (Young et al. 1985). Similar losses have been reported in isokinetic protocols (Poulin et al. 1992; Vandervoort et al. 1990) and in other lower extremity (e.g., Cunningham et al. 1987) and upper extremity (e.g., Bassey and Harries 1993) muscles. Furthermore, aging is associated with changes in the rate of fatigue during prolonged skeletal muscle activity via both central (Yoon et al. 2008) and peripheral (Baudry et al. 2007) mechanisms. In light of the impact of neuromuscular deficits on mobility, morbidity, and loss of independence in old adults, it is critical to gain some understanding of the mechanisms that contribute to these age-associated changes in neuromuscular output.

Age-associated reductions in twitch amplitude, twitch contraction, and relaxation times (Vandervoort and McComas 1986), twitch potentiation (Klein et al. 2002; Vandervoort and McComas 1986), muscle fiber number (Lexell et al. 1983, 1988), and an increased proportion of type 1 muscle fibers in some (Jakobsson et al. 1988; Larsson 1983) but not all (Grimby et al. 1982; Lexell et al. 1988) studies indicate that impaired neuromuscular function is due in part to changes within the muscle itself. However, these changes may be preceded or accompanied by age-induced changes in the motoneurons that innervate muscle such that impaired neuromuscular function is secondary to, or at least exacerbated by, the effects of aging on the motoneuron. It has been suggested that aging results in a selective loss of the high-threshold motoneurons that innervate fast twitch (type II) muscle fibers, followed by reinnervation of these muscle fibers by low-threshold motoneurons and subsequent conversion of reinnervated muscle fibers from a fast (type II) to a slow (type I) phenotype (Kanda and Hashizume 1989). However, it is also possible that aging alters the basic and rhythmic firing properties of motoneurons thereby impairing force production via suboptimal neural activation.

In other regions of the CNS, neuronal function appears to be compromised by cumulative damage from reactive oxygen species (Butterfield et al. 2001; Martin and Grotewiel 2006). This is the basis of the “free radical theory” of aging (Beckman and Ames 1998; Colavitti and Finkel 2005). According to this theory, a lifelong calorie-restricted diet should limit cellular damage by reactive oxygen species, delay age-related functional deficits, and even prolong life. In the CNS, caloric-restriction reduces an age-associated loss of spinal motoneurons (Kanda 2002), increases dopaminergic transmission (Diao et al. 1997), and offsets age-related declines in hippocampal function, memory, and spatial learning (Adams et al. 2008; Eckles-Smith et al. 2000; Fontan-Lozano et al. 2007; Martin and Grotewiel 2006; Okada et al. 2003). Therefore the objective of this study was to characterize the effects of aging on the biophysical properties of aged rat lumbar motoneurons after a lifetime ad libitum or caloric-restricted diet. We hypothesized that aging would alter the functional properties of motoneurons and that caloric restriction would offset these changes.

METHODS

Animal care and dietary regimen

Thirty female Fischer 355/Brown Norway (FBNF1) rats were obtained from the National Institute on Aging (NIA) aging colony at an age of 24
mo (Harlan Sprague Dawley, Indianapolis, IN). Of these animals, 15 were fed ad libitum (oldAL) with NIA-31 rat chow, and 15 were maintained on a calorie-restricted diet of National Institutes of Health-31/NIA-fortified rat chow beginning at 14 wk of age (oldCR). Caloric intake of the oldCR animals was decreased in a stepwise fashion over the first 3 wk to reach a daily intake of 60% of oldAL cohort. On arrival at our animal facility, oldAL animals continued to feed ad libitum, whereas oldCR animals were maintained on a static restricted diet of 15 g of National Institutes of Health-31 fortified rat chow per day according to previously published protocols of dietary restriction for the same breed of rat (Turturro et al. 1999). Animals were housed individually in standard plastic cages in an environmentally controlled room maintained at 23°C with a 12:12 h light:dark cycle. Pelleted food was placed in dishes within the cage rather than in overhead food hoppers, and water was provided ad libitum for both groups of animals. Twelve young (8.4 ± 4.6 mo) FBNF1 animals that were fed ad libitum were used as controls (young). OldAL and oldCR animals were maintained in our own facility prior to electrophysiological experimentation at ages ranging from 28 to 31 mo (oldAL), 28 to 31 mo (oldCR), and 4 to 14 mo (young). Animal characteristics are shown in Table 1.

Surgical preparation for electrophysiological recordings

ANESTHESIA. Rats were anesthetized with ketamine/xylazine (65 mg/kg ketamine, 6.5 mg/kg xylazine), resulting in a surgical plane of anesthesia that was verified by continuous monitoring of heart rate, expired CO₂, the absence of hindlimb withdrawal when testing bilateral pedal reflexes as well as mean arterial pressure once the femoral artery catheter was in place. Anesthesia was maintained via arterial infusion of ketamine/xylazine (7 mg/ml ketamine, 0.7 mg/ml xylazine) in a 5% dextrose 0.9% sodium chloride saline vehicle (Baxter, Mississauga, ON, Canada) at a rate of 0.7–0.9 ml/h through a catheter in the femoral artery. We used this anesthetic because we have previously shown that, unlike pentobarbital, ketamine-xylazine anesthesia does not affect motoneuronal persistent inward currents (PIC) or frequency-current (f-I) slopes when compared with motoneurons from decerebrate (unanesthetized) animals (Button et al. 2006).

SURGICAL PROCEDURES. Following the initial anesthetization, an atropine-dextrose solution was administered (50 μg/kg atropine in 5% dextrose and 0.9% sodium chloride solution ip) to minimize airway secretions and the following surgical procedures were performed prior to moving the animal to a stereotaxic unit: 1) tracheostomy for ventilation with oxygen-enriched and humidified room air (Harvard Apparatus, St. Laurent, QC, Canada), 2) femoral artery catheterization for continuous monitoring of mean arterial pressure and continuous infusion of anesthetic (Pump 11, Harvard Apparatus), 3) exposure of the left hindlimb sciatic nerve for electrical stimulation, 4) exposure of the thoracic and lumbar vertebrae. The animal was then moved to a stereotaxic unit where the head, thoracic and lumbar vertebrae, hips, and hindlimbs were immobilized with clamps. Oil baths were created around the sciatic nerve and spinal cord. To expose the left side of the cord in preparation for electrophysiological recording, a laminectomy was performed from L₅ to L₆, the dura mater was incised, dorsal roots were cut and reflected over the right cord, and an incision was made in the pia mater from L₅—L₆. A pneumothorax was performed on the left side of the thorax, and 0.2 mg/kg pancuronium bromide was infused via the femoral catheter to induce paralysis of respiratory and hindlimb muscles just prior to electrophysiological recording. Paralysis was maintained throughout the remainder of the experiment using supplemental doses of pancuronium bromide as required. Blood pressure was maintained between 80 and 110 mmHg, and respiration was kept at a tidal volume of 2.0–2.5 ml with a ventilation rate of 60–80 strokes/min. Expired CO₂ levels were maintained between 3.4 and 3.8% (CAPSTAR 100 CO₂ analyzer, CWE, Ardmore, PA). Rectal temperature was monitored and maintained near 37°C using a feedback homeothermic blanket (Harvard Apparatus, Canada) throughout the above procedures and during subsequent electrophysiological recordings.

Electrophysiological recording procedures

Thin-walled 1-mm glass microelectrodes (World Precision Instruments, Sarasota, FL) were pulled to impedances of ~10 MΩ (Kopf vertical pipette puller, David Kopf Instruments, Tujunga, CA) and filled with 2 M potassium citrate. The electrode was positioned above the incision in the pia mater and advanced through the cord in 5- to 10-μm steps (Burleigh Innchworm, Burleigh Products Group, Victor, NY). The sciatic nerve was stimulated (~1 Hz) with a bipolar silver electrode to elicit antidromic field potentials as the microelectrode was advanced through the cord. Field potential amplitude was used to locate regions of the cord that were rich in hindlimb motoneurons. A sudden decrease in membrane potential of ≥60 mV that was accompanied by a spike with a positive overshoot (spike height: >60 mV) and a reproducible latency of <4 ms from the stimulation artifact indicated successful impalement of a motoneuron. Once a motoneuron was successfully impaled, we made the following series of intracellular recordings of basic (Fig. 1) and rhythmic (Fig. 2) motoneuron properties using an intracellular amplifier system (Axoclamp 2B, Axon Instruments, Molecular Devices, Union City, CA) in either bridge or discontinuous current-clamp mode (DCC, 2–10 kHz).

Basic Properties (Fig. 1). Rheobase, or the minimum current required to elicit an action potential 50% of the time, was recorded in response to 50-ms intracellular current pulses in DCC mode. Spike threshold, or the membrane voltage at which an action potential was elicited, was also determined from these recordings (Fig. 1A). Input resistance was determined in DCC mode by recording the membrane response to an average of ~60 nA hyperpolarizing intracellular current pulses each lasting 150 ms (Fig. 1B). Spike amplitude, afterhyperpolarization amplitude (AHP₅₀₋₇₀), and afterhyperpolarization 1/2 decay time (AHP₅₀₋₇₀; Fig. 1C) were measured from an average of ~40 orthodromic spikes evoked by 0.5-ms supramaximal intracellular current pulses recorded in bridge mode.

Rhythmic Properties (Fig. 2). Minimum and maximum steady-state firing frequencies and the slope of the frequency-current relationship were determined using an incremental series of 500-mas intracellular current pulses at a rate of 1 pulse/1–2 s in DCC mode. Current was increased until a maximal steady-state firing rate was reached at which point steps of decreasing amplitude were administered until the current was subthreshold (Fig. 2A). Steady-state firing frequency elicited by each current was determined from the average instantaneous firing frequency of the last three spikes evoked at each current step. Linear regression was used to derive f-1slope from plots of state-firing frequency as a function of current. Spike frequency adaptation, or the decline in spike frequency during prolonged rhythmic firing at a constant

| TABLE 1. Characteristics of oldAL, oldCR, and young animals |
|-----------------|-----------------|-----------------|
|                  | oldAL (n = 9)   | oldCR (n = 11)  | young (n = 11) |
| Age (mo)         | 30.8 ± 1.3*     | 31.0 ± 1.04*    | 8.4 ± 4.6      |
| Diet             | Ad libitum      | Caloric-restricted | Ad libitum  |
| Body weight (g)  | 346.9 ± 46.8**  | 262.7 ± 14.4    | 233.3 ± 31.6  |
| Body weight:mass | 54.6 ± 5.7**    | 45.1 ± 12.1*    | 38.7 ± 2.9     |
| Number of cells recorded | 29 | 32 | 46 |

Body weight:mass ratio is derived from wet muscle weights of red and white vastus lateralis and rectus femoris of the left and right hindlimb as well as tibialis anterior, soleus, plantaris, medial and lateral gastrocnemius of the left hindlimb. The index was calculated as body weight in grams/combined weight of muscles in grams. One-way ANOVA was used to test for a main effect of group and Tukey post hoc analysis was used to test differences between group means. Statistical significance (P < 0.05) is denoted by * (different from young control) and ** (different from rats on a calorie-restricted diet from 14 wk of age (oldCR) and young controls). Data are presented as means ± SD. oldAL, rats fed ad libitum.
current, was assessed during a 30-s intracellular current pulse at 1.5 nA above rhythmic firing threshold in DCC mode (Fig. 2B). The decline in the number of spikes from the first 5-s bin of firing (0–5 s) to the fifth 5-s bin of firing (20–25 s) was used as to create an index of spike frequency adaptation (Button et al. 2007). Finally, persistent inward current amplitude (PIC) was estimated from the response of the motoneuron to a 10-s ramped intracellular current in DCC mode. The peak amplitude of the current was set to evoke between 10 and 75 spikes within a period of 0.5–2.5 s at the peak of the ramp. As described in greater detail previously (Bennett et al. 2001; Button et al. 2006; Lee and Heckman 1998), PIC amplitude was estimated by subtracting the current at which spikes were recruited from the amplitude at which spikes were derecruited (Fig. 2C). For each motoneuron, instantaneous firing frequency was plotted as a function of the current to characterize the f–I relationship according to the voltage threshold and resting membrane potential measurements within each animal. An example of intra-animal variability for rheobase current is shown in Fig. 3. The intra-animal variability for rheobase current in the oldCR was comparable to the oldAL group. Thus the differences in the motoneuron properties reported here were not due to inclusion of atypical experiments with higher yields of data.

**Statistical analyses**

One-way ANOVA was use to test for a main effect of group (oldAL, oldCR, young), and Tukey post hoc analysis was used to test for differences between means. Because ANOVA revealed no differences between oldAL and oldCR, these two groups of old animals were pooled. Independent t-test were then used to test for differences between young and old MNs. Linear regression was used to derive the slope of the f–I relationship and the $I_{\text{rheo}}$–input conductance relationship. $\chi^2$ analysis was used to determine whether significant differences were present in MN f–I relationship type determined by ramp current injections between old and young MNs. Where applicable, data are presented as mean ± SD. In all cases, $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using Statistica version 8 (Statsoft, Tulsa, OK).

**RESULTS**

Overall, we recorded the basic and active properties of 46 motoneurons from 11 young rats, 32 motoneurons from 11 oldCR rats, and 29 motoneurons from 9 oldAL rats. Typically, each rat yielded two to six motoneurons with acceptable data (see METHODS). There was high variability in motoneuron property measurements within each animal. An example of intra-animal variability for rheobase current is shown in Fig. 3. The intra-animal variability for rheobase current in the oldCR was comparable to the oldAL group. Thus the differences in the motoneuron properties reported here were not due to inclusion of atypical experiments with higher yields of data.
ANIMAL CHARACTERISTICS. As expected, oldCR animals had significantly lower body weights and had lower body weight to muscle mass ratios (Table 1). Muscle mass in this study is a rough estimate derived from the wet muscle weight of 11

Fig. 2. Measurement of rhythmic motoneuron properties. The frequency-current relationship was determined via an incremental series of 500-ms intracellular current pulses (A). Steady-state firing frequencies shown were determined from the average instantaneous firing frequency of the last 3 spikes evoked at each current step. Minimum and maximum steady-state firing and the slope of the f-I curve were made from these recordings as described in METHODS. Spike frequency adaptation, or the decline in firing frequency that occurs during prolonged rhythmic firing (B), was assessed using a 30-s intracellular constant-current injection (current channel not shown). Inset: the 1st and last second of firing are shown with expanded x-axes to illustrate the change in firing frequency. The number of spikes discharged in 1-s bins were used to calculate an index of spike frequency adaptation (Fig. 8) as described in METHODS. Persistent inward current was estimated by injecting a ramped intracellular current (C). Persistent inward current was calculated as current at spike recruitment-current at spike derecruitment) as illustrated both C and the inset. All of the preceding recordings were made in discontinuous current-clamp mode from an old motoneuron.
hindlimb muscles per animal dissected immediately after the experiment. These muscles included red and white vastus lateralis and rectus femoris of the left and right hindlimb as well as tibialis anterior, soleus, plantaris, medial and lateral gastrocnemius of the left hindlimb. As expected from previous reports, caloric restriction helped prevent the increase in body mass to muscle mass ratio that normally occurs in free-eating rats as they age.

BASIC MOTONEURON PROPERTIES. Caloric-restriction did not have an effect on any motoneuron properties. Results of the \( t \)-test (all old vs. young) are presented in all figures. Old motoneurons had 28.7% lower rheobase, 49.7% higher input resistance, 104% larger AHP\textsubscript{amp}, and 21.1% longer AHP\textsubscript{decay} compared with young motoneurons (Fig. 4) Old motoneurons also had longer latencies (71%) from the stimulus artifact to the initiation of the antidromic action potential, suggesting increased axon conduction velocities.

Rheobase was significantly correlated with input conductance when the data were fit with a linear equation in both the young \( (r = 0.53, P < 0.0001) \) and old \( (r = 0.62, P < 0.0001) \) motoneurons (Fig. 5). Although the old motoneurons had a greater range of input conductance, the regression line of the old motoneurons fell within the 95% confidence band of the young motoneurons. Thus aging did not significantly affect the relationship between rheobase and input conductance that is seen in young adult rats. The distributions of input resistance and AHP\textsubscript{decay} were more dispersed and included values suggestive of a population of old motoneurons exhibiting lower current thresholds than seen in young motoneurons (Fig. 6). Basic property coefficients of variation in this study were higher for old compared with young motoneurons (Table 2). There were no effects of age on resting membrane potential \( \text{old} = -68.5 \pm 7.0 \text{mV}, \text{young} = -70.2 \pm 6.1 \text{mV} \), voltage threshold \( \text{old} = -46.1 \pm 7.2, \text{young} = -47.7 \pm 7.2 \), or spike amplitude \( \text{old} = 72.9 \pm 11.6, \text{young} = 70.3 \pm 24.0 \).

RHYTHMIC FIRING PROPERTIES OF THE MOTONEURON. The incremental series of 500-ms intracellular current pulses revealed that old motoneurons had 30.2% lower minimum steady-state firing frequencies, 16.7% lower maximal steady-state firing frequencies, and 35.5% lower \( f_{\text{slopes}} \) compared with young motoneurons (Fig. 7).

Both old and young motoneurons displayed spike frequency adaptation during the sustained 30-s intracellular current pulse (Fig. 8A). Old motoneurons demonstrated 21.1% less spike fre-
Four types of frequency-current relationships were seen. Briefly, types 1 and 2 MN frequency-current relationships demonstrated a firing frequency slope that overlaps on the ascending and descending portions of the ramp current or a clockwise hysteresis (i.e., MN firing rate adaptation) where the firing frequencies were greater during the ascending versus the descending portion of the ramp at any given current, respectively. Types 3 and 4 MN f-I relationships demonstrated a linear regression line with some self-sustained firing (i.e., activation of PIC) and a counter-clockwise hysteresis or an acceleration of firing just after spike recruitment and below the linear regression line (i.e., activation of PIC), respectively. A χ² analysis revealed that there was a significant difference in the distribution of frequency-current relationship types between young and old motoneurons. Only 31.2% of young motoneurons demonstrated persistent inward current, whereas the incidence of persistent inward current increased to 78.2% in old motoneurons (Fig. 9).

**DISCUSSION**

This study is the first to document the effects of aging on the biophysical properties of rat hindlimb motoneurons. It has been proposed that aging results in a selective loss of high-threshold “fast-type” motoneurons, followed by the reinnervation of orphaned fast twitch muscle fibers by surviving low-threshold “slow-type” motoneurons, and subsequent fast to slow muscle fiber-type conversion (Kanda and Hashizume 1989). Consistent with this hypothesis is a decrease in total muscle fiber number and the presence of atrophic or angulated fibers dispersed throughout muscle (denervation), fiber-type grouping (reinnervation), and an increased proportion of slow muscle fibers (fiber-type conversion) (Kanda and Hashizume 1989).

**FIG. 5.** The relationship between rheobase and input conductance was not affected by age. Rheobase was plotted as a function of input conductance for both young motoneurons and old motoneurons (A). Each plot was fit with a linear equation shown with 95% confidence bands (B).

**FIG. 6.** Aging increased the range of input resistance and AHP 1/2 decay time. The distributions of input resistance (A) and AHP 1/2 decay time (B) of old motoneurons extended beyond the range of young motoneurons toward values consistent with smaller and slower motoneurons. When input resistance is plotted against AHP 1/2 decay time for young (C) and old (D) motoneurons, it is evident that these properties are more heterogeneous in the old motoneuron pool.
portion of lower-threshold (smaller and slower) motoneurons. This is, in fact, consistent with our observations of lower rheobase, higher input resistance, larger and longer AHPs, and less spike frequency adaptation in old motoneurons. However, a closer look at the data indicates that other factors must contribute to the differences in biophysical properties between old and young motoneurons. For properties of rheobase, input resistance, AHP_{amp} and AHP_{decay} coefficients of variation were consistently higher for old motoneurons when compared with young motoneurons from this study, and, with the exception of AHP amplitude, when compared with young motoneurons from our previous studies (Table 2). The relatively larger coefficient of variation for AHP amplitude in our previous studies combined, compared with the young sample in the present data set, may be related to the more selective criteria for keeping data in the present study (cells had to be capable of rhythmic firing, and have resting potentials \textless{} 60 mV), which may have allowed for a wider range of AHP amplitudes in previous studies. In addition, we found that the ranges of input resistance and AHP_{decay} in old motoneurons extend beyond the ranges seen in young motoneurons toward values expected of smaller (higher input resistance) and slower (longer AHP)

That tetanic tension is greater in slow motor units and lower in fast motor units in old rats when compared with middle-aged rats has been cited as further evidence of the acquisition of extra muscle fibers denervated by high-threshold motoneuron death (Kanda and Hashizume 1989; Kanda et al. 1986). This hypothesis would predict a decrease in the diversity of basic and rhythmic properties that vary somewhat systematically across the spectrum of low- to high-threshold motoneurons. However, our data suggest that aging does not cause a simple shift from one motoneuron type to another. Instead it causes diverse changes in the biophysical properties of motoneurons. Specifically, old motoneurons have lower current thresholds (i.e., lower rheobase and higher input resistances) compared with young motoneurons. Old motoneurons also have changed rhythmic firing properties which include; lower \( f_{\text{f-slope}^-} \), less spike frequency adaptation, and a higher incidence of PICs. These alterations in old motoneuron rhythmic firing properties may be due to voltage-dependent differences in active conductances. Surprisingly, there was no effect of caloric restriction on any of the basic or rhythmic properties that we assessed.

Morales et al. (1987) report a 40% increase in input resistance and a 20% decrease in rheobase in motoneurons of old cats compared with young controls, changes that are nearly identical in magnitude and direction to the changes in rheobase and input resistance that we report in old rat motoneurons. Morales et al. (1987) concluded that if rheobase declined because of an age-associated increase in membrane excitability, then the decline should have been greater relative to the change observed in input resistance. On this basis, Morales argued that the reduction in rheobase does not reflect an increase in motoneuron excitability but is more likely the net effect of changes in multiple factors, such as total membrane area and membrane resistivity, that may occur in opposing directions. We demonstrate that the relationship between rheobase and input conductance is virtually superimposed for young and old motoneurons using a linear plot. From this, we conclude that old motoneurons do indeed have lower current thresholds when activated with a brief depolarizing current and that this increase is consistent with their smaller size as estimated by input conductance.

If old motoneurons have lower current thresholds than young motoneurons, could this be due to a selective loss of high-threshold and/or shrinkage of existing low-threshold motoneurons? At the surface, much of our data appears to support this notion. If there was a selective loss of high-threshold motoneurons, we might expect to sample from a greater proportion of lower-threshold (smaller and slower) motoneurons. This is, in fact, consistent with our observations of lower rheobase, higher input resistance, larger and longer AHPs, and less spike frequency adaptation in old motoneurons. However, a closer look at the data indicates that other factors must contribute to the differences in biophysical properties between old and young motoneurons. For properties of rheobase, input resistance, AHP_{amp} and AHP_{decay} coefficients of variation were consistently higher for old motoneurons when compared with young motoneurons from this study, and, with the exception of AHP amplitude, when compared with young motoneurons from our previous studies (Table 2). The relatively larger coefficient of variation for AHP amplitude in our previous studies combined, compared with the young sample in the present data set, may be related to the more selective criteria for keeping data in the present study (cells had to be capable of rhythmic firing, and have resting potentials \textless{} 60 mV), which may have allowed for a wider range of AHP amplitudes in previous studies. In addition, we found that the ranges of input resistance and AHP_{decay} in old motoneurons extend beyond the ranges seen in young motoneurons toward values expected of smaller (higher input resistance) and slower (longer AHP)

### Table 2. Coefficients of variation

<table>
<thead>
<tr>
<th>Age</th>
<th>Previous Reports, %</th>
<th>Young, %</th>
<th>Old, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 mo</td>
<td>4–14 mo</td>
<td>28–31 mo</td>
</tr>
<tr>
<td>Rheobase (nA)</td>
<td>40.2</td>
<td>44.2</td>
<td>48.4</td>
</tr>
<tr>
<td>Input resistance (Ω)</td>
<td>46.2</td>
<td>45.2</td>
<td>54.2</td>
</tr>
<tr>
<td>AHP amplitude (mV)</td>
<td>68.5</td>
<td>46.1</td>
<td>60.5</td>
</tr>
<tr>
<td>AHP half-decay time (ms)</td>
<td>25.2</td>
<td>32.5</td>
<td>50.5</td>
</tr>
</tbody>
</table>

Previous reports include control group data from Button et al. (2006–2008), recorded from Sprague-Dawley rats. In all cases except for AHP amplitude, coefficients of variation ((SD/mean) \times 100) were similar in comparing previous control motoneuron data with those for the young group in this study and were highest in the old group.

FIG. 7. Minimum and maximum steady-state firing frequencies and \( f_{\text{f-slope}^-} \) are lower in old animals. Minimum and maximum steady-state firing frequencies are plotted for young and old (A). Vertical and horizontal bars represent \( \pm SD \) for firing frequencies and current, respectively. The slope drawn in A is drawn from minimum to maximum for clarity. The more accurate \( f_{\text{f-slope}^-} \) data derived from linear regression of the 500-ms pulse series is shown below (B). Data are plotted as means \( \pm SD \). *, a significant difference between young and old as detected by independent \( t \)-test.
cells. This is best illustrated in Fig. 6. Thus rather than seeing an increase in the homogeneity of motoneurons with respect to these two properties, we see greater diversity. It is worth noting that, in our previous published reports of young adult (Sprague-Dawley) rat motoneuron properties (n = 100), motoneurons seldom had input resistances >3 MΩ (10%) and even less had resistances >4 MΩ (4%), similar to the young sample in this study, while these proportions were increased in the old motoneurons in this study (13 and 22%, respectively). Perhaps the best evidence that age-associated differences in motoneuronal properties are not due solely to a selective loss of high-threshold or shrinkage of low-threshold motoneurons, is that the motoneuron f-I slope is lower in the old motoneurons. This age-related decline in motoneuronal gain cannot be explained by a shift in motoneuron type because f-I slope does not differ between fast and slow rat motoneurons (Cormery et al. 2005).

One possible explanation for the differences between old and young motoneurons is a decline in descending neuromodulatory input to the motoneuron with age. Degenerative changes in descending serotonergic pathways of aged rats are most pronounced in the lumbosacral cord where these tracts contact lower limb motoneurons (Johnson et al. 1993). Studies of bladder function have also revealed similar declines in monoaminergic input to the autonomic and somatic nuclei of the lumbosacral spinal cord (Ranson et al. 2003). Following spinal cord transection (ablation of descending input to motoneuron) and spinal cord isolation (ablation of descending, ascending, and afferent input) motoneurons have lower rheobase, higher input resistance, larger AHPs, higher incidence of PIC, slower minimum and maximum steady-state firing frequencies, and lower f-I slope (Button et al. 2008), similar findings to those of the present study. An age-associated reduction in serotonergic input to the motoneuron pool is consistent with the reduction in f-I slope that we observed in old motoneurons and complements our finding of an increased incidence of persistent inward current in old motoneurons. Bennett’s group has shown on many occasions that chronic spinal cord transected motoneurons have distinct differences in their ability to activate PIC channels and show larger PICs (Bennett et al. 2001; Harvey et al. 2006c; Li et al. 2007) possibly due to an increase in 5-HT and NA receptor sensitivity to residual endogenous monoamines (Harvey et al. 20061,b). It may be that there are changes in the expression of 5-HT receptor subtypes, in the downstream signaling actions of 5-HT receptors, or in the calcium and sodium channels that contribute to PIC. In any event, age-related changes in neuromodulatory input to the

FIG. 8. Old motoneurons exhibit less spike frequency adaptation (SFA). SFA is shown as the number of spikes discharged in 1-s bins over 30 bins. To derive these frequencies, the number of spikes over 5 in the last bin was subtracted from all other bins—all firing therefore terminated at 5 in the final bin (A). Overall young motoneurons have different patterns of SFA compared with old motoneurons. Because more spikes were discharged in the first few seconds in young motoneurons than old motoneurons, the spike frequency adaptation index (1-bin5/bin1) (Button et al. 2007) was significantly lower (i.e., less adaptation) in old motoneurons (B). Data are plotted as means ± SD. *, a significant difference between young and old SFA ratio 1-bin5/bin1.
motoneuron do not offer a simple, stand-alone explanation for the effects of aging observed in the present study.

Age-related changes in neurons in some parts of the brain are associated with modifications in ion channel expression. For example, age-dependent changes in the distribution of voltage-gated sodium channel (subunits Nav1.1 and Nav1.2) (Chung et al. 2003), potassium channel (subunits Kv1.1 and Kv1.2) (Chung et al. 2001a), and the α1D subunit of L-type voltage-gated calcium channels (Chung et al. 2001b) are observed in the rat cerebellum. Modeling suggests that alterations in the expression of fast sodium channels and delayed rectifier potassium channels may contribute to alterations in the biophysical properties of motoneurons following increases and decreases in physical activity (Gardiner et al. 2006). Thus some of the changes in basic and rhythmic firing properties seen in old motoneurons may be due to age-related changes in ion channel expression.

We saw no effect of caloric-restriction on any of the basic and rhythmic properties that we assessed. The standard caloric-restriction protocol (Turturro et al. 1999) that we used was effective given its effects on body weight and body weight relative to muscle mass compared with the ad libitum animals. We recorded from cells that had a resting membrane potential \( \leq -60 \text{ mV} \) and a spike with a positive overshoot that may have introduced some selection bias, whereby those motoneurons most affected by the detrimental effects of aging were excluded. Caloric restriction appears to enhance antiapoptotic mechanisms in the brains of old Fischer 344 rats (Hiona and Leeuwenburgh 2004); this may offset apoptotic neuronal loss that has been associated with normal brain aging (Morrison and Hof 1997). Furthermore, alternate day food restriction reduces the extent of motoneuron loss in rat lumbar spinal cord (Kanda 2002). Thus it was surprising to find no difference between oldCR and oldAL motoneuron biophysical properties. While we saw no effect of caloric restriction on the functional properties of those motoneurons that survived and from which we were able to record, it is possible that caloric restriction offset an age-associated decline in motoneuron number in the present study. One limitation in this study was the fact that caloric restriction started at 14 wk of age, a time period in which rats are reaching physiological maturation. Perhaps differences in motoneuron biophysical properties may have occurred between the old groups if the old CR rats were caloric restricted following weaning.

In summary, old rat hindlimb motoneurons have lower current thresholds when activated from rest by a single brief depolarizing input but have lower firing frequencies and \( f-I_{\text{slopes}} \) during prolonged depolarizing inputs compared with young controls. An increase in the incidence of persistent inward current may be a compensatory mechanism which counteracts the age-associated decline in firing frequencies and \( f-I_{\text{slopes}} \). The age-associated changes to the basic and rhythmic motoneuron properties suggest that aging results in both size-dependent changes in recruitability and/or voltage-dependent differences in active conductances. Many differences between young and old motoneuron properties are consistent with a selective loss of high-threshold motoneurons. However, the increase in heterogeneity of motoneuron properties and the reduction in the slope of the frequency-current relationship indicate that motoneurons are not merely being lost at one end of the continuum. Rather aging is associated with changes in the biophysical properties and firing behavior of motoneurons that may in turn contribute to age-related changes in muscle output. Unlike the case with skeletal muscle and other tissues, the progress of age-associated changes in hindlimb motoneurons is unaffected by life-long caloric restriction.

FIG. 9. A greater proportion of old motoneurons exhibit persistent inward current compared with young motoneurons. \( \chi^2 \) analysis revealed a significant difference in the distribution of the \( f-I \) relationships among the MN groups. The percentage of old motoneurons that exhibit PIC \((\bullet)\) is greater than the percentage of young motoneurons that exhibit PIC \((\circ)\). Numbers of motoneurons are shown in text over each bar. Motoneurons were considered to demonstrate PIC if their frequency-current relationship during ramp current injection could be categorized as type 3 or type 4 according to Button et al. (2006).

ACKNOWLEDGMENTS

The authors thank Dr. Chris MacDonell for assistance in data analysis and G. s. Detillieux and M. Ellis at the University of Manitoba for technical assistance.

GRANTS

This research was supported by grants from Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Institute for Health Research, and the Canada Research Chairs program. D. C. Button and J. M. Kalmar were funded by NSERC PGS-B and PDF scholarships, respectively.

REFERENCES


