

# Effects of 4-Aminopyridine on Muscle and Motor Unit Force in Canine Motor Neuron Disease

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Hereditary Canine Spinal Muscular Atrophy (HCSMA) is an autosomal dominant disorder of motor neurons that shares features with human motor neuron disease. In animals exhibiting the accelerated phenotype (homozygotes), we demonstrated previously that many motor units exhibit functional deficits that likely reflect underlying deficits in neurotransmission. The drug 4-aminopyridine (4AP) blocks voltage-dependent potassium conductances and is capable of increasing neurotransmission by overcoming axonal conduction block or by increasing transmitter release. In this study, we determined whether and to what extent 4AP could enhance muscle force production in HCSMA. Systemic 4AP (1–2 mg/kg) increased nerve-evoked whole muscle twitch force and electromyograms (EMG) to a greater extent in older homozygous animals than in similarly aged, symptomless HCSMA animals or in one younger homozygous animal. The possibility that this

difference was caused by the presence of failing motor units in the muscles from homozygotes was tested directly by administering 4AP while recording force produced by failing motor units. The results showed that the twitch force and EMG of failing motor units could be significantly increased by 4AP, whereas no effect was observed in a nonfailing motor unit from a symptomless, aged-matched HCSMA animal. The ability of 4AP to increase force in failing units may be related to the extent of failure. Although 4AP increased peak forces during unit tetanic activation, tetanic force failure was not eliminated. These results demonstrate that the force outputs of failing motor units in HCSMA homozygotes can be increased by 4AP. Possible sites of 4AP action are considered.

*Key words:* neuromuscular disease; synaptic transmission; transmitter release; muscle force; potassium channel; calcium influx

Hereditary Canine Spinal Muscular Atrophy (HCSMA) is an autosomal dominant disorder of motor neurons in which affected animals exhibit progressive weakness and eventually become paraparetic (Cork et al., 1979, 1982; Sack et al., 1984). The progression of muscular weakness exhibits a distinct spatiotemporal gradient: proximal and caudal muscles (such as tail muscles) become weak early, and more distally and rostrally located muscles become weak at later stages. The defective gene in HCSMA has not yet been identified. The pathological features of HCSMA include chromatolysis of motor neurons, motor axon terminal degeneration, neuronophagia, denervation atrophy of skeletal muscle, accumulation of neurofilaments in proximal motor axons, and late in the disorder, motor neuron cell death (Cork et al., 1982; Alderson and Cork, 1992). These features are also found in human motor neuron diseases (including amyotrophic lateral sclerosis) (Hirano, 1988, 1991). The similarities between HCSMA and human motor neuron diseases suggest that similar pathological mechanisms may operate.

An important advantage of HCSMA is that the properties of functionally isolated motor units can be studied and related to

pathological features at the cellular level as well as to the clinical status of the animal. By exploiting this access, we recently found a type of motor unit failure in which unit force was not sustained during repetitive activation, a phenomenon we call tetanic failure (Pinter et al., 1995). Tetanic failure was observed in motor units of the medial gastrocnemius (MG) muscle (an ankle extensor) of older homozygote animals that had become extensively weakened by disease progress. A similar phenomenon occurs in human motor neuron disease, where it is observed as decrementing EMG potentials during repetitive activation of motor units (Mulder et al., 1959; Stålberg et al., 1975; Denys and Norris, 1979; Bernstein and Antel, 1981; Maselli et al., 1993). Understanding the underlying mechanisms and finding ways to inhibit tetanic failure or increase unit force are of particular interest, because tetanic failure unquestionably contributes to weakness and weakness is the major problem in motor neuron disease.

We have suggested that defective neuromuscular synaptic transmission or axonal conduction in motor axon terminal arbors underlies tetanic failure in older HCSMA homozygotes (Pinter et al., 1995), and it seems likely that similar defects underlie decrementing motor unit EMG responses in human motor neuron disease (Maselli et al., 1993). We thus reasoned that increasing neuromuscular transmission might improve motor unit performance in older homozygotes and provide a strategy for the treatment of symptoms. The drug 4-aminopyridine (4AP) has been shown to increase the release of acetylcholine (ACh) from motor terminals (Harvey and Marshall, 1977; Lundh and Thesleff, 1977; Molgo et al., 1977; Illes and Thesleff, 1978; Lundh, 1978; Thesleff, 1980; Burley and Jacobs, 1981; Thomsen and Wilson, 1983; Ar-

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gentieri et al., 1992). In addition, 4AP can eliminate axonal conduction failure under some conditions (Bostock et al., 1981; Targ and Kocsis, 1985). We therefore examined whether 4AP could affect muscle and motor unit performance in HCSMA. We found that systemic administration of 4AP increases nerve-evoked whole muscle twitch force and that the effect is greatest for MG muscles in older homozygotes in which motor unit tetanic failure is also observed. Examination of several individual motor units indicated that the 4AP effects are greatest among motor units exhibiting the highest degree of tetanic failure.

## MATERIALS AND METHODS

All animals used in this study were obtained from the HCSMA breeding colony maintained at Stanford University. HCSMA animals presumed to be homozygous were identified by the appearance of weakness in tail muscles beginning about 6–8 weeks of age and by the rapid progression of muscular involvement thereafter. At 80–190 d of age, HCSMA heterozygotes cannot be distinguished from normal HCSMA animals, because HCSMA heterozygotes generally do not begin showing symptoms until ~1 year of age. Data from these younger animals are therefore grouped into a “symptomless” category for purposes of comparison with data from homozygotes.

### Experimental procedures

**Surgical preparation.** All dogs were prepared for study in terminal experiments as described in detail by Pinter et al. (1995) and briefly as follows. Each animal was initially anesthetized by intravenous pentobarbital (35 mg/kg) and deeply anesthetized throughout the experiment by supplementary intravenous infusions. Typically, 20–25 mg/hr of intravenous pentobarbital was required to maintain a depth of anesthesia characterized by a complete absence of corneal and paw-pinch withdrawal reflexes. Vital signs were monitored continuously and maintained within these limits: mean arterial blood pressure at 80–100 mmHg, end-tidal CO<sub>2</sub> between 2.8 and 3.5%, and rectal temperature at 37–38°C. The MG muscle and nerve were exposed by dissection of the left hindlimb, and ventral roots containing MG motor axons and spinal segments containing MG motor neurons were exposed by laminectomy of lumbosacral vertebrae (L4–S1).

**Recording procedures.** Forces produced by isometric contractions of the whole MG muscle or of individual MG motor units were recorded by strain gauge transducers connected to conventional amplifiers and attached to the cut distal tendon of the muscle. Additionally, fine stainless steel wires (four pairs distributed across the muscle) were used to record differentially the evoked EMG potentials. When recording from single motor units, we switched between these pairs to locate the largest EMG signal. The digitized records of both force and EMG were stored on computer for later analysis of twitch and EMG amplitudes as described below. Some experiments were devoted to studying twitch contractions and EMG produced by the whole MG muscle in response to supramaximal stimulation of the MG nerve via bipolar hook stimulating electrodes (0.2 msec pulses delivered at 1 Hz). In other studies, single motor units were isolated by impaling antidromically identified MG motor axons in the ventral rootlets (L7 or S1), as described by Cope and Clark (1991). Motor unit mechanical responses were recorded after injection of suprathreshold depolarizing current pulses (0.5 msec duration). Mechanical properties studied included twitch time-to-peak, twitch amplitude, and maximum force amplitude at stimulation frequencies of 50, 100, 150, and 200 Hz.

**4AP protocol.** After data collection from several trials of whole muscle contraction or from several single motor units, 4AP was injected intravenously at doses of 1–2 mg/kg body weight. This dose level was selected on the basis of previous *in vivo* work in adult cat spinal cord that demonstrated the ability of 4AP to increase postsynaptic potential amplitudes recorded in normal motor neurons (Jankowska et al., 1982). For dogs, 4AP was administered over a 1–2 min interval in two equal volumes, each followed by a saline wash. We did not attempt to determine a minimum effective dose; however, on several occasions, motor unit force effects appeared immediately after or during injection of the first half of the total dose, suggesting a minimal effective dose of <1 mg/kg. The injections occurred while unit or whole muscle twitch forces and EMG signals were being recorded and produced at 1 Hz. Subsequently, twitch and EMG peak-to-peak amplitudes were measured and are shown in Results as time series records that include a predose control, injection,

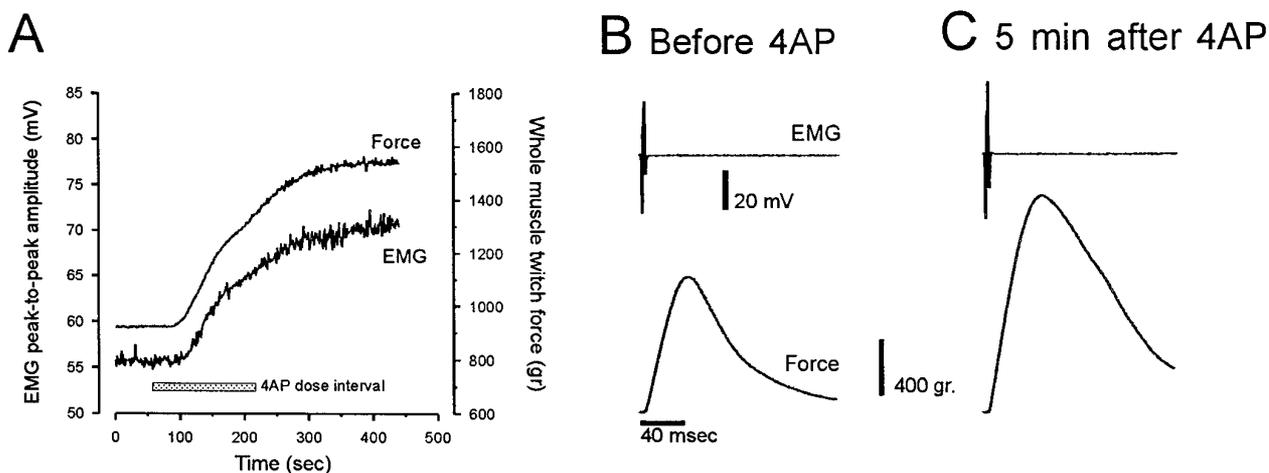
and postdose time intervals. Effects of 4AP on whole muscle or single motor unit force and EMG were generally monitored for 10–15 min after a single dose. To study 4AP effects at the motor unit level, we considered sampling as many motor units as possible after 4AP injections. Although this approach could have produced more unit sampling per animal, variable and uncontrollable delays that inevitably occur during the search for antidromically activated MG motor axons would have introduced a confounding variable, because drug effects diminish with time. Another difficulty with simply sampling motor units after 4AP is that there is no decisive way to distinguish between a normally functioning motor unit and a dysfunctional unit whose capabilities might have been improved by the drug. Because of these concerns, we studied 4AP effects only in individual motor units so that their properties could be directly determined before 4AP and thus serve as control for any possible drug effects. We also restricted sampling to only one motor unit in three of four animals. This was done out of concern for the possibility that longer-term drug effects (Jankowska et al., 1982) might influence results obtained from subsequently tested units. In one older homozygote, a single dose of 4AP was given for each of two units studied, spaced at an interval of 3 hr. By selecting the first unit for the absence of high-frequency failure, we were able to verify that the expected negative result was not attributable to any persistent action of a previous dose (see Results). Selection of a second unit that failed allowed us to determine in the same animal whether a second dose could enhance force production.

In addition to the effects described in Results, 4AP increased mean arterial blood pressure by 10–20 mmHg for ~30 min. In some animals, 4AP also induced spontaneous contractions in the muscle, which interfered with motor unit force recording. In these cases, the effects of 4AP were assessed by examining motor unit EMG records.

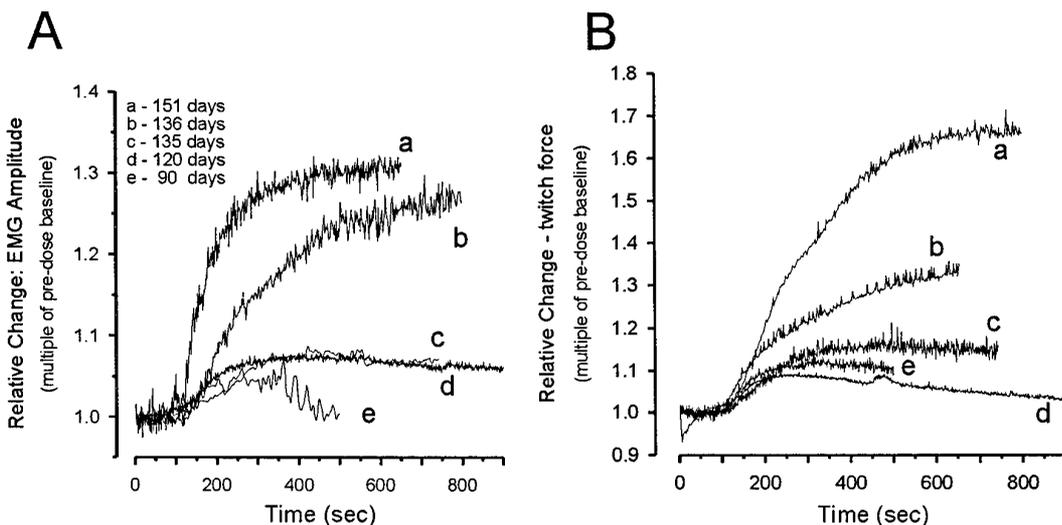
## RESULTS

### Effects of 4AP at the whole muscle level

To obtain an indirect indication of whether 4AP could increase the forces of failing motor units, we first compared the effects of intravenously administered 4AP (1–2 mg/kg) on nerve-evoked whole MG muscle force between older homozygotes and other HCSMA animals in which failing motor units are not found (Pinter et al., 1995). An example obtained from a homozygote aged 151 d that showed extensive weakness is illustrated in Figure 1. Time series plots of whole muscle twitch and EMG peak-to-peak amplitudes (obtained at 1 Hz) before and after a 2 mg/kg 4AP dose (dose interval indicated by shaded region) are shown (Fig 1A). Near the conclusion of the injection, both force and EMG began to increase and reached values ~60% greater than predose baseline levels in ~5 min (B). A comparison of time series records from two symptomless, age-matched HCSMA animals and a younger homozygote showed that 4AP increased whole muscle twitch force or EMG to the greatest extent in older homozygotes in whom weakness was most developed. This may be seen in Figure 2 (A, EMG amplitude time series; B, force amplitude time series); the records showing the largest relative changes were obtained from older homozygotes aged 151 (trace a) and 136 d (trace b). In another extensively weakened older homozygote (186 d) in which we did not obtain the full time course (because of simultaneous motor unit recording; see below), we observed a 67% increase of the whole muscle twitch EMG peak-to-peak amplitude 10 min after 4AP administration. The other time series records shown in Figure 2A,B were obtained from similarly aged but symptomless HCSMA littermates (traces c and d) and from one younger homozygote aged 90 d (trace e) that had not yet developed extensive weakness. In each of these cases, twitch force and EMG amplitudes increase maximally no more than ~5–10% and begin to decline within a few minutes after the onset of the effects. These smaller effects may have been related to increases of blood pressure observed after 4AP, because the time course of the blood pressure changes were similar. A similar absence of 4AP effects was found in the MG muscle of one older



**Figure 1.** Effect of 4AP on whole muscle twitch force and EMG. Data obtained from an HCSMA homozygote aged 151 d. *A*, Time series plots of MG whole muscle maximum twitch force and EMG peak-to-peak amplitudes evoked by electrical stimulation of the MG nerve. 4AP was administered intravenously (2 mg/kg) over the interval indicated by the shaded region, followed by a saline wash. Note that the postdose increase of twitch force is associated with an increase of EMG amplitude. *B*, Single sweep records of whole muscle twitch force and EMG taken before administration of 4AP. *C*, Muscle and EMG records as in *B*, but obtained 5 min after 4AP dose.



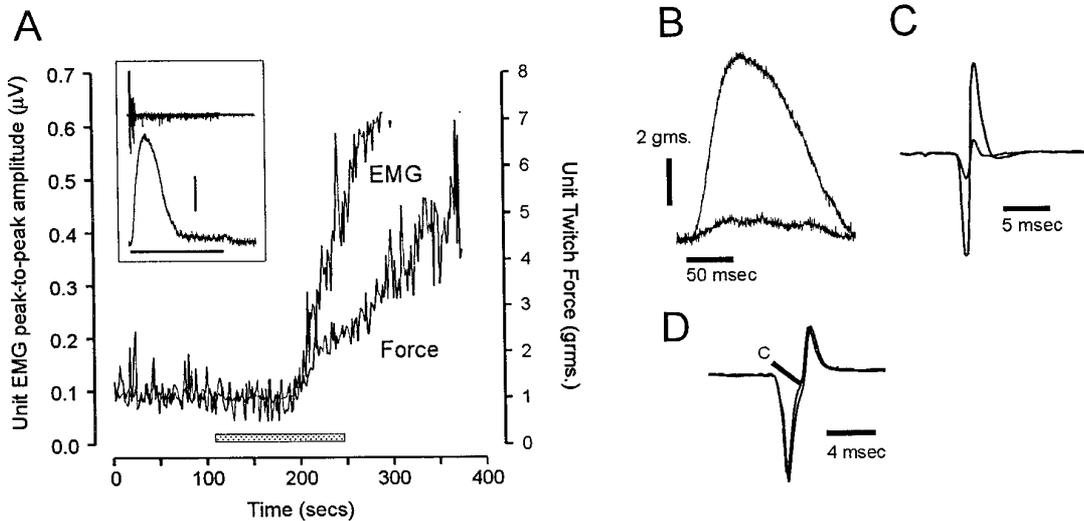
**Figure 2.** Time series plots of whole muscle maximum twitch force and EMG peak-to-peak amplitudes obtained from five HCSMA animals after intravenous 4AP administration. Muscle force and EMG peak-to-peak amplitudes are expressed as multiples of predose averages of at least 25 sweeps. To enable comparison of time series, all records are plotted so that the beginning of 4AP effects are aligned in time. In *A* and *B*, records labeled *a* and *b* were obtained from two older homozygotes aged 151 and 136 d, as indicated by inset in *A*. These animals exhibited extensive weakness. Records labeled *c* and *d* were obtained from two symptomless animals, whereas record *e* was obtained from a younger homozygous individual (aged 90 d) that did not exhibit extensive weakness. Note that 4AP effects on muscle force and EMG are greatest for the two older homozygotes.

(>1 year) normal dog from the HCSMA (data not shown). These results demonstrate that MG muscle twitch force and EMG amplitudes of the most severely affected HCSMA animals (older homozygotes) exhibit the largest postdose increases after intravenous 4AP.

#### Effects of 4AP at the motor unit level

The most likely explanation for the increased 4AP responsiveness of MG muscles from older homozygotes is that 4AP increases force production in failing motor units. To determine this directly, we isolated failing MG motor units in three additional older homozygotes (all aged ~180 d) so that their properties could be examined before and after 4AP administration. In addition, we studied the effects of 4AP in a nonfailing MG motor unit obtained from a symptomless HCSMA animal aged 156 d.

Intravenous 4AP increased twitch forces and/or EMG potentials in all failing units that were studied. The inset of Figure 3*A* shows an example of the tetanic force and EMG profiles (100 Hz) of one of these units and demonstrates that force was nearly absent at the end of the motor axon stimulation train (duration indicated by time calibration bar). After systemic administration of 4AP (2 mg/kg), the unit EMG peak-to-peak and twitch force amplitudes increased with a time course similar to that seen in older homozygote whole MG muscle (Fig. 3*A*). In this case, average twitch and EMG peak-to-peak amplitudes increased more than fivefold after 4AP (Fig. 3*B,C*). Similar results were obtained from two additional failing units from two other older homozygotes. In contrast with the clear 4AP effects on failing motor units from older homozygotes, a nonfailing unit isolated in

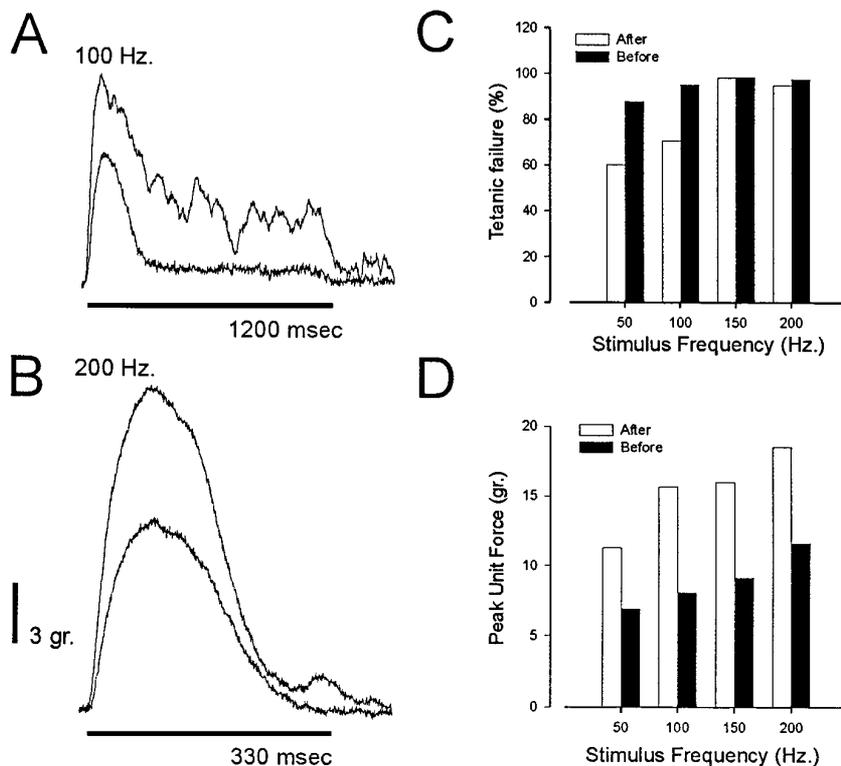


**Figure 3.** Twitch force and EMG amplitudes increase after 4AP in a failing motor unit. *A*, Time series plots of unit maximum twitch force and EMG peak-to-peak amplitude before and after intravenous administration of 2 mg/kg 4AP. Duration of 4AP dose administration is indicated by shaded bar. *Inset*, Demonstration of failure of unit to maintain force at a stimulation rate of 200 Hz. *EMG*, top trace; *Force*, bottom trace. Duration of stimulus train indicated by calibration bar: 620 msec. Vertical calibration: 3 gm. *B*, Averaged records (5 sweeps) of unit twitch before (smaller twitch amplitude) and ~10 min after 4AP dose. *C*, Averaged records (5 sweeps) of unit EMG potentials before (smaller EMG potential) and ~10 min after a single 4AP (1 mg/kg) dose. This unit was obtained from a normal, symptomless HCSMA animal.

one symptomless HCSMA pup (aged 156 d) showed no effect after 4AP. Averaged EMG records obtained from this unit before (labeled *c*) and ~10 min after 4AP administration are shown in Figure 3*D* and demonstrate that no difference was caused by 4AP.

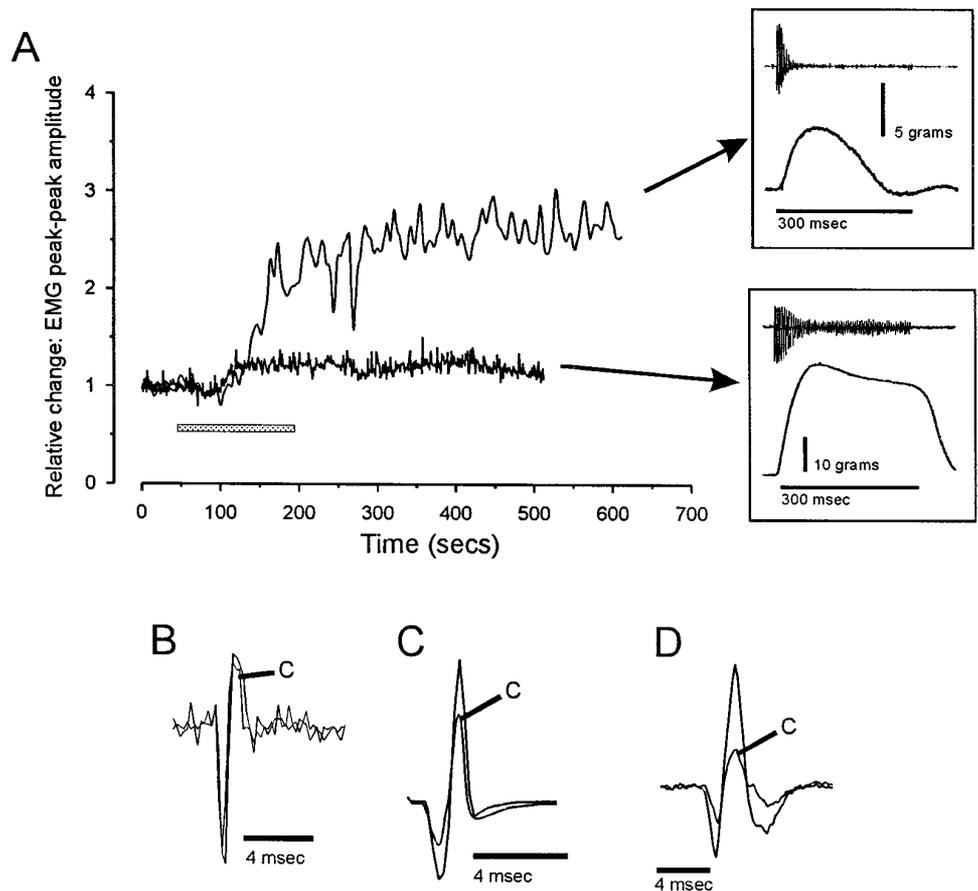
Spontaneous muscle contraction caused by the effects of 4AP on the CNS (Lemeignan, 1973; Jankowska et al., 1982) prevented accurate measurement of the forces produced by single motor units during high frequency stimulation in all but one case. In this

case, 4AP administration decreased tetanic force failure but to a limited extent and only at lower activation frequencies. Comparisons of tetanic force records obtained before and after 4AP (Fig. 4*A*) demonstrate an improvement in the ability of the unit to maintain tetanic force at 100 Hz, although failure was still clearly present. At 200 Hz, no postdose improvement was evident (Fig. 4*B*). Figure 4*C* summarizes the effects of 4AP on tetanic failure at all four tested frequencies for this unit. These data indicate that



**Figure 4.** 4AP effects on motor unit tetanic force production. Data obtained from the unit shown in Figure 3. In *A* and *B*, the smaller force profiles were obtained before 4AP at 100 (*A*) and 200 Hz (*B*), and the larger profiles were obtained ~10 min after 4AP. In each case, peak force production is increased after 4AP, but force failure remains present. *C*, Comparison of motor unit tetanic force failure at indicated frequencies before and after 4AP. Tetanic failure is the difference between peak force and force present at the end of the stimulus train expressed as a percentage of the peak force. Note decrease of failure at lower stimulus frequencies. *D*, Comparison of peak tetanic force before and after 4AP at indicated stimulus frequencies. Maximum unit force production is increased at all tested frequencies. Force calibration in *B* also applies to *A*.

**Figure 5.** 4AP motor unit effects are related to the extent of tetanic failure. Data in *A–D* were obtained from a single HCSMA homozygote aged 188 d that exhibited pronounced weakness. *A*, Time series of motor unit EMG peak-to-peak amplitude before and after 4AP dose (1 mg/kg, administered during interval indicated by shaded bar) for two motor units that differed in the extent of tetanic failure. *Insets* show tetanic force and EMG profiles (200 Hz) for each unit, with duration of stimulus train indicated by time calibration bars. The unit with greater tetanic failure was sampled during a second dose of 4AP given 3 hr after the first dose, which was given while recording from the unit with less failure. Note that the increase of EMG amplitude after 4AP is greater for the unit with greater tetanic failure. *B*, Comparison of average EMG signals (5 sweeps) obtained before (*c*) and 10 min after 4AP for unit with less failure. *C*, EMG signal comparison as in *B* but for whole MG muscle. These data were obtained during sampling from unit with less tetanic failure and demonstrate that 4AP increased whole muscle EMG amplitude. *D*, EMG signal comparison as in *B* but for unit showing more tetanic failure.



4AP can decrease tetanic failure for activation frequencies below 100 Hz but does not eliminate tetanic failure under the present experimental conditions. After 4AP, peak motor unit force production at each tested frequency was increased, as illustrated in Figure 4*D*.

The possible dependence of 4AP action on the extent of motor unit failure was examined in two units obtained from one of the older homozygote animals. In this experiment, a unit that displayed as little tetanic failure as possible was isolated first, as illustrated by the bottom right panel in Figure 5*A*. After 4AP administration, this particular animal developed levels of background motor unit firing in the MG muscle that made it impossible to obtain accurate postdose estimates of unit force; however, unit EMG potentials could still be clearly identified as being linked to the axonal stimulus. As may be seen in the time course plot of Figure 5*A*, the EMG peak-to-peak amplitude for this unit exhibited a postdose increase of ~10%. A comparison of single sweeps of the unit EMG before and ~7 min after 4AP administration (Fig. 5*B*) also showed little effect. One possible explanation for this limited effect is that 4AP did not have access to the MG muscle or simply was without effect for other reasons; however, comparison of whole muscle twitch EMG records obtained before and ~8 min after 4AP administration demonstrated a clear postdose increase of ~70% (Fig. 5*C*). This result is compatible with whole muscle effects in other older homozygotes (Fig. 2) and indicates that the limited effects on this motor unit were not attributable to a generalized failure of 4AP action.

After a delay of ~3 hr to allow some of the effects of the first 4AP dose to diminish, we located a motor unit that displayed greater tetanic force failure; records from this unit are shown in

the top right panel of Figure 5*A*. This unit was more responsive to 4AP, exhibiting a greater than twofold increase in EMG peak-to-peak amplitude (top time course record, Fig. 5*A*). It is possible that the full extent of the response of this unit to 4AP may have been underestimated because of the preceding dose. Comparison of average EMG waveforms (5 sweeps) before and after 4AP (Fig. 5*D*) demonstrates that only peak-to-peak EMG amplitude was affected; no changes in the various times-to-peak or overall potential duration are evident. This indicates that 4AP did not affect muscle fiber electrical properties and that the increase of EMG amplitude occurred as the result of activation of additional muscle fibers. These results suggest that the extent to which 4AP can increase the force production of failing motor units is directly related to the extent of failure that is present before 4AP.

## DISCUSSION

The present results show that 4AP can increase the force production of motor units that exhibit tetanic failure. This effect explains the ability of 4AP to increase nerve-evoked whole MG muscle force in older HCSMA homozygotes, because these are the only members of the pedigree in which failing motor units have been found (Pinter et al., 1995). The relative absence of 4AP effects on whole MG forces of two age-matched symptomless HCSMA animals, a young homozygote, and one normal adult can thus be explained by the absence of failing motor units in these muscles and by a lack of 4AP effects on nonfailing motor units (Fig. 3*D*). These results most likely reflect the ability of 4AP to increase ACh release from the motor terminals of failing motor units and provide further evidence for the existence of defective or insufficient transmitter release.

#### 4AP mechanism and site of action in failing motor units

Because 4AP blocks voltage-sensitive  $K^+$  channels (Choquet and Korn, 1992; Kirsch et al., 1993), our results may reflect drug actions in the axons, synapses, or muscle fibers of failing motor units. Because the effects on twitch records observed in failing motor units and in whole muscle feature simultaneous increases of EMG and force amplitude, it seems likely that 4AP increases the number of muscle fibers activated by single motor axon action potentials rather than increases the force generated by muscle fibers that are already activated by nerve stimulation before 4AP administration. Therefore, our results cannot be explained simply by a direct 4AP effect on muscle contractility (Harvey and Marshall, 1977). Although it is also possible that some muscle fibers in older homozygotes fail to generate action potentials in response to suprathreshold depolarization and that 4AP somehow transiently eliminates this deficit, intracellular recordings from normal muscle fibers in other animals have failed to reveal any direct effects of 4AP on muscle fiber resting potential (Molgo et al., 1977). Moreover, the aminopyridine drugs do not affect the time course of endplate potentials (Molgo et al., 1977; Lundh, 1978) or ACh receptors (Thesleff, 1980). It remains possible that the HCSMA disease process directly affects muscle fiber excitability and that this is somehow associated with an emergent 4AP sensitivity; however, we have not yet detected evidence for direct muscle fiber involvement in HCSMA. Comparisons of motor unit mechanical properties between affected and nonaffected HCSMA individuals show, for example, that the contraction speed of motor units is not influenced by HCSMA (Pinter et al., 1995). Differences that have been observed chiefly involve the amount of motor unit force generated and seem most likely related to increased motor neuronal involvement among affected HCSMA individuals (Pinter et al., 1995).

On the basis of various evidence, it seems more likely that the 4AP effects we observed reflect a presynaptic site of action. One possibility is that by blocking voltage-sensitive potassium channels in axons and increasing the duration of axonal action potentials, 4AP eliminates axonal conduction blockade (Bostock et al., 1981; Targ and Kocsis, 1985; Kocsis, 1986). Evidence of conduction blockade exists for MG motor neurons in HCSMA, and this is most likely located in motor axon terminal arbors (Pinter et al., 1995). This evidence, however, has been observed only in younger homozygotes (<100 d) that possess few if any motor units that exhibit tetanic failure (Pinter et al., 1995). Moreover, there was a comparably small 4AP effect in the single young homozygote (90 d) we tested at the level of the whole muscle. Our results indicate further that 4AP effects on the main axon are unlikely to contribute to increased neurotransmission in older HCSMA homozygotes, because force and EMG were present before 4AP administration in each motor unit studied. This shows that action potentials were propagated along the main axon before 4AP administration and means that if the effect of 4AP is to decrease or eliminate conduction blockade in failing motor units, it would likely occur within intramuscular branches or within the terminal arbors themselves. One condition that might be associated with focal conduction blockade at these sites is demyelination (Bostock et al., 1981). Although a more detailed analysis of the myelination status of intramuscular motor axons in HCSMA is needed, preliminary evidence does not suggest the existence of demyelination

that might give rise to conduction blockade in motor axon terminal arbors (our unpublished observations).

The other way 4AP might act presynaptically in failing motor units is by increasing release of ACh from motor terminals. 4AP and an analog, 3,4-diaminopyridine (3,4 DAP), are known to increase release of transmitter at neuromuscular junctions in normal animals (Harvey and Marshall, 1977; Lundh and Thesleff, 1977; Molgo et al., 1977; Illes and Thesleff, 1978; Lundh, 1978; Thesleff, 1980; Thomsen and Wilson, 1983), at regenerating junctions (Argentieri et al., 1992), and in other neuromuscular disorders (Lundh et al., 1977; McEvoy et al., 1989). This effect is related to an increased duration of the presynaptic action potential, which increases the duration of motor terminal calcium currents (Thesleff, 1980; Thomsen and Wilson, 1983; Mallart, 1985; Wheeler et al., 1996), or a direct effect on  $Ca^{2+}$  transport (Illes and Thesleff, 1978). In this respect, it is of interest to note how closely the effects of 4AP on force production in failing motor units resemble the effects of a single tetanic stimulus (Pinter et al., 1995), because tetanic stimuli are also thought to increase levels of motor terminal  $Ca^{2+}$  and perhaps levels of  $Ca^{2+}$ -bound molecules involved in the release process (Swandulla et al., 1991; Delaney and Tank, 1994). Both manipulations increase twitch force and EMG simultaneously and increase peak force reached during tetani, but have limited effects on the force failure that occurs during the tetani (compare Fig. 4 with Fig. 5) (Pinter et al., 1995). Moreover, our present data suggest that the extent to which 4AP increases force production may be directly related to the extent of failure; this relationship exists between tetanic failure and potentiation in failing motor units (Pinter et al., 1995). To achieve these effects on unit force, both 4AP and tetanic stimuli would need to increase release or activate silent or nonreleasing synapses (Argentieri et al., 1992) or both. Our data thus suggest the hypothesis that a defective synaptic release mechanism underlies tetanic failure and that the defect, in part, involves defective  $Ca^{2+}$  processing. Possible mechanisms include a decrease in the number of motor terminal  $Ca^{2+}$  channels, a decrease in the calcium affinities or concentration of molecules involved in the release process, or perhaps a relative increase in voltage-dependent  $K^+$  currents associated with motor terminal action potentials. The latter possibility might occur if some motor terminals in failing units belong to motor axon terminal sprouts. Because they are usually poorly or lightly myelinated, sprouts may be particularly susceptible to the actions of 4AP, because voltage-dependent K channels are more uniformly distributed (Anguat-Petit and Mallart, 1985) or are relatively more exposed in a manner analogous to regenerating axonal sprouts (Kocsis, 1986; Kocsis and Waxman, 1987) or immature myelinated axons (Eng et al., 1988). We had proposed previously that tetanic failure might be associated with motor axon or terminal sprouting (Pinter et al., 1995); however, recent confocal microscopy observations of MG muscles taken from older homozygotes in which many motor units exhibited tetanic failure have not revealed any terminal or nodal sprouting (unpublished observations). This suggests that neither tetanic failure nor the 4AP effects observed in this study are related to the presence of motor axon terminal sprouting. Further characterization of the mechanisms underlying tetanic failure and how they are affected by 4AP will require *in vitro* study of neuromuscular synaptic function in HCSMA.

### Possible use of aminopyridine drugs in human motor neuron disease

There are at present no effective therapies available for human motor neuron diseases. It is thus useful to consider briefly whether aminopyridine drugs such as 4AP or 3,4 DAP might be useful for symptomatic treatment. A recent paper described a trial using 3,4 DAP in humans with amyotrophic lateral sclerosis and reported some benefits, but it used evaluation methods that are difficult to interpret in terms of motor unit performance (Aisen et al., 1995). Supporting the possibility that 4AP or 3,4 DAP might have some benefit are descriptions of EMG decrementing responses during repetitive motor unit activation in human motor neuron disease (Mulder et al., 1959; Stålberg et al., 1975; Denys and Norris, 1979; Bernstein and Antel, 1981; Maselli et al., 1993), a phenomenon very similar to that seen among failing motor units in HCSMA in which, as we showed above, 4AP can increase force production. We do not know at present whether the mechanisms of motor unit failure in human and HCSMA motor units are the same. Limited available evidence indicates that decreased transmitter release (Maselli et al., 1993) and/or conduction failure in motor terminals (Stålberg and Thiele, 1972) might play a role in the human motor unit failure, in which case the aminopyridine drugs might be useful for enhancing motor unit performance. Any therapeutic benefit, however, will also depend on the number of motor units that are sensitive to 4AP as well as their location. In a disorder that features involvement of limited numbers of motor units at any time, less benefit on overall motor performance would be expected. Greater benefit might be expected with increased numbers of 4AP-sensitive units located in larger muscles that serve as prime movers. It is also important to note that the use of aminopyridine drugs in motor neuron disease might involve risks. If the neurotransmission deficits in human and canine motor neuron disease reflect the occurrence of degenerative changes at the molecular level of motor terminals, it is conceivable that the changes could extend to the terminal calcium buffering capacity (Alexianu et al., 1994). If this capacity is decreased, increasing calcium influx might accelerate degenerative changes. Recent evidence for mediation of postaxotomy axonal degeneration by specific calcium channels (George et al., 1995) argues for caution when considering use of compounds such as the aminopyridines in degenerative disorders. Further work with the HCSMA model will help decide the limits of usefulness of drugs such as 4AP in motor neuron disease.

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