Huntington disease skeletal muscle is hyperexcitable owing to chloride and potassium channel dysfunction

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Huntington disease is a progressive and fatal genetic disorder with debilitating motor and cognitive defects. Chorea, rigidity, dystonia, and muscle weakness are characteristic motor defects of the disease that are commonly attributed to central neurodegeneration. However, no previous study has examined the membrane properties that control contraction in Huntington disease muscle. We show primary defects in ex vivo adult skeletal muscle from the R6/2 transgenic mouse model of Huntington disease. Action potentials in diseased fibers are more easily triggered and prolonged than in fibers from WT littermates. Furthermore, some action potentials in the diseased fibers self-trigger. These defects occur because of decreases in the resting chloride and potassium conductances. Consistent with this, the expression of the muscle chloride channel, ClC-1, in Huntington disease muscle was compromised by improper splicing and a corresponding reduction in total Clcn1 (gene for ClC-1) mRNA. Additionally, the total Kcnj2 (gene for the Kir2.1 potassium channel) mRNA was reduced in disease muscle. The resulting muscle hyperexcitability causes involuntary and prolonged contractions that may contribute to the chorea, rigidity, and dystonia that characterize Huntington disease.

trinucleotide repeat | myotonia | myopathy | channelopathy | electrophysiology

Huntington disease (HD) is a debilitating and progressive disorder that develops in most patients during middle age; the more extreme form of the disease develops in juveniles (1, 2). There is currently no cure for the disease, which is characterized by severe motor and cognitive defects. The motor symptoms include chorea (irregular jerky movements), rigidity or rigor, and dystonia (abnormal tonicity often resulting in abnormal positioning of the head and limbs). These defects are the result of involuntary and prolonged contractions. Examinations of HD skeletal muscle have demonstrated atrophy, metabolic and histological abnormalities, and a loss of strength (3–12). Far more studies of HD have focused on the central nervous system, and the motor defects are widely considered to be the result of neurodegeneration (1, 2, 8). However, no previous study has examined the basic membrane properties in HD muscle that control action potential initiation and propagation. A normal skeletal muscle contraction requires that action potentials initiated at the neuromuscular junction propagate along the surface membrane and into the interior of the muscle fiber through the transverse tubular system. The responsiveness of the muscle to neuronal stimulation and the shape of each action potential repolarization are determined by potassium and chloride conductances, which set and maintain the resting membrane potential of skeletal muscle (13). In effect, the inward rectifying potassium (Kir) and chloride conductances buffer the membrane potential at rest. If they are reduced, the membrane becomes hyperexcitable, and normally subthreshold events can initiate action potentials and involuntary contractions. Most of the resting conductance in skeletal muscle is mediated by chloride through the muscle chloride channel (CIC-1), and a large decrease in this conductance results in the hyperexcitability, involuntary contractions, rigidity, and persistent contractions that characterize general myotonia at the cellular level (14–20).

We examined action potentials, as well as CIC-1 and Kir conductances, in dissociated ex vivo adult skeletal muscle fibers from transgenic HD mice and from age-matched WT littermates. The HD muscle came from the R6/2 transgenic mouse line, which carries the human HD gene (21) and exhibits many of the motor and cognitive defects found in HD patients (2, 22). In humans, HD is caused by an expanded CAG trinucleotide repeat in the huntingtin gene (IT15) (22). Patients with >40 CAG repeats develop the disease during middle age; those with >50 CAG repeats develop a more extreme juvenile form of the disease (1, 2). Because of the rapid onset of reduced activity at 4.5 wk of age (22, 23) and overt motor defects at 8 wk of age (24–26), the R6/2 line most closely models the juvenile form of HD. The R6/2 line is often considered the model of choice for preclinical trials of potential HD therapeutics owing to its rapidly developing and well-described phenotype (1). Last, because an expanded trinucleotide repeat has been shown to disrupt Clcn1 (gene for CIC-1) mRNA processing in myotonic dystrophy type 1 (27–36), we determined whether there was a disruption in Clcn1 mRNA processing in HD muscle. We reveal defects in HD muscle that cause hyperexcitability and may contribute to the severe involuntary and prolonged contractions that are hallmarks of the disease.

Results

We performed all electrophysiological experiments on individual dissociated HD and WT flexor digitorum brevis or interosseus muscle fibers using two intracellular microelectrodes (Fig. S1). The HD mice averaged 12 wk of age, and the WT mice averaged 13 wk of age.

Action Potentials. An action potential is the basis of excitability and the physiological signal that initiates muscle contraction. We measured action potentials in HD (n = 12) and WT fibers (n = 17) by injecting a series of depolarizing current pulses through a current-passing electrode and measuring the membrane potential with a voltage-sensing electrode (Fig. 1). This simulates the physiological condition whereby an inward current through acetylcholine receptors at the neuromuscular junction triggers an action potential. Our series of 0.5-ms current pulses ranged in amplitude from subthreshold to suprathreshold. There was a striking prolongation of the repolarization phase in HD compared with WT fibers (Fig. L4). The slower time constant of the repolarization to the resting membrane potential of HD fibers (15.0 ± 2.1 ms) was nearly double that of WT (7.7 ± 0.4 ms) (Fig. 1B and Table 1). The HD fibers were also hyperexcitable compared with WT: the minimum current pulse required to trigger an action potential was significantly less in HD than in WT fibers.

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(Fig. 1C). We also observed an after-depolarization in four of the HD fibers following an apparent subthreshold current pulse, which resulted in a spontaneous action potential (Fig. 1D). The spontaneous action potentials occurred at an average of 6.4 ± 1.7 ms after the termination of the stimulating current pulse. In all of the action potentials, there were no significant differences in the maximum rate-of-rise or peak amplitude, suggesting that the effects we measured were not due to changes in the function and/or density of the fast voltage-gated sodium channels in HD fibers (Table 1).

The prolonged repolarization, hyperexcitability, and spontaneous action potentials would be expected if there was a reduction in the resting muscle chloride conductance through ClC-1 (G_{ClC-1}). The presence of self-triggered action potentials after only subthreshold stimuli indicates a pronounced hyperexcitability in HD muscle. This could occur if there was also a reduction in the inward rectifying potassium conductance (G_{Kir}). The slight but significant increase in the initial repolarization time constant of the HD action potentials could also be the result of decreases in G_{ClC-1} and G_{Kir} (Table 1).

**Chloride Channels.** We measured chloride currents (I_{Cl}) from 9 HD and 14 WT fibers using a three-pulse voltage clamp protocol (Fig. 2A) that accounted for the voltage- and time-dependent deactivation of ClC-1 (37–40). To ensure large inward currents, we used high intracellular chloride (70 mM). From a holding potential of −20 mV, the chloride equilibrium potential, we applied a depolarizing conditioning pulse (P1) that was followed by two consecutive test pulses (P2 and P3). P1 fully activated the chloride channels. We then used P2 to determine the voltage dependence of the instantaneous or peak currents through the open channels. After the channels deactivated, we used the currents at the onset of P3 to determine the relative open probability of the chloride channels. The resulting average specific currents (µA/cm²) are shown in Fig. 2B–D. To isolate the chloride currents, we blocked the major Na⁺, K⁺, and Ca²⁺ channels with tetrodotoxin, Cs⁺ substitution, and nifedipine, respectively. The specific I_{Cl} (Fig. 2D) was the difference between the currents recorded before (Fig. 2B) and during (Fig. 2C) exposure to the chloride channel blocker anthracene-9-carboxylic acid (9AC).

There was a clear reduction in the specific I_{Cl} and G_{ClC-1} of HD compared with WT fibers (Fig. 2D). The peak I_{Cl} at −140 mV during P2 in HD fibers (−360 ± 48 µA/cm²) was significantly smaller than the mean value in WT (−1,133 ± 72 µA/cm²) (P < 0.001). To determine G_{ClC-1} we examined the current–voltage (IV) relationship of the peak I_{Cl} values during P2 (Fig. 2E). The peak G_{ClC-1}, the slope of the IV relationship from −100 to −140 mV, was significantly reduced in HD fibers (4.0 ± 0.5 mS/cm²) compared with WT (11.9 ± 0.8 mS/cm²) (P < 0.001).

We also examined the average ClC-1 steady-state values, deactivation kinetics and the corresponding outward Cl⁻ movement, and the peak I_{Cl} kinetics (Fig. S2). Generally the results indicate that any change in ClC-1 function in HD fibers was minor compared with the marked reduction total ClC-1 currents. This was also demonstrated by the chloride channel relative open probability, which we determined by plotting the peak currents at the onset of P3 (normalized to the maximum current) as a function of the steady-state voltages from P2 (Fig. 2F). The relative open probability data were fitted with a Boltzmann curve. The resulting mean V_{0.5} values for the HD (−41 ± 4 mV) and WT (−41 ± 3 mV) fibers were not significantly different (P = 0.88). The small difference in slope factors (k values) for HD (27 ± 1 mV) and WT (24 ± 1 mV) fibers (P = 0.02) suggests a minor change in ClC-1 function in HD fibers.

**Inward Rectifying Potassium Channels.** We measured the specific Kir currents (I_{Kir}) from HD (n = 11) and WT (n = 12) fibers (Fig. 3). Kir channels are unique because their opening depends on the membrane potential and the extracellular [K⁺] (41). At potentials negative to the potassium equilibrium potential (E_K), the Kir channels open and generate inward currents; whereas, at potentials positive to E_K, the channels are mostly closed. To generate large inward currents, we used high extracellular K⁺ (130 mM). The E_K was −9.5 mV. We measured I_{Kir} from a holding potential of 0 mV by applying large negative and small positive pulses (Fig. 3A). To isolate the potassium currents, we blocked the major Na⁺, Cl⁻, and Ca²⁺ channels with tetrodotoxin, 9AC, and nifedipine, respectively. The specific I_{Kir} (Fig. 3B) increased by 2.1 ± 0.8 µA/cm².

### Table 1. Action potential properties of HD and WT fibers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Resting membrane potential (mV)</th>
<th>Max rate-of-rise (mV/ms)</th>
<th>Max ∆voltage (mV)</th>
<th>Decay τ₁ (ms)</th>
<th>Decay τ₂ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 17)</td>
<td>−87 ± 1</td>
<td>420 ± 33</td>
<td>127 ± 3</td>
<td>0.62 ± 0.04</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>HD (n = 12)</td>
<td>−86 ± 1</td>
<td>465 ± 47</td>
<td>125 ± 2</td>
<td>0.82 ± 0.06*</td>
<td>15.0 ± 2.1*</td>
</tr>
</tbody>
</table>

Average values (±SEM) of the resting or baseline membrane potential, the maximum rate-of-rise of the depolarization, the peak change in membrane potential, the initial repolarization time constant (τ₁), and the slower repolarization time constant (τ₂).

* indicates a significant difference compared to WT fibers (P < 0.05).
was the difference between the currents recorded before and during exposure to the Kir channel blocker, Ba$^{2+}$ (Fig. S3).

The IV relationship of the peak $I_{Kir}$ values is shown in Fig. 3C. The peak $I_{Kir}$ at −60 mV in HD (−87 ± 11 μA/cm$^2$) was significantly less than in WT fibers (−216 ± 12 μA/cm$^2$) ($P < 0.001$). The peak $G_{Kir}$, the slope of the peak $I_{Kir}$ values from −40 to −60 mV, was significantly less in HD (1.8 ± 0.2 mS/cm$^2$) than in WT fibers (4.3 ± 0.2 mS/cm$^2$) ($P < 0.001$). The decline in $I_{Kir}$ at large negative voltage pulses (Fig. 3B) was likely the result of K$^+$ depletion in the transverse tubular system (42). A decrease in $G_{Kir}$ should slow the rate at which the $I_{Kir}$ declines. Accordingly, $I_{Kir}$ declines at a slower rate in HD than WT fibers (Fig. S3E). Our results are consistent with a previous study showing a reduced Kir current density in striatal medium-sized spiny neurons in R6/2 HD mice (43).

To more fully assess the decreases in $G_{ClC-1}$ and $G_{Kir}$ as well as the membrane properties, we analyzed the capacitance. The amount of plasma membrane is generally considered to be directly proportional to the capacitance of the fiber. Thus, current...
levels are often normalized to total fiber capacitance and reported as current densities.

**Specific Capacitance and Current Densities.** We measured capacitance by integrating capacitive transients in the same fibers used to study $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$, under conditions whereby the chloride and potassium channels were blocked (Fig. S4). This minimized the errors due to voltage-activated channels. The capacitance of HD and WT fibers were compared by normalizing to total fiber surface area to obtain the specific capacitance ($C_m$). The specific chloride and $K_r$ currents listed above were obtained above the same way. For each fiber, we estimated the surface area using an image that included the fiber length and diameter (Fig. S1) and assuming the fiber was cylindrical. If the surface membrane accounted for all of the plasma membrane, the $C_m$ of muscle would be $\sim 1 \mu$F/cm$^2$. In muscle, the $C_m$ is higher because of the transverse tubular system, a series of invaginations of the surface membrane that spread radially into the interior of the fiber.

$C_m$ was significantly lower in HD ($3.4 \pm 0.2 \mu$F/cm$^2$, $n = 20$) than in WT fibers ($5.1 \pm 0.2 \mu$F/cm$^2$, $n = 26$) ($P < 0.001$). A smaller average diameter of the HD fibers may contribute to, but does not explain, the full decrease in $C_m$ (Table S1). The decreased $C_m$ of HD fibers suggests there was a reduction of the transverse tubular system (detubulation) in the diseased muscle. Because detubulation could cause decreases in membrane conductance, we also normalized the CIC-1 and Kir results to total fiber capacitance. The HD $G_{\text{ClC-1}}$ ($1.0 \pm 0.1$ mS/μF) and $G_{\text{Kir}}$ ($0.6 \pm 0.1$ mS/μF) values normalized to capacitance were significantly less than the WT $G_{\text{ClC-1}}$ ($2.4 \pm 0.2$ mS/μF, $P < 0.001$) and $G_{\text{Kir}}$ ($0.9 \pm 0.1$ mS/μF, $P = 0.008$). The decreases in $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$ (when normalized to capacitance) should be independent of fiber diameter. Thus, the density of functional CIC-1 and Kir channels in HD fibers was reduced.

**mRNA Analysis.** The loss of chloride channels in myotonic dystrophy is thought to be due to an accumulation of RNA with CUG or CCUG repeats in the nucleus that disrupt the function of RNA binding proteins, such as muscleblind-like 1 and 2; consequently, aberrantly spliced Clcn1 mRNA that contains exon 7a is degraded via nonsense-mediated decay (27–36). We tested for this mechanism in HD interosseous muscle (Fig. 4). We found a nearly threefold increase in the proportion of aberrantly spliced Clcn1 mRNA (containing exon 7a) in HD compared with WT muscle (Fig. 4 A and B). A similar increase was found in extensor digitorum longus muscle (Fig. S5). Additionally, the total level of normal Clcn1 mature mRNA, determined using two separate primer sets, was significantly reduced in HD compared with WT muscle (Fig. 4 C and D). Similarly, the level of mRNA for the gene that encodes the Kir 2.1 potassium channel ($Kcnj2$) was significantly reduced in the HD interosseous muscle (0.186 ± 0.005, $n = 2$) relative to WT (1.000 ± 0.181, $n = 3$) ($P = 0.04$) and in the HD extensor digitorum longus muscle (0.495 ± 0.074, $n = 2$) relative to WT (1.000 ± 0.071, $n = 3$) ($P = 0.02$).

**Discussion**

We examined the fundamental membrane properties that govern the responsiveness of HD muscle to neuronal stimulation and initiate contraction. There were significant decreases in $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$, which account for most of the resting conductance in muscle and keep the membrane potential near resting levels (13). For example, triggering an action potential requires that excitatory currents overcome $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$. Thus, the reductions in $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$ explain the reduced current required to trigger an action potential in HD fibers. The decreased conductances also explain the prolonged falling phase of the action potentials in HD fibers: currents through chloride and Kir channels help drive the membrane potential back to resting levels during the repolarization phase. For the same reasons, the decreases in $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$ explain the increased input resistance and membrane time constant previously reported for flexor digitorum brevis fibers from R6/2 mice (6). We confirmed those results using a similar procedure (Table S2).

A previous study found reductions in muscle CIC-1 (likely mediated by myogenic factors) that occurred 2 d after denervation (17). Could the defects we report be related to denervation or motor neuron degeneration? This seems very unlikely because a previous study, using the same muscle tissue, demonstrated that neuromuscular innervation was normal throughout the life of the R6/2 mice, which occurred despite significant, uniform, and progressive muscle atrophy (6). Moreover, the same study found that the R6/2 mice maintained a normal ability to regenerate motor axons and functional neuromuscular junctions after crushing the tibial nerve. The normal innervation of HD muscle indicates that the defects reported in this study were independent of denervation and motor neuron degeneration.

We found greater percent decreases in $G_{\text{ClC-1}}$ and $G_{\text{Kir}}$, when normalized to surface area rather than to capacitance. This could occur if some of the CIC-1 and Kir channels were lost simply because of a decrease in the transverse tubular system. Indeed, a partial detubulation in the HD fibers was suggested by the decrease in $C_m$ and the faster time-to-peak of the CIC-1 and Kir channel currents (Figs. S2E and S3F and Table S1). Whereas there are conflicting reports about the location of CIC-1 in muscle (37, 40), it is established that Kir channels are expressed in the transverse tubular system (44, 45). A definitive confirmation of a partial detubulation will require optical methods that include electron microscopy.
In summary, a key finding of this study is that the decreased resting conductances cause hyperexcitability in HD muscle. The degree of hyperexcitability was high enough to cause self-trig-
ger action potentials that occurred after apparent sub-
threshold stimuli. We found minimal disruption of CIC-1 and Kir 
function in HD fibers (Figs. S2 and S3), suggesting that the 
decreased conductances were caused by a reduced expression of 
CIC-1 and Kir channels. Consistent with this, we found reduced 
levels of normal mature Clcn1 and Kcnj2 mRNA. A disruption of 
mRNA splicing has been shown to cause a decrease in CIC-1 
expression in myotonic dystrophy type 1, another trinucleotide 
repeat disorder (27–36). We measured elevated levels of aber-
rant Clcn1 mRNA containing exon 7a in HD muscle, which 
indicates a similar disruption in Clcn1 pre-mRNA splicing. At 
the cellular level, we have found striking biophysical and 
molecular similarities between HD and myotonic dystrophy muscle, 
suggesting, to some degree, that there is a common pathophys-
iology that involves a disruption in RNA processing. It will be 
interesting in future studies to determine the factors that result 
in similar but distinct clinical phenotypes in the diseases. The 
hyperexcitability and decreased conductances that we identified 
reveal a primary myopathy that may contribute to the motor 
defects of HD.

**Materials and Methods**

**Animal Care and Use.** All animal procedures were performed in accordance with the policies of the Animal Care and Use Committee of the California State Polytechnic University, Pomona. Five-week-old female R6/2 [B6CBA-Tg (Hdh exon1)62Gqp/1J hemizygous] and WT sibling mice were ordered from The Jackson Laboratory and cared for according to published reports (6, 21). We used a total of 14 HD mice that ranged in age from 78 to 88 d (average age of 12 wk) and 15 WT mice that ranged in age from 73 to 100 d (average age of 13 wk). The date of birth information from The Jackson Laboratory was ±3 d.

Upon arrival animals were housed with like-genotype littermates in cages supplied with filtered air within an isolation rack (Innovive). The cages contained irradiated ¾-inch corn cob bedding (Harlan Teklad 7902) and environmental enrichment (mouse house and cotton nestlet). Mice were supplied with dry chow (Irradiated Rodent Diet; Harlan Teklad 2918) and water ad libitum. At 10 wk of age, the HD mice were supplied with supplemented food and water in the form of Hydrogel and mash (dry mash moistened until paste-like in consistency) placed on the cage floor. Envi-
ronmental conditions were maintained with a 12-h day/night cycle and 
constant temperature (21–23 °C) and humidity (55% ± 10%). Cages were 
changed every 2 wk or as needed.

The behavior and physical condition of the mice were tested weekly until 
10 wk of age and then daily until the animals were selected for experiments. 
Testing of physical condition, approximate respiratory rate and 
activity level, consumption of water and food, weight loss, and hind 
limb clamping. Each category was rated on a scale of 0–3, with 0 marking 
normal condition and 3 representing extremely poor condition. Mice were 
selected for experiments when the sum of the first five category scores 
was greater than or equal to 6 or if the score of any individual category 
was equal to 3. All HD mice used experienced weight loss of 10–20% of 
their maximum weight. Mice were killed by inhalation of a saturating dose of 
isoflurane for at least 1 min, followed by cervical dislocation.

**Electrical Recordings.** *Flexor digitorum brevis* and interosseus muscle fibers were surgically removed, pinned to Syglogg-bottomed Petri dishes, and 
enzymatically dissociated at 35–36 °C under mild agitation for ∼1 h using 1,000 
U/ml of collagenase type IV (Worthington Biochemical). Collagenase was 
dissolved in the extracellular solution used for recording action potentials 
and EGTA, pH 7.2 with CsOH (190 K+). Internal solution for GClC-1 (in mM) was as follows: 17 aspartate, 30 HCl, 30 
CaCl2, 10 MgCl2, 5 glucose, 1 NaH2PO4, 10 Mops, and pH 7.2 
with NaOH.

Extracellular GClC-1 solution (in mM) was as follows: 140 HCl, 10 CsOH, 5 
CaCl2, 2 MgCl2, 5 glucose, 1 NaH2PO4, 10 Mops, 0.0002 tetrodotoxin, 0.02 
nifedipine, and pH 7.2 with tetraethylammonium hydroxide. 0.4 anthracene-
9-carboxylic acid was added for GClC-1 blocking solution.

Extracellular Gk channel solution (in mM) was as follows: 130 NaCl, 2.5 KCl, 5 CaCl2, 2 MgCl2, 5 glucose, 1 NaH2PO4, 10 Mops, and pH 7.2 
with NaOH.

mRNA Analysis. Total muscle RNA was isolated and 1 μg of RNA was reverse 
transcribed (46). To quantify total Clcn1 and Kcnj2 mRNA levels, real-time 
quantitative RT-PCR was performed with a BioRad DNA engine Opticon 2. 
Clcn1 was quantified using primers that spanned either exons 10–14 
or exons 16–19 as previously described (31). Kcnj2 was quantified using a Taq-
man assay (Mm00434616.m1, Applied Biosystems). Clcn1 and Kcnj2 were 
normalized to β-2-microglobulin (Taqman assay Mm00437762.m1, Applied 
Biosystems) and analyzed using the ΔΔCT method. For analysis of alterna-
tively spliced gene products (inclusion of exon 7a) of Clcn1, PCR was per-
formed as previously described (31), using primers that spanned the exon 7a 
site. PCR products were separated and stained with ethidium bromide. 
The amplicon containing exon 7a (420 bp) was quantified relative to the total 
including the normal adult Clcn1 amplicon without exon 7a (341 bp).

**Curve Fitting.** Data analysis was accomplished with pCLAMP 10 and Sig-
maPlot 11. The action potential repolarizing phase was fitted with a double exponential, 

$$f(x) = y_o + A_1e^{-x/t_1} + A_2e^{-x/t_2},$$

where $y_o$ is the offset; $A_1$ and $A_2$ are amplitudes for components 1 and 2; 
and $t_1$ and $t_2$ are the decay constants of components of 1 and 2. Chloride 
channel relative open probability data were fitted with a Boltzmann curve (47),

$$P_o = \frac{1}{1 + e^{(V_m - V_{50})/k}},$$

where $P_o$ is the relative open probability, $V_m$ is the test potential, $V_{50}$ is 
the voltage at half-maximal activation, and $k$ is the slope factor. Linear fits 
were used to determine conductance values.


Fig. S1. Dissociated muscle fiber impaled with two intracellular microelectrodes. (Scale bar, 50 μm.)

Fig. S2. Average muscle chloride channel (ClC-1) steady-state current–voltage (IV) relationship, deactivation kinetics, outward charge movement, and peak current kinetics from WT (n = 14) and Huntington disease (HD) (n = 9) fibers. (A–E) All of the data were generated using the average chloride current (I_{Cl}) records in Fig. 2D. (A and B) IV relationships of the steady-state I_{Cl}, normalized to fiber surface area (cm^2), at the end of P2 for (A) WT and (B) HD fibers. Other than the significant difference in I_{Cl} magnitude, the steady-state I_{Cl}–voltage relationships in HD and WT fibers are very similar. One slight difference may be a slightly greater relative inhibition of the steady-state I_{Cl} at positive voltages in HD compared with WT fibers. (C) The time course of I_{Cl} deactivation in P2, shown as fractional currents, for HD and WT fibers at −140, −120, −100, and −80 mV (legend for C and D is in the bottom right of Fig. S2). Time 0 is the peak I_{Cl}. At −140 and −120 mV there was no apparent difference in the kinetics of I_{Cl} deactivation in HD compared with WT fibers. At −100 and −80 mV there was a slight decrease at later time points in the rate of I_{Cl} deactivation in HD compared with WT fibers. This may suggest a slight change in function of ClC-1 in HD fibers, or, if ClC-1 is located in the transverse tubular system (TTS), the change in kinetics may be related to the accumulation of Cl\(^{−}\) in the TTS of WT fibers (D). (D) The outward movement of Cl\(^{−}\) (nC/cm\(^2\)) during the deactivation of I_{Cl} in HD and WT fibers, obtained by integrating the specific I_{Cl} records. Because of the rapid and more complete inhibition of ClC-1 at large negative potentials, at later time points there was more net outward Cl\(^{−}\) at −100 and −80 mV than there was at −140 and −120 mV. This was particularly apparent in WT fibers. Thus, an accumulation of Cl\(^{−}\) in the TTS may cause a faster rate of I_{Cl} deactivation in WT fibers. (E) Faster time-to-peak I_{Cl} at −140 mV during P2 in HD compared with WT fibers.
Fig. S3. Kinetics and full set of average inward rectifying potassium currents of WT \((n = 12)\) and HD \((n = 11)\) fibers. (A–D) Voltage clamp traces for WT (Left) and HD (Right) fibers. (A) Voltage protocol with a holding potential of 0 mV and a series of 700-ms steps from −60 to +20 mV in +10-mV increments. (B) Total currents composed of the inward rectifying potassium current \((I_{\text{Kir}})\) plus leak and capacitive currents. (C) Leak and capacitive currents measured by blocking \(I_{\text{Kir}}\) channels with 5 mM Ba\(^{2+}\). For scaling purposes, the full capacitive transients in B and C are not shown. (D) Specific \(I_{\text{Kir}}\) records obtained by subtracting the leak and capacitive currents (C) from the total currents (B). (E) Time course of \(I_{\text{Kir}}\) (fractional) at −60 mV for HD and WT fibers. (F) Faster time-to-peak \(I_{\text{Kir}}\) at −60 mV in HD compared with WT fibers.

Fig. S4. Capacitive transients. (A and B) Representative voltage clamp traces used to obtain fiber capacitance recorded from WT (Left) and HD (Right) fibers. Each trace is from fibers used to obtain chloride currents under blocking conditions (in the presence of anthracene-9-carboxylic acid). (A) Voltage protocol. (B) Specific currents. The capacitance of the WT fiber was 5.9 \(\mu F/cm^2\) and the HD fiber was 3.8 \(\mu F/cm^2\). To determine the capacitance, we integrated the transients at the onset (on phase) and termination (off phase) of voltage steps. In all data used, there was no significant difference in the capacitance measurements from the on or off phases for HD or WT fibers. We used the average result from an equal number of positive and negative voltage steps (of equal magnitude) from the off phase and normalized the value to the surface area to obtain the specific capacitance \((C_m)\).

Fig. S5. Atypical splicing of the Clcn1 gene product in the extensor digitorum longus muscle of HD mice. (A) Gel showing aberrantly spliced Clcn1 mRNA that contains exon 7a (exon 7a+, 420 bp) and normal adult Clcn1 mRNA that lacks exon 7a (exon 7a−, 341 bp) in WT (Left) and HD (Right) muscle. (B) Aberrant Clcn1 mRNA with exon 7a was expressed at higher proportional levels in the HD \((n = 2)\) compared with WT \((n = 3)\) muscle. *Significant difference compared with WT muscle \((P < 0.05)\).
Table S1. Fiber capacitance and dimensions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Capacitance per surface area $C_m$ (μF/cm$^2$)</th>
<th>Diameter (μm)</th>
<th>Length (μm)</th>
<th>Surface area (×10$^{-4}$ cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 26)</td>
<td>5.1 ± 0.2</td>
<td>52 ± 2</td>
<td>527 ± 9</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>HD (n = 20)</td>
<td>3.4 ± 0.2*</td>
<td>43 ± 2*</td>
<td>519 ± 13</td>
<td>7.3 ± 0.4*</td>
</tr>
</tbody>
</table>

The average values (±SEM) of the normalized fiber capacitance and the fiber physical dimensions. In skeletal muscle, values normalized to surface area depend on fiber diameter; this is because the proportion of plasma membrane that is in the transverse tubular system depends on the surface-to-volume ratio (1). As a result, the $C_m$ of skeletal muscle changes in a roughly linear manner with diameter. For example, in WT mouse flexor digitorum brevis muscle, a 10-μm decrease in fiber diameter correlates with a 0.74 μF/cm$^2$ decrease in capacitance (2). This does not fully explain the results of this study, because we found a 1.7-μF/cm$^2$ decrease in the $C_m$ of HD fibers but had only a 9-μm decrease in diameter. Additionally, if detubulation is occurring in the HD fibers, the recently published radial cable model of mammalian skeletal muscle (3) predicts that the peak $I_{Cl}$ should occur more rapidly in HD compared with WT fibers. Indeed, the time-to-peak $I_{Cl}$ (Fig. S2E) and $I_{Cl}$ (Fig. S3F) were faster in HD compared with WT fibers. Thus, the decrease in $C_m$ of the HD fibers is likely the result of a decrease in fiber diameter along with a partial detubulation.

*Significant difference compared with WT fibers ($p < 0.05$).

Table S2. Input resistance and membrane time constant of HD and WT fibers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Input resistance (MΩ)</th>
<th>Membrane time constant (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 13)</td>
<td>1.8 ± 0.1</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>HD (n = 11)</td>
<td>6.1 ± 0.7*</td>
<td>9.7 ± 0.8*</td>
</tr>
</tbody>
</table>

Average values (±SEM). Data were obtained using the internal and extracellular solutions for action potentials, with tetrodotoxin (0.001 mM) and nifedipine (0.02 or 0.04 mM) added to the extracellular solution. Signals were low-pass filtered at 1 kHz. As with action potentials, the baseline membrane potential was maintained between −80 and −90 mV by applying a constant holding current of absolute magnitude <−5 nA for HD fibers and <−15 nA for WT fibers. We measured input resistance and membrane time constant by injecting a series of 300-ms step current pulses and measuring the resulting changes in membrane potential (±10 mV in magnitude). The input resistance was the slope of the steady-state change in membrane potential plotted as a function of injected current. The membrane time constant was obtained from the time course of the membrane potential change after the termination of the step current pulse, the falling phase. These results confirm the finding from a previous study (1). The same previous study also measured a depolarized resting membrane potential in the R6/2 muscle. This could be explained by the decreases in $G_{Cl}$ and $G_{Kv}$ that we measured, assuming that the density of the background Na$^+$ leak was not decreased in the HD muscle. Because we adjusted the resting or baseline membrane potential to similar values to minimize voltage-dependent changes, we did not confirm the previous measurements of the resting membrane potential.

*Significant difference compared with WT fibers ($p < 0.05$).