Striatal Potassium Channel Dysfunction in Huntington’s Disease Transgenic Mice

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1Mental Retardation Research Center and 2Department of Physiological Sciences, David Geffen School of Medicine, University of California, Los Angeles, California; 3Department of Medicine, Division of Endocrinology, Massachusetts Medical Center, Worcester, Massachusetts; 4Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts; and 5Department of Neuroscience, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois

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Ariano, Marjorie A., Carlos Cepeda, Christopher R. Calvert, Jorge Flores-Hernández, Elizabeth Hernández-Echeagaray, Gloria J. Klapstein, Scott H. Chandler, Neil Aronin, Marian DiFiglia, and Michael S. Levine. Striatal potassium channel dysfunction in Huntington’s disease transgenic mice. J Neurophysiol 93: 2565–2574, 2005. First published December 29, 2004; doi:10.1152/jn.00791.2004. Huntington’s disease (HD) is an autosomal dominant, neurodegenerative disorder that mainly affects the striatum as well as cortical neurons (Vonsattel and DiFiglia 1993). The HD mutation produces an expanded polyglutamine (CAG) sequence within the encoded protein huntingtin (Huntington’s disease Collaborative Research Group 1993). Mutated huntingtin causes neuronal dysfunction long before cell death (DiFiglia et al. 1997; Levine et al. 2004), and this is sufficient to induce behavioral symptoms. Generation of HD transgenic mice (Laforet et al. 2001; Mangiarini et al. 1996; Reddy et al. 1998; Schilling et al. 1999) enables direct tests of development of cellular dysfunction. In previous studies, we demonstrated morphological and electrophysiological changes in MSNs that included, among others, alterations in passive and active membrane properties, corticostriatal inputs and responses to N-methyl-D-aspartate (NMDA) (Cepeda et al. 2001a, 2003; Klapstein et al. 2001; Laforet et al. 2001; Levine et al. 1999). Using the R6/2 model, we detected increased membrane input resistances and depolarized resting membrane potentials as the behavioral phenotype developed. In the striatum, the expression of K+ channel subunits proteins has been cloned that contribute to its expression, Kir1, Kir2, and Kir3 (ROMK, IRK and GIRK, respectively) (Gutman et al. 2003; Karschin et al. 1996; Lesage et al. 1994). Kir channels assemble as heteromeric complexes in the membrane. Kir1 channels are only “mildly” rectifying, whereas Kir2 channels underlie “strong” inward rectification and are the principal contributors of measured inward K+ currents detected in the striatum (Karschin et al. 1996; Mermelstein et al. 1998).}

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant, neurodegenerative disorder that affects the projection neurons of the striatum as well as cortical neurons (Vonsattel and DiFiglia 1993). The HD mutation produces an expanded polyglutamine (CAG) sequence within the encoded protein huntingtin (Huntington’s Disease Collaborative Research Group 1993). Huntingtin is ubiquitous in the body and brain, but major degeneration occurs in the striatum and cortex. Theories explaining selective neuronal damage include excitotoxicity and abnormal energy metabolism (Beal et al. 1993; DiFiglia 1990). The excitotoxicity theory proposes that subpopulations of striatal medium-sized spiny projection neurons (MSNs) are hypersensitive to corticostriatal and thalamostriatal glutamate, or excessive glutamate is released by these afferents, while striatal interneurons are less affected (DiFiglia 1990). In addition, defects in energy metabolism and impairment of mitochondrial function contribute to the cellular pathology in HD (Beal et al. 1993).

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Another family of channel proteins mediates depolarization-activated $K^+$ currents (Hille 2001). Molecular cloning has revealed numerous families of voltage-gated $K^+$ channels, with multiple variants in each subgroup (Gutman et al. 2003). Three types of outward $K^+$ currents have been described in MSNs (Nisenbaum et al. 1996; Surmeier et al. 1991). Two of these outward currents are fast or slowly inactivating conductances and are sensitive to 4-aminopyridine. The third outward current, the delayed rectifier, is noninactivating or persistent, is detected at relatively depolarized potentials, and can be blocked by the addition of tetrathylammonium (TEA) (Ashcroft 2000). A major protein composing this channel is the Kv2.1 subunit (Baranauskas et al. 1999; Murakoshi and Trimmer 1999).

The present experiments examined electrophysiological characteristics of inward and outward rectification and alterations in $K^+$ channel subunit expression of MSNs in two mouse models of HD. R6/2 transgenics, which carry exon 1 of the human HD gene with $\sim$155 CAG repeats, have an aggressive disease, with a progressive neurological syndrome overtly beginning at $\sim$40 days of age and death occurs at $\sim$100 days (Mangiarini et al. 1996). The TgCAG100 model has a larger transgene encoding the first one-third of the huntingtin protein expressed solely in neurons. It has a smaller polyglutamine expansion (100 CAG repeats) (Laforet et al. 2001). The TgCAG100 shows a longer disease progression compared with the R6/2. Inward and outward $K^+$ conductances were characterized in R6/2 MSNs at the beginning and after full development of the behavioral phenotype. TgCAG100 mice only were examined after appearance of behavioral abnormalities.

**Methods**

Experimental procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of California, Los Angeles. For electrophysiology, wild-type (WT), R6/2, and TgCAG100 transgenic mice were used. Two different age groups of R6/2 transgenics were examined (Table 1), defined according to development of the overt behavioral phenotype (5–7 wk, corresponding to its onset; 11–15 wk, displaying the full behavioral phenotype) (Carter et al. 1999; Lione et al. 1999; Mangiarini et al. 1996). One age group of the TgCAG100 mice was used (8–14 mo of age) corresponding to behaviorally symptomatic animals (Laforet et al. 2001). Morphological studies were based on a minimum of four age-matched WT and transgenic animals.

**Slice preparation**

Patch electrodes (3–6 $M\Omega$) were filled with the following internal solution [in mM]: 112.5 $K^+$ glutconate, 4 NaCl, 17.5 KCl, 0.5 CaCl$_2$, 1 MgCl$_2$, 5 K$_2$ATP, 5 EGTA, 10 HEPES (Na$^+$ salt), and 1 GTP. An Axopatch 200B (Axon Instruments, Foster City, CA) was used for recording. A 3 M KCl agar bridge was inserted between the extracellular solution and the Ag-AgCl indifferent electrode. Tight seals (2–5 GΩ) were obtained by applying negative pressure. The membrane was disrupted with additional suction to obtain the whole cell configuration. Recordings were not corrected for junction potentials, which ranged from 2 to 3 mV. Access resistances ranged from 10 to 25 MΩ and were compensated 60–85%. In some cells, the voltage-clamp protocol was switched to current-clamp mode to measure RMPs. Cells were initially held at $-70$ mV to minimize contributions of voltage-gated currents. Voltage-gated currents also were blocked using the following drugs in the bath: 1 mM tetraethylammonium (TTX); 100 mM Cd$^{2+}$; 3 mM Cs$^+$, or 20 mM TEA. The inward $K^+$ current protocol consisted of 300-ms step voltage commands (from $-140$ to $-50$ mV in 10-mV steps) from a holding potential of $-50$ mV. Because MSN are more hyperpolarized than $-50$ mV, a positive holding current was injected through the patch pipette during the voltage protocol. Inward currents were measured from the average current between 280 and 300 ms after the start of the step. In some cases, a slow ramp voltage command (4-s duration, from $-120$ to $-30$ mV) was also used. The protocol for outward currents consisted of 80-ms step voltage commands ($-80$ to $+10$ mV in 10-mV steps) from a holding potential of $-70$ mV. Steady-state currents were measured 70–80 ms after the initiation of the step.

**Acute neuron dissociation**

Brains were removed and slices obtained as described in the preceding text. Slices were then transferred to the following solution for 1 h [(in mM): 126 NaCl, 2.5 KCl, 2 MgCl$_2$, 2 CaCl$_2$, 26 NaHCO$_3$, 1 Na$_2$HPO$_4$, 1 pyruvic acid, 0.005 glutathione, 1 $N^\prime$-nitro-l-arginine, 1 kynurenic acid, 10 glucose, and 15 HEPES (pH 7.4) at 300–304 mOsM/kg]. The slices were then placed into low-Ca$^{2+}$ isothionate solution [in mM] 140 Na isethionate, 2 KCl, 4 MgCl$_2$, 0.1 CaCl$_2$, 23 glucose, and 15 HEPES], and the dorsal striatum was dissected and placed in an oxygenated cell-stir chamber (Wheaton, Millville, NJ) containing protease (0.7 mg/ml; Calbiochem, La Jolla, CA) in HEPES-buffered Hanks’ balanced salt solution (HBSS, Sigma Chemical, St. Louis, MO) at 35°C. After 20–40 min of enzyme digestion, the tissue was rinsed three times with the low-Ca$^{2+}$ isothionate solution and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was plated into a 35-mm NUNC dish containing HBSS on the microscope stage.
Recordings of K⁺ currents in dissociated striatal neurons were obtained with borosilicate glass electrodes (3–4 MΩ resistance). The internal solution consisted of (in mM) 140 K⁺ gluconate, 10 NaCl, 5 HEPES, 3 MgCl₂, 0.001 spermine, 2 ATP, 0.4 GTP, 0.5 EGTA, 12 phosphocreatine, and 0.1 leupeptine (pH 7.3 adjusted with NaOH and HCl, 270 mOsm/l). The external solution consisted of (in mM) 154 N-methyl-d-glucamine (NMDG), 20 K⁺ gluconate, 2 MgCl₂, 0.5 CaCl₂, 10 glucose, 56 sucrose, 10 HEPES, and phenol red 1% (pH 7.35, adjusted with KOH or sulfonic acid as needed, 300 mOsm/l). TTX (1 μM), Cd²⁺ (100 μM), and Cs⁺ (3 mM) were applied through a 150-μm-diam capillary positioned close (~200 μm) to the recorded cell. The same voltage protocol used in slices to examine inwardly rectifying currents was applied to dissociated cells. Based on somatic size and passive membrane properties, all recordings in acutely dissociated cells were from MSNs.

Electrophysiology data analyses

Passive membrane properties of cells in slices or dissociated cells were determined in voltage-clamp mode by applying a depolarizing step voltage command (10 mV) and using the membrane test function integrated in the pClamp8 software (Axon Instruments, Foster City, CA). This function reports membrane capacitance (in pF), input resistance (in MΩ or GΩ) and time constant (in micro- or millisecond). The time constant was obtained from a single-exponential fit to the decay of the capacitive transients. Values in the figures and text are presented as means ± SE. Differences in mean current densities at various voltage commands were assessed with a two-way ANOVA with one repeated measure followed by multiple comparisons using Bonferroni t-test; t-tests alone were used when only two group means were compared. Differences between means were considered statistically significant when P < 0.05.

Immunohistochemistry

Specific primary antisera for Kir2.1, Kir2.3, and Kv2.1 were purchased from Chemicon (Temecula, CA), Alomone Labs (Jerusalem, Israel), and Upstate Biotech (Waltham, MA). Slide-mounted tissue sections were cut fresh frozen from mouse brains at 10 μm in the coronal plane. Sections were immersion-fixed in freshly prepared cold 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) for 5 min and then rinsed in PBS. Primary antisera were diluted in PBS (dilutions: Kir2.1, 1:100; Kir2.3, 1:100; Kv2.1, 1:200). Sections were incubated overnight at 4°C in a humidified environment. The following day, unbound primary antisera were rinsed off in PBS, and secondary fluorescently coupled antisera (donkey anti-rabbit or donkey anti-mouse conjugated to Cy3 or Cy5) were applied to sections. After washing with PBS, sections were mounted in 60% glycerol and 4% DABCO in PBS. Sections were examined using an epifluorescence microscope. The signal intensity value of the K⁺ channel protein studied (Kir2.1, Kir2.3, Kv2.1) was determined for each image from the WT and the transgenics. Specific sequence in the coronal sections, from dorsolateral, dorsomedial, ventrolateral, to ventromedial quadrants of the striatum. Image files were stored for later analysis as previously described (Ariano et al. 2002).

Fluorescence intensities that provided cellular staining 20% brighter than the background signal were counted; intensities dimmer than this value were omitted. The background fluorescence signal was established from the signal intensity emitted in fiber bundles of the internal capsule that penetrate the striatum and were averaged from three randomly chosen areas in the bundles. The median value of the fluorescent signal within a 30-pixel-diam circle area was calculated using the histogram function in Adobe Photoshop (Lehr et al. 1999). This “background signal” was subtracted from the staining intensity values of individual MSN fluorescence. To randomly choose MSN, a counting grid was overlaid on each image acquired, and MSNs meeting the criteria of size (enlarged by 30 pixels) and location in the dorsal striatum that intersected the counting grid were then encircled by the 30-pixel circular marquee. The signal intensity value of the K⁺ channel subunit protein was averaged, the background signal was subtracted, and the numbers stored in Excel spreadsheets. Means of individual cellular luminosities and the SE for MSNs evaluated in each image from the WT and the transgenics were computed. Results were expressed as the percentage change in mean cellular luminosity values for the K⁺ channel protein in the transgenic versus the WT. Immunofluorescence comparisons were evaluated using paired two-tailed t-test with a conservative criteria for differences achieving statistical significance only if P < 0.001. At least four different pairs of R6/2 and TgCAG100 mice and their age-matched WT controls were evaluated for each K⁺ channel protein studied (Kir2.1, Kir2.3, Kv2.1), corresponding to ~2,500 MSNs for each antigen investigated in each age range.

RESULTS

Recordings were made from 28 MSNs from WT (n = 9) and 19 neurons from R6/2 (n = 7) in the 5- to 7-wk-old group and 14 neurons from WT (n = 6) and 15 neurons from R6/2 (n = 8) in the 11- to 15-wk-old group. In the 5- to 7-wk age range, the R6/2 transgenics began to show subtle electrophysiological changes. Whereas the mean RMPs, capacitances, and time constants of MSNs were similar, the input resistances were significantly higher than those of neurons from WT (P = 0.002; Table 1). In the 11- to 15-wk age range, after overt expression of the HD behavioral phenotype in R6/2 transgenics, the RMPs of MSNs were depolarized significantly compared with those of WT (P = 0.009). Input resistances remained significantly higher (P < 0.001), capacitance was significantly lower (P < 0.001), while time constants were slightly shorter, but the difference was not statistically significant (Table 1). Some of these functional alterations such as decreased membrane capacitance and increased input resistance may correlate with the reduced cross-sectional somatic areas, dendritic thinning and spine losses, documented previously in older R6/2 transgenics using IR videomicroscopy, intracellular markers like biocytin and Golgi impregnation (Klapstein et al. 2001; Levine et al. 1999).

Inward K⁺ currents were attenuated in 11- to 15-wk-old R6/2 transgensics

K⁺ currents in slices were examined using an external K⁺ concentration of 3 mM and in the presence of TTX (1 μM) and Cd²⁺ (100 μM). The voltage protocol used to examine inwardly rectifying channels was adapted from that used in MSNs from nucleus accumbens and has been demonstrated to

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minimize the contribution of depolarization-activated K⁺ channels (Mermelstein et al. 1998). Hyperpolarizing step voltage commands from a holding potential of −50 mV produced a large inward current that increased with the magnitude of the voltage command (Fig. 1A). To normalize for differences in cell size for quantitative analysis, current was divided by capacitance to examine changes in current density (Alzheimer et al. 1993) because MSNs from 11- to 15-wk mice were smaller than those of WTs. At 5–7 wk of age, there were no differences in the evoked current densities at any voltage step (Fig. 1, A and B1, top). At 11–15 wk, inward K⁺ current densities were reduced significantly in R6/2 MSNs compared with WTs, primarily at more hyperpolarized voltage steps from −140 to −90 mV (Fig. 1B2, top).

Voltage-dependent inwardly rectifying K⁺ currents (I_{Kir}) are sensitive to Cs⁺ and Ba²⁺ (Nisenbaum and Wilson 1995; Reyes et al. 1998; Uchimura et al. 1989). In the present study, Cs⁺ was chosen as the main blocker of inward rectification based on previous reports demonstrating that TEA and Ba²⁺ mainly affect linear conductances (Reyes et al. 1998). Thus striatal slices were exposed to Cs⁺ (3 mM) and the Cs⁺-sensitive current densities were isolated by digital subtraction from the total K⁺ current densities (Fig. 1, A and B1, bottom). The Cs⁺-sensitive current-voltage relationship demonstrated a large conductance at hyperpolarized potentials that reversed around −90 mV, close to the estimated Nernstian equilibrium potential (approximately −97 mV based on the external and internal K⁺ concentrations). At more depolarized potentials, a small outward current was also evident. Cs⁺-sensitive K⁺ current densities were similar in 5- to 7-wk transgenics and WTs but reduced significantly in the R6/2 transgenics at 11–15 wk at potentials of −140 to −100 mV (Fig. 1B2, bottom). When the differences were compared across the two age groups examined, it was apparent that at 5–7 wk, current densities were lower than the corresponding densities from 11–15 wk WTs. Thus it is possible that K⁺ channels increase in density with age but this elevation did not occur in the R6/2 transgenic, possibly because of reductions in cell size and loss of dendritic processes and spines (Klapstein et al. 2001; Levine et al. 1999).

The voltage dependence of activation for the Cs⁺-sensitive current at 11–15 wk was expressed as the relative conductance (G/G_{max}) at the different voltage steps and was fitted using Boltzmann functions (Fig. 2A). Half-maximal voltage activations were calculated from these curves. Voltage dependence of I_{Kir} showed an initial activation at about −70 to −80 mV. Activation curves from WT and R6/2 transgenics overlapped with mean half-activation at −106 and −108 mV, respectively, indicating that the primary alteration was a reduction in the density of Kir channels without a change in conductance or activation parameters.

We also examined inward K⁺ current densities in response to ramp voltage commands and the effects of Cs⁺ in the 11- to 15-wk MSNs (Fig. 2B). Slow ramp commands in the presence of TTX and Cd²⁺ produced a large inward current in MSNs from WTs but a much smaller inward current in MSNs from
R6/2 transgenics. Subtracted currents also were reduced in the transgenics in the presence of Cs/H11001. The differences in mean Cs/H11001-sensitive current densities between the current-voltage relationships were statistically significant from 120 to 100 mV (Fig. 2).

To minimize potential space-clamp limitations associated with the use of whole cell patch recordings from neurons with extended processes in the striatal slices, we examined changes in acutely dissociated MSNs from 11- to 15-wk-old animals. The dissociation process eliminated most of the secondary and tertiary dendrites and left only the soma and a few primary and occasionally secondary dendrites. Based on differences in cell capacitance, a reflection of membrane area, it was estimated that cells in slices had ~10 times more membrane area than dissociated cells (109 ± 8 vs. 8.3 ± 1.3 pF in WT from slices and acutely dissociated neurons, respectively, and 65 ± 5 vs. 6.2 ± 0.7 pF in R6/2 transgenics from slices and acutely dissociated neurons, respectively). In dissociated MSNs, the external K+ concentration was increased to 20 mM to set the equilibrium potential at approximately ~50 mV (cf. Mermelstein et al. 1998). Cs+-sensitive inward K+ currents and densities were examined with the same voltage protocol used in slices (n = 5 and 6 additional neurons from WTs and R6/2 transgenics, respectively). There was a significant reduction in both the inward current density (Fig. 3A, significant differences from −120- to −90-mV holding potentials) and the Cs+-sensitive inward current density (Fig. 3B, significant differences from −120 to −80 mV) in dissociated MSNs, mirroring results obtained in striatal slices in 11- to 15-wk-old transgenics.

**Outward K+ conductances were reduced in 11- to 15-wk R6/2 transgenic mice**

We next examined the differences in the depolarization-activated K+ currents in MSNs in striatal slices. We focused on the delayed rectifying current because it functions to hyperpolarize or repolarize membrane potentials (Nisenbaum and Wil-

![Fig. 2. Voltage dependence of activation of Kir from step voltage commands (A) and inward K+ currents evoked by a ramp voltage command in striatal slices obtained from 11- to 15-wk R6/2 transgenics and WTs (B and C). A: voltage dependence of activation for the Cs+-sensitive current at 11–15 wk expressed as the relative conductance (G/Gmax) at the different voltage steps (Boltzmann function fit). See text for further description. B: examples of inward K+ currents evoked by slow ramp commands (voltage protocol in inset). Top: control and currents in the presence of Cs+ (−). Bottom: subtracted Cs+-sensitive currents. C: graphs show means ± SE for current-voltage relationships of Cs+-sensitive current densities for MSNs in response to ramp voltage commands. Statistical significance occurred from −120 to −100 mV (*).](http://jn.physiology.org/)

![Fig. 3. Current-voltage relationships for inward K+ current densities (A) and Cs+-sensitive K+ current densities (B) obtained from acutely dissociated MSNs from 11- to 15-wk R6/2 transgenics and WTs. In these experiments, a 20 mM K+ concentration in the external solution was used to set the equilibrium potential at approximately −50 mV, corresponding to the holding potential.](http://jn.physiology.org/)
son 1995; Wilson and Kawaguchi 1996). The protocol and examples of these currents are shown in Fig. 4A. The delayed rectifier was blocked by TEA (20 mM), and the TEA-insensitive current densities were subtracted digitally from the whole cell current densities (Reyes et al. 1998). TEA-sensitive currents began to activate at about −30 mV in both genotypes and at both ages. There were no differences in current density or TEA-sensitive current density at 5–7 wk between genotypes (Fig. 4B, top and bottom). In contrast, at 11–15 wk, R6/2 MSNs showed statistically significant decreases in outward K\(^+\) current densities and TEA-sensitive current densities at −10- to +10-mV voltage steps (Fig. 4B, top and bottom). Similar changes were observed in dissociated MSNs (data not shown).

**Inward and outward K\(^+\) conductances were reduced in symptomatic TgCAG100 transgenics**

Similar outcomes were found in whole cell recordings from MSNs in striatal slices from the TgCAG100 model. Older, behaviorally symptomatic animals were used in these experiments because the behavioral phenotype of this HD model develops later and is less pronounced than in the R6/2 (Laforet et al. 2001). Basic membrane properties and K\(^+\) current analyses were obtained from animals slightly over a year in age \([n = 11 \text{ cells from } 3 \text{ WT (378 ± 78 days)} \text{ and } 15 \text{ cells from } 4 \text{ TgCAG100 mice (496 ± 40 days)}]\). Unlike R6/2 transgenics, the RMPs of MSNs from TgCAG100 were not significantly different from their age-matched WTs (Table 1). Cell capacitances were reduced, and input resistances were increased significantly in TgCAG100 MSNs, analogous to the outcomes obtained in 11- to 15-wk R6/2 transgenics (Table 1). As shown in Fig. 5A, inward current densities and Cs\(^+\)-sensitive current densities were reduced significantly in TgCAG100 mice but only at very hyperpolarized holding potentials (−140 and −120 mV for inward current density and −140 mV for Cs\(^+\)-sensitive current density). Outward current densities and TEA-sensitive current densities were reduced significantly (Fig. 5B) but only at the most depolarized potentials (0 and +10 mV for outward current density and TEA-sensitive current density).

**Expression of Kir and Kv channel proteins was decreased in HD models**

We employed two age groups of R6/2 mice, one prior to the onset and the second following development of the behavioral HD phenotype, complementing the electrophysiological studies. We examined Kir2.1 and Kir2.3 channel staining in the HD transgenics and age-matched WTs as benchmarks of the inward K\(^+\) currents characterized physiologically. The rationale for selecting these subunits was based on earlier studies indicating abundant expression of Kir2.1 and Kir2.3 subunits in striatum (Karschin et al. 1996), whereas expression of Kir 2.2...
is only moderate and Kir2.4 is preferentially expressed in cholinergic interneurons (Prüß et al. 2003). Strong Kir2.1 staining was detected in MSNs of R6/2 and WT's complementing previous physiological assessments of normal MSN (Mermelstein et al. 1998). A strong Kir2.1 signal was seen throughout the cytoplasm of WT MSNs (Fig. 6, top left) and throughout the neuropil but was lacking within the myelinated fiber bundles that penetrate the striatum that were visible as dark ovoid structures within the stained neuropil. No differences in cellular luminosity values were detected in the R6/2 versus WT at 5–7 wk. By comparison, a noticeable loss of fluorescence signal intensity occurred at 11–15 wk (Fig. 6, top right). Mean cellular luminosity for the WT was 77.5 ± 0.64, whereas for the R6/2s it was 40.4 ± 0.5 (P < 0.001). This correlates to ~50% loss of the Kir2.1 signal. Similar attenuation of the channel signal was observed at 14 mo in the TgCAG100 (data not shown).

Kir2.3 K⁺ channel protein staining mirrored the data for the Kir2.1 channel (Fig. 6, middle). The Kir2.3 K⁺ channel protein was prevalent within MSNs and to a lesser extent within the neuropil. Staining was absent in the myelinated fiber bundles of the internal capsule. No substantial differences in staining intensity or distribution were evident in 5- to 7-wk R6/2 transgenics (data not shown) but were clearly evident at 11–15 wk (Fig. 6, middle). Differences in cellular luminosity values in MSNs for Kir2.3 were statistically significant at 11–15 wk. The mean value for the WT was 74.2 ± 1.1, whereas for the R6/2, it was 54.7 ± 1.1 (P < 0.001); a loss of ~25% in the transgenics. A 30–40% attenuation of the channel signal was expressed at 14 mo in the TgCAG100 (data not shown).

Kv2.1 protein staining was detected readily surrounding the peripheral rim of cytoplasm and for some distance along the initial processes of MSNs as discrete punctate staining (Fig. 6, bottom). Fiber bundles of the internal capsule also were not reactive. Kv2.1 protein staining was equivalent between the WT and transgensics at 5–7 wk. Again there were statistically significant reductions in staining intensity for the Kv2.1 channel at 11–15 wk in the R6/2. Cellular luminosities in the WT averaged 60.4 ± 2.4 compared with 31.8 ± 2 in the R6/2, leading to a 47% loss in Kv2.1 staining. TgCAG100 showed analogous loss of the Kv2.1 staining compared with the age-matched WTs at over 1 yr of age (data not shown).

**DISCUSSION**

Genetic mouse models of HD are providing important information about early morphological and electrophysiological cellular alterations and demonstrate that these dysfunctions can support symptoms even in the absence of prominent cell death (Klapstein et al. 2001; Laforet et al. 2001; Levine et al. 2004). Morphological and electrophysiological changes have been found in several of these models. Reductions of somatic areas and dendritic fields, as well as loss of spines, occur in striatal and cortical projection neurons in the R6/2 and other mouse models (Klapstein et al. 2001; Laforet et al. 2001; Levine et al. 1999). Concurrent electrophysiological changes have been reported and encompass three principal areas: alterations in receptor sensitivity, in synaptic communication, and in some voltage-gated conductances.

Our laboratory first reported increased striatal sensitivity to NMDA receptor activation, whereas responses to α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) were not affected (Levine et al. 1999). This observation was further supported by electrophysiological and Ca²⁺ imaging evidence demonstrating increased responsiveness to NMDA in a subset of MSNs (Cepeda et al. 2001a; Zeron et al. 2002). Changes in postsynaptic receptor sensitivity were probably related to changes in synaptic activity. Our own studies demonstrated that MSNs from R6/2 transgenics require more intensity of stimulation to induce postsynaptic potentials (Klapstein et al. 2001; Cepeda et al. 2001a; Zeron et al. 2002).
This is likely due to the loss of pre- and postsynaptic protein markers and synaptic contacts leading to a significant reduction in spontaneous synaptic activity as the disease progresses (Cepeda et al. 2003). Increases in GABA synaptic activity occur concomitantly in R6/2 mice (Cepeda et al. 2004). Alterations in voltage-gated Ca\(^{2+}\)/H\(^{+}\) channels have also been found in the R6/2 model. For example, there is a significant reduction in the amplitude of voltage-gated Ca\(^{2+}\)/H\(^{+}\) currents as the disease progresses (Bibb et al. 2000; Cepeda et al. 2001a). Less is known about changes in other voltage-gated channels. The present study demonstrated that reductions in inward and outward K\(^{+}\)/H\(^{+}\) conductances, as well as in expression of specific K\(^{+}\) channel subunits, occurred in MSNs of transgenic mouse models of HD. These abnormal K\(^{+}\) conductances may contribute to altered passive and active membrane properties in the MSN, providing evidence for membrane disturbances likely resulting directly or indirectly from the insertion of mutated huntingtin. Depolarized membrane potentials were observed in the R6/2 but not in the TgCAG100, whereas other measures such as capacitance and input resistance were similarly altered. Examination of early time points (5–7 wk in the R6/2) did not demonstrate changes in the HD transgenic model compared with control littermates. The reductions in Kir2.1 and Kir2.3 channel proteins contributing to the inwardly rectifying K\(^{+}\) currents, plus attenuation of Kv2.1 protein forming a component of the ionophore of the delayed rectifying K\(^{+}\) currents were contemporaneous with aberrant electrophysiology associated with the transgenic HD models. Abnormal K\(^{+}\) conductances were more severe in the R6/2 than in the TgCAG100 model, indicating that CAG repeat number and length and expression of the transgene have an effect. This may relate to the more protracted nature and reduced severity of symptoms in the TgCAG100 model (Laforet et al. 2001; Levine et al. 2004).

K\(^{+}\) conductances have diverse functions that regulate membrane potentials and the excitability of neurons (Hille 2001; Lesage et al. 1994; Neusch et al. 2003). The decrease in K\(^{+}\) currents in HD mice should have major effects on the functioning of MSNs. One effect of reduced Kir channels would be an increase in membrane input resistance observed in most mouse models of HD (Cepeda et al. 2001a; Klapstein et al. 2001; Laforet et al. 2001). It has been demonstrated that Cs\(^{+}\) blockade of inward rectifier conductances is sufficient to explain the increase in dendritic input resistance (Reyes et al. 1998). Depressive thinning and loss of spines, which are characteristic findings in MSNs from symptomatic HD mice (Klapstein et al. 2001), also would contribute to the increase in input resistance measured.

K\(^{+}\) channels also help preserve the membrane potential of MSNs at a highly hyperpolarized state (Nisenbaum et al. 1996) and the Kir current accounts for ~50% of the resting conductance of MSNs within the striatal complex (Jiang and North 1991; Uchimura et al. 1989). Thus decreases in inward rectification would contribute to depolarized RMPs of MSNs in the
R6/2 model (Klapstein et al. 2001). This decrease, in conjunction with the attenuation of delayed rectifying K\(^+\) currents, would affect the excitability of MSNs (Nisenbaum and Wilson 1995) and alter their normal ability to oscillate between the “up” and “down” states (Wilson and Kawaguchi 1996). Thus the “up” state is produced by excitatory synaptic inputs from the cortex, but the membrane potential during the “up” state is partially determined by dendritic K\(^+\) channels (Wilson and Kawaguchi 1996).

The outcome of K\(^+\) channel alterations on cellular excitability and synaptic integration in MSNs are very complex because many other cellular parameters are also altered, at least in the R6/2 model. A reduction in inward and outward rectification, leading to or in conjunction with increased input resistance, would predict increased cellular excitability. Indeed, previous studies demonstrated that the current necessary to evoke action potentials, rheobase, is reduced in R6/2 animals (Klapstein et al. 2001). However, the finding that voltage-gated Ca\(^2+\) potentials, rheobase, is reduced in R6/2 animals (Klapstein et al. 2001). This decrease, in conjunction with the attenuation of delayed rectifying K\(^+\) currents, would affect the excitability of MSNs (Nisenbaum and Wilson 1995) and alter their normal ability to oscillate between the “up” and “down” states (Wilson and Kawaguchi 1996). Thus the “up” state is produced by excitatory synaptic inputs from the cortex, but the membrane potential during the “up” state is partially determined by dendritic K\(^+\) channels (Wilson and Kawaguchi 1996).

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Another consequence of K\(^+\) channel impairment is that affected MSNs may be more sensitive to glutamate, activating not only AMPA/kainate receptors, but also NMDA receptors that would no longer be as voltage dependent because of depolarized RMPs. As indicated in the preceding text, we have demonstrated enhancements in NMDA receptor responses in subpopulations of MSNs in multiple mouse models of HD (Cepeda et al. 2001a; Laforet et al. 2001; Levine et al. 1999). In parallel, the immunofluorescent staining of the obligatory NR1 subunits of the NMDA receptor was elevated in MSNs in HD transgenics (Cepeda et al. 2001a).

At present, little is known about the possible molecular mechanisms leading to altered K\(^+\) channel function in these mouse models of HD and more studies are needed. However, recent data suggest a novel mechanism by which pathological-length polyglutamine proteins disrupt feedback mechanisms regulating the polyamine production pathway, leading to the reduction of free intracellular spermine levels (Colton et al. 2004). The inward rectification property of the K\(^+\) channels is conferred by Mg\(^{2+}\) (Matsuda 1988; Vandenberg 1987) and by cytoplasmic polyamines such as spermine (Ficker et al. 1994; Lopatin et al. 1994).

In conclusion, our interpretation of the present data is that the HD-vulnerable MSNs would be more receptive to excitotoxicity due to loss of intrinsic voltage regulation mechanisms produced by simultaneous attenuation of K\(^+\) fluxes and enhanced NMDA receptor responsiveness. This cellular dysfunction would precipitate the behavioral phenotype associated with the HD transgenic models. Because the MSNs are a vital component of the basal ganglia circuitry, decreases in K\(^+\) conductances also would affect afferent and efferent information processing upstream and downstream to the neuron. Dysfunctions in other parts of the basal ganglia would exacerbate the HD abnormalities.

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