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BRAIN RESEARCH

Research Report

Normal electrical properties of hippocampal neurons modelling early Huntington disease pathogenesis

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ABSTRACT

Huntington disease (HD) is a neurodegenerative disorder caused by an unstable and progressive expansion of a CAG trinucleotide repeat tract in the HD gene. Previous studies using truncated forms of the HD gene have shown pronounced deficits in synaptic transmission and plasticity but rather modest changes in intrinsic cellular properties, despite overt pathology. The knock-in mice carrying a 72–80 CAG repeat mutation is an accurate genetic model of early stage HD, displaying a more subtle disease phenotype. To relate full-length HD gene expression and differential polyglutamine expansion with possible pathophysiological changes in salient electrophysiological properties of neurons that may underlie early symptoms of HD, including mood and cognitive impairments, we have conducted whole-cell recordings from hippocampal area CA1 pyramidal cells in Hdh6/Q72 and Hdh4/Q80 knock-in mice. Electrophysiological characterisation of cells obtained from young adult (<4 months) HD mice harbouring an expanded CAG repeat stretch and age-matched wild type (WT) mice revealed no significant differences in any of the active or passive membrane properties investigated. Similar findings, showing a lack of significant differences in cellular electrical properties, were obtained from cells of aged (>18 months) HD mice and WT controls, despite modest levels of repeat length variability demonstrated by single cell PCR. Thus, the current study indicates a lack of overt changes in the electrical membrane properties of pyramidal cells in HD mice accurately modelling early stage HD pathology. Furthermore, together with our previous work, these findings point to a synaptic rather than cellular locus of HD-related pathology.

1. Introduction

An unstable expansion of a normally occurring trinucleotide (CAG) repeating sequence in the *HD* gene located at 4p16.3 results in the expression of a debilitating neurodegenerative disorder called Huntington disease (Huntington's Disease Collaborative Research Group, 1993). Unaffected chromosomes typically have a short (<30) polymorphic CAG repeat sequence in the *HD* gene that encodes a polyglutamine tract at the N-terminus of the huntingtin protein. In HD the CAG repeat number exceeds 36 and demonstrates significant length changes ('mosaicism' or 'instability') in both somatic and germline tissues The size of the inherited CAG repeat correlates strongly with age at onset of the disease so that individuals with repeats of >65 often develop symptoms during childhood.

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Abbreviations: HD, Huntington disease; WT, wild type; AHP, afterhyperpolarisation

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The pathology of Huntington disease is associated most strongly with the unique pattern of degeneration found in the striatum in which selective loss of medium spiny neurons (Graveland et al., 1985) accounts for end-stage reductions in striatal volume of up to 90% (Vonsattel et al., 1985). However, the striatum is not the only region of the brain to experience neuronal loss as end-stage HD pathology is associated with degeneration in the basal ganglia (Lange et al., 1976; Mann et al., 1993), cerebral cortex (Tellez-Nagel et al., 1974; Cudkowicz & Kowall, 1990; Macdonald & Halliday, 2002; Rosas et al., 2003), cerebellum (Jeste et al., 1984) and hippocampus (Spargo et al., 1993). In addition, reductions in the volume of white matter in the brain (de la Monte et al., 1988) suggest possible changes to axonal connections within and between different brain structures. The time course of extra-striatal pathology and the events leading up to neuronal death are not well understood. However, hippocampal-related cognitive defects, including memory and information-processing deficits, mood changes, aggressive behaviour and disruptions in spatial working memory, are among the earliest symptoms experienced by some HD patients, and can occur before the movement disorder (Lawrence et al., 1996, 1998; Jason et al., 1997). Little is known about the functioning of neurons in the brains of HD patients prior to degeneration and how the disease process alters or affects their functioning.

In a previous study we developed a 'knock-in' HD mouse model carrying ~80 CAG repeats inserted into an endogenous copy of the mouse *Hdh* gene (Shelbourne et al., 1999) to demonstrate that full length mutant huntingtin protein disturbs hippocampal synaptic transmission and plasticity (Usdin et al., 1999). Subsequent studies, focusing upon models of HD using mice harbouring a shortened (truncated) form of the HD gene, have reported similar impairments in hippocampal synaptic plasticity but also additionally report rather subtle alterations in the intrinsic electrophysiological properties of hippocampal neurons (Murphy et al., 2000). Alterations in intrinsic electrophysiological properties have also been shown in striatal neurons of mice encoding the first one-third of mutant huntingtin protein (Laforet et al., 2001; Klapstein et al., 2001). Such mice exhibit a rapidly progressive phenotype and thus it remains to be established whether alterations in intrinsic biophysical properties can be observed in full-length HD mice which more accurately model early-stage HD. Furthermore, it remains to be established whether CAG repeat length expansion which occurs with the full-length mutant allele (Kennedy and Shelbourne, 2000; Kennedy et al., 2003) may expedite cell-specific alterations in neuronal properties at the level of the single cell.

The current study reports a combined electrophysiological/molecular approach to investigate the impact of the full-length mutant huntingtin protein on the salient electrophysiological properties of principal cells in area CA1 of the hippocampus.

2. Results

In this study the electrophysiological properties of CA1 pyramidal neurons were investigated using brain slices acutely prepared from both young adult (<17 weeks of age) and aged adult (>76 weeks) knock-in HD mice and their age-matched WT littermates. The HD and WT cohorts were indistinguishable in terms of appearance and gross behavioural signs. However, previous work has shown that the HD mice exhibit subtle but progressive deficits in motor performance (Kennedy et al., 2003).

2.1. *Electrophysiological investigations of hippocampal CA1 neurons in HD mice*

Current-clamp recordings were obtained from 62 CA1 pyramidal cells in brain slices prepared from HD and age-matched WT mice. All cells reported here displayed electrophysiological characteristics consistent with those of pyramidal neurons. Cells displaying fast spiking activity, little spike frequency adaptation or fast, deep afterhyperpolarisations characteristic of cell body layer interneurons (Buhl et al., 1995) were excluded from further analysis. The first cohort of HD mice studied ranged in age from 11–17 weeks (mean 14±1.8 weeks, $n=12$). Electrical properties (passive and active membrane characteristics) were compared with those obtained from similarly aged (7–16 weeks) WT littermates (mean 11.6±0.6 weeks, $n=21$). The range of active and passive properties assessed, including membrane time constant, input resistance, resting membrane potential, action potential threshold, action potential rise and decay kinetics and action potential discharge in response to depolarising current injection are summarised in Table 1 and shown in graphical format in Fig. 1.

Overall, the scatter of data was very similar for pyramidal cells derived from both HD and WT mice across all parameters measured, with statistical analyses showing no significant differences (ANOVA, $P>0.05$ for all comparisons) between cohorts.

As the HD mice display age-dependent changes in phenotype (Li et al., 2000, Li et al., 2001, Kennedy et al., 2003), an older cohort of HD mice was also investigated. These mice ranged in age from 76–109 weeks (mean 97±2.5 weeks, $n=18$). Cellular electrophysiological properties were again compared with cells obtained from similarly aged WT littermates (81–110 weeks, mean 99.67±3.1 weeks, $n=11$) as shown in Table 1 and Fig. 1. As with the neurons derived from the younger mice, statistical comparison of the salient electrophysiological properties revealed no significant difference between HD and WT groups (ANOVA, $P>0.05$ for all comparisons).

Finally, age-related changes in the electrophysiological properties of young and old sample groups were investigated. The pooled population of cells derived from old HD and WT animals showed a significantly larger amplitude afterhyperpolarisation (3.7±0.5 mV at peak; 15 cells at −74±1.6 mV) when compared to the population of cells derived from young HD and WT animals (2.1±0.3 mV peak amplitude, 16 cells at −75±1.6 mV, $P=0.0009$, ANOVA). This age-related increase in AHP amplitude following single action potentials mirrors the commonly reported age-related increase of the slow AHP that follows bursts of action potentials (Landfield and Pitler, 1984; Moyer et al., 1992; Power et al., 2002). No significant age-related alterations were detected in any of the other electrophysiological properties investigated (all $P>0.05$, ANOVA).