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Regional and genotypic differences in intrinsic electrophysiological properties of cerebellar Purkinje neurons from wild-type and dystrophin-deficient *mdx* mice

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ABSTRACT

Cerebellar subregions are recognized as having specialized roles, with lateral cerebellum considered crucial for cognitive processing, whereas vermal cerebellum is more strongly associated with motor control. In human Duchenne muscular dystrophy, loss of the cytoskeletal protein dystrophin is thought to cause impairments in cognition, including learning and memory. Previous studies demonstrate that loss of dystrophin causes dysfunctional signaling at γ-aminobutyric acid (GABA) synapses on Purkinje neurons, presumably by destabilization of $GABA_A$ receptors. However, potential differences in the intrinsic electrophysiological properties of Purkinje neurons, including membrane potential and action potential firing rates, have not been investigated. Here, using a 2 × 2 analysis of variance (ANOVA) experimental design, we employed patch clamp analysis to compare membrane properties and action potentials generated by acutely dissociated Purkinje neurons from vermal and lateral cerebellum in wild-type (WT) mice and *mdx* dystrophin-deficient mice. Compared to Purkinje neurons from WT mice, neurons from *mdx* mice exhibited more irregular action potential firing and a hyperpolarization of the membrane potential. Firing frequency was also lower in Purkinje neurons from the lateral cerebellum of *mdx* mice relative to those from WT mice. Several action potential waveform parameters differed between vermal and lateral Purkinje neurons, irrespective of dystrophin status, including action potential amplitude, slope (both larger in the vermal region), and duration (shorter in the vermal region). Moreover, the membrane potential of Purkinje neurons from the vermal region of WT mice exhibited a significant hyperpolarization and concurrent reduction in the frequency of spontaneous action potentials compared to Purkinje neurons from the lateral region. This regional hyperpolarization and reduction in spontaneous action potential frequency was abolished in *mdx* mice. These results from mice demonstrate the presence of differential electrophysiological properties between Purkinje neurons from different regions of the WT mouse cerebellum and altered intrinsic membrane properties in the absence of dystrophin. These findings provide a possible mechanism for the observations that absence of cerebellar dystrophin contributes to deficits in mental function observed in humans and mouse models of muscular dystrophy. Moreover, these results highlight the importance of distinguishing functional zones of the cerebellum in future work characterizing Purkinje neuron electrophysiology and studies using the model of dissociated Purkinje neurons from mice.

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive human genetic disorder that abolishes expression of dystrophin (Hoffman, Brown, & Kunkel, 1987). In muscle, dystrophin localizes along the sarcolemma where it forms a key component of the dystrophin-associated glycoprotein complex (DGC) (Ervasti, Ohlendieck, Kahl, Gaver, & Campbell, 1990). In DMD, the lack of dystrophin in muscle leads to muscle necrosis (Hoffman et al., 1987) that is ultimately fatal. In addition to the 427 kDa full-length isoform of dystrophin present in muscle (M-type dystrophin), there are two other promoter-specific full-length isoforms in the central nervous system (CNS) (Gorecki et al., 1992): B-type dystrophin is found in pyramidal neurons of the cerebral cortex and hippocampus, and P-type dystrophin resides exclusively in cerebellar Purkinje neurons (Gorecki, Abdulrazzak, Lukasiuk, &

Abbreviations: ANOVA, analysis of variance; CNS, central nervous system; CV, coefficient of variation; DGC, dystrophin-associated glycoprotein complex; DMD, Duchenne muscular dystrophy; EGTA, ethylene glycol tetraacetic acid; GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; MW, Mann–Whitney; RT, room temperature; WT, wild-type.

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Barnard, 1997; Gorecki et al., 1992). Truncated isoforms of dystrophin (Dp71, Dp116, Dp140, and Dp260) are also located in the periphery and CNS (as reviewed in Perronnet & Vaillend, 2010).

In addition to well-described muscle pathology (as reviewed in Wallace & McNally, 2009), cognitive deficits are also associated with DMD, presumably related to loss of dystrophin from the CNS (Kim, Wu, & Black, 1995; Uchino, Teramoto, et al., 1994). Among those with DMD, full-scale IQ scores follow a normal distribution that is shifted one standard deviation below the population mean, with a higher prevalence of mental retardation (Cotton, Voudouris, & Greenwood, 2001). As well, general academic achievement is lower among boys with DMD compared to their unaffected siblings (Hinton, De Vivo, Fee, Goldstein, & Stern, 2004; Hinton, De Vivo, Nereo, Goldstein, & Stern, 2001). Specific cognitive deficits are observed in those with DMD, including deficits in verbal working memory, as measured by performance on the Digit Span subtest (Anderson, Routh, & Ionasescu, 1988; Dorman, Hurley, & D'Avignon, 1988; Hinton, De Vivo, Nereo, Goldstein, & Stern, 2000; Leibowitz & Dubowitz, 1981; Ogasawara, 1989; Whelan, 1987), and impairments in executive function (Donders & Taneja, 2009; Mento, Tarantino, & Bisiacchi, 2011; Wicksell, Kihlgren, Melin, & Eeg-Olofsson, 2004). Deficits in verbal working memory persist when analysis accounts for general intelligence (Hinton et al., 2000).

Given the strong presence of dystrophin in cerebellar Purkinje neurons relative to other brain regions (i.e., the cerebral cortex and hippocampus) (Lidov, Byers, Watkins, & Kunkel, 1990) and the particular cognitive deficits associated with DMD (i.e., verbal working memory), there is speculation that dystrophin, specifically the lack of P-type dystrophin (herein referred to as "dystrophin") in Purkinje neurons, may account for impaired verbal working memory (Cyrulnik & Hinton, 2008). This notion is based on the idea that lack of dystrophin would disrupt rehearsal of information in the cerebrocerebellar loops that emanate from the lateral cerebellum (Cyrulnik & Hinton, 2008). Additional evidence to support the role of dystrophin in cognition comes from studies of *mdx* mice, the murine model of DMD in which both brain and muscle full-length dystrophin isoforms are absent (Uchino, Yoshioka, et al., 1994). These mice display cognitive impairments, including deficits in passive-avoidance (Muntoni, Mateddu, & Serra, 1991) and spatial learning (Vaillend, Billard, & Laroche, 2004), as well as memory deficits (Vaillend, Rendon, Misslin, & Ungerer, 1995).

Although the cerebellum is traditionally viewed as a structure dedicated to the regulation of motor output (i.e. balance, coordination, posture), this view is changing to include regulation of several non-motor abilities, including attention (Allen & Courchesne, 2003), reading ability (Fulbright et al., 1999), and working memory (Chen & Desmond, 2005). The diverse functions subserved by the cerebellum are anatomically confined to specific circuits within the cerebellum, where functional zones have been characterized based on both anatomical and functional distinctions (i.e. the vestibulocerebellum, consisting of the flocculonodular lobe; the spinocerebellum, which includes the vermis, and the cerebrocerebellum, consisting of the lateral cerebellar hemispheres (Dow, 1961)). Located medially, the vermal region projects mainly to the spinal cord and is associated with motor function (Joyal et al., 1996; Nyberg-Hansen & Horn, 1972), whereas the bilateral hemispheres ("lateral cerebellum") receive input from and project to the cerebral cortex (Middleton & Strick, 1997). Specifically, the lateral hemispheres are implicated in cerebellar-mediated cognition and learning (Allen, Buxton, Wong, & Courchesne, 1997; Decety, Sjoholm, Ryding, Stenberg, & Ingvar, 1990; Joyal, Strazielle, & Lalonde, 2001; Joyal et al., 1996; Nyberg-Hansen & Horn, 1972; Ryding, Decety, Sjoholm, Stenberg, & Ingvar, 1993).

In support of a cerebellar cause for the mental deficits observed in the absence of dystrophin, expression of the dystrophin protein is strongest in the cerebellum (Lidov et al., 1990) where it is restricted to the cytoplasmic surface of somatic and dendritic membranes of Purkinje neurons. Moreover, although dystrophin is present in Purkinje neurons of both vermal and lateral regions, punctal density is higher in the lateral vs. the vermal region in mice (Snow, Fry, & Anderson, 2013). Immunohistochemical studies demonstrate extensive colocalization of dystrophin and $GABA_A$ neurotransmitter receptor subunits in the postsynaptic membrane (Knuesel et al., 1999). Although absolute levels of $GABA_A$ receptor subunit protein are not affected in the *mdx* mouse brain (Kueh, Head, & Morley, 2008), the number of $GABA_A$ receptor subunits at postsynaptic sites is decreased in the *mdx* mouse cerebellum (Knuesel et al., 1999), with an increase in the number of extrasynaptic $GABA_A$ receptor subunits (Kueh, Dempster, Head, & Morley, 2011). These findings strongly implicate dystrophin in stabilization and maintenance of $GABA_A$ receptors in Purkinje neurons. Further to this, electrophysiological studies have demonstrated reductions in the inhibitory drive to Purkinje neurons (Anderson, Head, & Morley, 2003; Kueh et al., 2008, 2011) and in the magnitude of expression of long-term depression (LTD) (Anderson, Head, & Morley, 2004) in *mdx* mice relative to WTs.

Intriguingly, in addition to a putative role in $GABA_A$ receptor anchoring, dystrophin in the CNS forms a multi-protein DGC similar to that seen in muscle (Waite, Tinsley, Locke, & Blake, 2009). In brain tissue, members of the DGC, including dystrophin and syntrophin, interact with several ion channels, including voltage-gated Na^+ channels (Gee et al., 1998) and inward rectifier K^+ channels (Connors, Adams, Froehner, & Kofuji, 2004; Leonoudakis et al., 2004). Loss of dystrophin then could easily disrupt the intrinsic membrane properties of Purkinje neurons and disrupt output from the cerebellum.

In the typical cerebellum, differences in electrophysiological properties of Purkinje neurons based on functional region are only beginning to be recognized. Recently, differences in Purkinje neuron electrophysiology were noted in various lobules of the vermis as a function of their afferent input (vestibular vs. spinal cord) (Kim et al., 2012). While the findings of Kim et al. (2012) suggest that Purkinje neurons in the lateral cerebellum may differ from those of the vermal region, given their separable functional roles and their distinct connectivity with other brain regions, patch clamp analysis has not been carried out on neurons from the two regions. Therefore, in order to investigate the role of dystrophin in determining electrical properties of Purkinje neurons and gain an understanding of potential differences in contributions of dystrophin in vermal vs. lateral cerebellum, we used a 2×2 ANOVA experimental design to simultaneously investigate differences in intrinsic electrical properties of acutely dissociated Purkinje neurons based on cerebellar region (i.e., vermal and lateral) and dystrophin status (i.e., WT and *mdx* mice).

2. Materials and methods

2.1. Purkinje neuron dissociation

Dystrophic *mdx* mice ($n = 20$) and WT mice from the same background strain, C57BL10 ($n = 15$) (Jackson Laboratories, Bar Harbor, ME, USA) were used. All procedures were carried out using methods approved by the institutional Animal Care and Use Protocol Review Committee at the University of Manitoba. The dissociation protocol was modified from that used by Raman and Bean (1997). Given that the window for obtaining quality recordings from dissociated Purkinje neurons is limited to approximately 6 h, tissue was extracted from only one region/mouse. Mice at postnatal day (P) 16–21 were decapitated, and the head immediately placed in oxygenated Tyrode's solution