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BRAIN RESEARCH

Research Report

Loss of neuronal projections in the dystrophin-deficient *mdx* mouse is not progressive

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ARTICLE INFO

Article history:
Accepted 17 May 2008
Available online 4 July 2008

Keywords:
Dystrophin
Neuronal loss
Duchenne's muscular dystrophy
Red nucleus
Trigeminal sensory complex
Aging

ABSTRACT

Lack of dystrophin is known to reduce several cerebral fiber systems. To investigate if the loss of fibers is progressive, we analyzed projections of the trigeminal sensory system to the red nucleus in 3, 6, and 12 month old dystrophin-deficient *mdx* mice. The retrograde tracer fluorogold was injected in the magnocellular part of the red nucleus, and the number of labeled neurons in the oral part of the spinal trigeminal nucleus (Sp5O) was counted. We found that the number of labeled Sp5O neurons was reduced by 50% in *mdx* mice compared to age-matched control mice. The number of labeled Sp5O neurons did not change significantly between 3 and 12 months neither in *mdx* nor in control mice. In addition, the number of labeled neurons in the interstitial system of the trigeminal nerve was reduced by 43% in *mdx* mice. We conclude that fiber loss did not continue beyond the age of 3 months. Our data suggest that lack of full-length dystrophin impairs neuronal migration or axonal outgrowth, or increases neuronal death during fetal or early life.

1. Introduction

Duchenne muscular dystrophy (DMD) is a muscle disease caused by a recessive mutation in the dystrophin gene, localized in the Xp21 region of the X chromosome. Dystrophin is part of a protein complex that connects the actin cytoskeleton to the extracellular matrix. Loss of dystrophin increases contraction-induced damage to the sarcolemma (Straub et al., 1997), leading to progressive muscle necrosis.

DMD patients also quite commonly present mild cognitive defects, including memory deficiencies and reduced reading and math skills (Anderson et al., 2002; Cyrulnik and Hinton, 2008), and increased incidence of psychiatric problems, including depression, hypochondria, and anxiety (Sekiguchi, 2005). These defects do not appear to be progressive (Lidov, 1996). The lack of dystrophin also reduces cortical excitability and affects cerebral and cerebellar metabolic rate (Mehler, 2000; Anderson et al., 2002).

There are several ways in which the lack of dystrophin can affect the brain. First, dystrophin is expressed in the developing brain (Houzelstein et al., 1992; Sogos et al., 2002), and mutations that affect the dystrophin complex can affect neuronal migration and differentiation (Mehler, 2000; Moore et al., 2002; Montanaro and Carbonetto, 2003; Endo and Toda,

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Abbreviations: DMD, duchenne muscular dystrophy; Sp5O, oral part of the spinal trigeminal nucleus

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doi:10.1016/j.brainres.2008.05.059

2003; Haenggi and Fritschy, 2006). Second, lack of dystrophin affects neuronal excitability. Dystrophin is found in the postsynaptic apparatus (Kim et al., 1992), serving to anchor receptors, including the $GABA_A$ receptor (Yoshihara et al., 2003; Haenggi and Fritschy, 2006). Lack of dystrophin also affects long-term synaptic plasticity (Vaillend and Billard, 2002). Third, loss of dystrophin may lead to neuronal death (Jagadha and Becker, 1988). It leaves the neuron more susceptible to metabolic and physiological insults (Menke and Jockusch, 1991; Culligan and Ohlendieck, 2002). Motorneurons may die when their dystrophin-deficient targets degenerate (Sogos et al., 2002).

The *mdx* mouse has a mutation that prevents the production of the full-length dystrophin molecule, although the production of shorter isoforms is intact. This mutant has relatively mild but widespread changes in the brain, including a reduced number of projections to the spinal cord from the cerebral cortex (Sbriccoli et al., 1995) and red nucleus (Carretta et al., 2001), and changes in the number of certain types of interneurons in the cerebral cortex (Carretta et al., 2003; Carretta et al., 2004).

We recently described that the number of neurons in the trigeminal sensory complex that is labeled by injection of the retrogade tracer fluorogold in the red nucleus is reduced by 50% in 3 month old *mdx* mice (Pinto et al., 2007a). This suggests that the number of neurons in the trigeminal sensory complex that project to the red nucleus is reduced in 3 month old *mdx* mice. To examine if neuronal loss in *mdx* mice is progressive, we injected fluorogold in the red nucleus of 6 and 12 month old *mdx* and control mice, and counted the number of neurons in the oral part of the spinal trigeminal nucleus that project to the red nucleus. Data were compared with data from 3 month old mice published previously (Pinto et al., 2007a).

2. Results

The caudal (magnocellular) part of the red nucleus receives the large majority of the projections from the trigeminal sensory complex to the red nucleus. We selected 3, 6, and 12 month old *mdx* and control mice that had similar fluorogold injections (between 3.4 and 3.8 mm rostral of the bregma) in the magnocellular part of the red nucleus, and compared the number of labeled neurons in the oral part of the contralateral spinal trigeminal nucleus (Sp5O). The number of fluorogold-labeled cell bodies in the oral subnucleus of the spinal trigeminal nucleus was determined as the total of labeled cells in each section that contained this subnucleus (8 sections per mouse). Fig. 1 shows that the number of labeled Sp5O neurons in *mdx* mice was much lower than in control mice ($p < 0.001$, two-way ANOVA). The number of labeled Sp5O neurons did not fall significantly with age, neither in control ($p > 0.4$, one-way ANOVA) nor in *mdx* mice ($p > 0.2$). Thus, the difference between *mdx* and control mice of the same age was constant (50.7%, 48.9%, and 48.9% at ages of 3, 6, and 12 month).

Labeling also appeared reduced in the other components of the trigeminal sensory complex of *mdx* mice (mesencephalic trigeminal nucleus, principal sensory trigeminal nucleus, and intermediate and caudal region of the spinal trigeminal nucleus, Fig. 2), similar to results described previously for 3 month old mice (Pinto et al., 2007a).

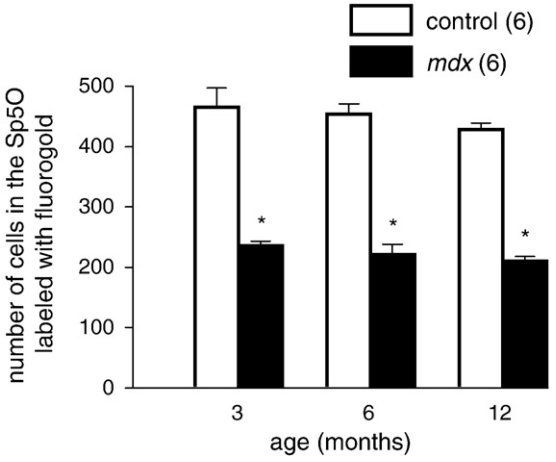

Fig. 1 – Number of neurons in the oral part of the spinal trigeminal nucleus (Sp5O) labeled by injection of fluorogold in the magnocellular red nucleus in control and dystrophic (*mdx*) mice of various ages. Error bars are SEM. *Difference between *mdx* and age-matched control ($p < 0.001$, Bonferroni's test, all groups $n = 6$).

The magnocellular part of the red nucleus also receives projections from the interstitial system of the trigeminal tract (Pinto et al., 2007b), a region that includes the paratrigeminal nucleus, the dorsal paramarginal nucleus, the insular trigemeo-lateral cuneate nucleus, the trigeminal extension of the parvocellular reticular formation, and lamina I and II neurons (Phelan and Falls, 1989). In the present study, we quantified the number of fluorogold-labeled neurons in this system in 3 month old *mdx* and control mice with comparable fluorogold injections (Table 1). The number of labeled neurons in the interstitial system of the trigeminal tract was reduced by 43% in *mdx* mice (48 ± 2 vs. 27 ± 2 cells, both groups $n = 6$, $p < 0.001$, t test).

3. Discussion

Our data show that the loss of Sp5O neurons that project to the red nucleus is not progressive in *mdx* mice, at least with ages between 3 and 12 months. The trigeminal sensory complex normally contains full-length dystrophin (Lidov et al., 1993), although the level is less than in the cerebral and cerebellar cortex. It has been suggested that the loss of hippocampal neurons in *mdx* mice could be due to cumulative insult-induced damage (Mehler et al., 1992), but our results suggest that other mechanisms may contribute to loss of neurons. Our data are in accordance with the non-progressive course of the mental impairments in human DMD patients (Lidov, 1996). Our results also are compatible with MRI studies that show that N-acetyl aspartate levels in the brain of *mdx* mice (age 2–5 months) are normal (Tracey et al., 1996), suggesting that extensive neuronal loss does not occur at this age in the *mdx* brain. Finally, even old *mdx* mice do not have gross brain