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Research Report

Quantitative changes of nicotinic receptors in the hippocampus of dystrophin-deficient mice

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ABSTRACT

Lack of dystrophin in Duchenne muscle dystrophy (DMD) and in the mutant *mdx* mouse results in progressive muscle degeneration, structural changes at the neuromuscular junction, and destabilization of the nicotinic acetylcholine receptors (nAChRs). One-third of DMD patients also present non-progressive cognitive impairments. Considering the role of the cholinergic system in cognitive functions, the number of nAChR binding sites and the mRNA levels of α4, β2, and α7 subunits were determined in brain regions normally enriched in dystrophin (cortex, hippocampus and cerebellum) of *mdx* mice using specific ligands and reverse-transcription polymerase chain reaction assays, respectively. Membrane preparations of these brain regions were obtained from male control and *mdx* mice at 4 and 12 months of age. The number of [^{3}H]-cytisine (α4β2) and [^{125}I]-α-bungarotoxin ([^{125}I]-αBGT, α7) binding sites in the cortex and cerebellum was not altered with age or among age-matched control and *mdx* mice. A significant reduction in [^{3}H]-cytisine (48%) and [^{125}I]-αBGT (37%) binding sites was detected in the hippocampus of *mdx* mice at 12 months of age. When compared with the age-matched control groups, the *mdx* mice did not have significantly altered [^{3}H]-cytisine binding in the hippocampus, but [^{125}I]-αBGT binding in the same brain region was 52% higher at 4 months and 20% lower at 12 months. mRNA transcripts for the nAChR α4, β2, and α7 subunits were not significantly altered in the same brain regions of all animal groups. These results suggest a potential alteration of

Abbreviations: αBGT, α-Bungarotoxin; nAChR, Nicotinic acetylcholine receptor; CA1, Cornus ammonis 1 of hippocampus; cDNA, Complementary DNA; CNS, Central nervous system; DGC, Dystrophin glycoprotein complex; DMD, Duchenne muscle dystrophy; dNTPS, Deoxynucleotide triphosphates; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic acid; $GABA_AR$, γ-Aminobutyric acid subtype A receptor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; mRNA, Messenger ribonucleic acid; NMDA, N-methyl-D-aspartate; PMSF, Phenylmethyl sulphonyl fluoride; RNAse, Ribonuclease; RT-PCR, Real-time polymerase chain reaction; Tris–HCl, Tris(hydroxymethyl)aminomethane hydrochloride

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the nicotinic cholinergic function in the hippocampus of dystrophin-deficient mice, which might contribute to the impairments in cognitive functions, such as learning and memory, that have been reported in the dystrophic murine model and DMD patients.

1. Introduction

Duchenne muscle dystrophy (DMD) is an X-linked muscle disease that manifests as a progressive and irreversible muscle degeneration with a high incidence in boys (Emery, 2002). The myopathy is caused by mutations in the dystrophin gene resulting in a lack of protein expression (Hoffman et al., 1987). One-third of DMD patients also present non-progressive cognitive impairment, as well as behavioral and neuropsychiatric disorders of variable intensities (Cyrulnik et al., 2008; Hendriksen and Vles, 2008; Mehler, 2000).

Dystrophin is a 427-kDa protein expressed in striated muscles and the central nervous system (CNS). It is localized at the cytoplasmic face of the plasma membrane linking the intracellular cytoskeleton with the extracellular matrix (Ervasti, 2007; Pilgram et al., 2010). Three full-length dystrophin (Dp427) isoforms, which are transcribed from distinct promoters of the *DMD* gene, are expressed in muscles (Dp427M), throughout the brain (Dp427B), and in cerebellar Purkinje cells (Dp427P). Four other shorter isoforms (Dp260, Dp140, Dp116, and Dp71), regulated by internal promoters, are expressed in the CNS and other tissues (Blake and Kroger, 2000; Perronnet and Vaillend, 2010). The mutant *mdx* mouse is a well-studied model of DMD that lacks the expression of full-length dystrophin in both the muscles and brain (Sicinski et al., 1989). At the cell membrane, dystrophin is associated with a complex of glycoproteins (DGC) comprised of dystroglycans, syntrophins, dystrobrevins, sarcoglycans, and sarcospan (Blake et al., 2002; Ervasti, 2007). In striated muscles, the lack of dystrophin disrupts the macromolecular complex and damages the plasma membrane, resulting in muscle degeneration and necrosis (Petrof, 2002). At the neuromuscular synapse, dystrophin and some components of the DGC are necessary for the maturation of post-junctional folds and the regulation of nicotinic acetylcholine receptors (nAChRs) (Ghedini et al., 2008; Grady et al., 2000; Huh and Fuhrer, 2002). The protein complex also plays a role in Ca^{2+} homeostasis and cell signaling pathways involved in the maintenance of cell membrane integrity (Batchelor and Winder, 2006).

In normal brains, dystrophin is abundant in the cerebral cortex, hippocampus, cerebellum, and amygdala, where it is located at postsynaptic densities of the neuronal synapses (Lidov, 1996; Sakamoto et al., 2008; Sekiguchi et al., 2009). In the hippocampus, cerebellum (Knuesel et al., 1999), and amygdala (Sekiguchi et al., 2009) of the *mdx* mouse, the lack of dystrophin and DGC has been shown to cause a reduction in the size and number of $GABA_A$ receptor ($GABA_AR$) clusters containing the α1 and α2 subunits. A decrease in kainate-type glutamate receptor density has also been described in different brain regions of dystrophin-deficient mice (Yoshihara et al., 2003). These observations suggest that dystrophin and the DGC play a role in the stability of receptors and synaptic function in the brain, indicating that cognitive impairment in the murine model and DMD patients might be related to dysfunction in synaptic transmission (Perronnet and Vaillend, 2010; Pilgram et al., 2010). Moreover, compared with control animals, *mdx* mice have been shown to exhibit a decreased response to nicotine in the passive avoidance test, suggesting a possible downregulation of nAChRs in the CNS (Coccurello et al., 2002). Decreased mRNA expression of the α3 nAChR subunit has also been observed in the cortex and hippocampus of *mdx* mice (Wallis et al., 2004), suggesting possible dysfunctions in nicotinic cholinergic transmission in the brain.

nAChRs are widely distributed in the brain, where they play a role in cognitive functions, such as attention, memory and learning. They are also involved in pathological conditions, such as Alzheimer's disease, Parkinson's disease, schizophrenia, anxiety, depression, and epilepsy (Newhouse et al., 2004; Sacco et al., 2004). These receptors are ligand-gated cationic channels formed by the pentameric combination of different α (α2–α10) and β (β2–β4) subunits expressed in the nervous system (Dani and Bertrand, 2007). In the brain, the most frequent nAChR subtypes are α4β2 (heteropentamers), which bind nicotine with high affinity (Flores et al., 1992), and α7 (homopentamers), which bind nicotine with low affinity and α-bungarotoxin with high affinity (Séguéla et al., 1993).

In view of the importance of the cholinergic system in cognitive functions (Gold, 2003), which are impaired in some DMD patients, the aim of this work was to quantify the binding sites for α4β2 and α7 nAChRs subtypes and to measure their mRNA levels in both whole brain tissue and in brain regions normally enriched in dystrophin (i.e., the cortex, hippocampus and cerebellum) of *mdx* mice. Considering that the progression of muscle disease in *mdx* mice is very slow compared to that in DMD patients, and that behavioral alterations and biochemical abnormalities in the brain are more evident in older (>6 months) *mdx* mice (Rae et al., 2002), the analysis was conducted at two stages of muscle disease: in young adults (4 months old), after maximal muscle degeneration has occurred (DiMario et al., 1991), and in old (12 months old) *mdx* mice, when the murine model exhibits some features of the muscle disease (Pastoret and Sebille, 1995).

2. Results

2.1. Binding sites for [^{3}H]-cytisine

The specific binding of [^{3}H]-cytisine to whole brain membranes from control and *mdx* mice was saturable and represented 80–85% of total ligand binding. The number of [^{3}H]-cytisine binding sites (B_{max}) in these membrane preparations decreased from 4 to 12 months of age by 20% in the control, and by 35% in *mdx* mice. However, there were no significant changes in the affinity of the ligand binding (K_d) in