Brain dystrophin-glycoprotein complex: Persistent expression of beta-dystroglycan, impaired oligomerization of Dp71 and up-regulation of utrophins in animal models of muscular dystrophy

ABSTRACT

This suggests an association of β-dystroglycan with membranes at the vascular-glial interface in the forebrain. In contrast to dystrophic skeletal muscle fibres, dystrophin deficiency does not trigger a reduction of all dystroglycans in the brain, and utrophins may partially compensate for the lack of brain dystrophins. Abnormal oligomerization of the dystrophin isoform Dp71 might be involved in the pathophysiological mechanisms underlying abnormal brain functions.

INTRODUCTION

Background The main hypotheses of how deficiency in dystrophin triggers muscular dystrophy suggest that the lack of this membrane cytoskeletal component weakens the sarcolemmal integrity, causes abnormal Ca2+-homeostasis and/or impairs proper clustering of ion channel complexes. Extensive biochemical and cell biological studies have demonstrated that one of the major functions of muscle dystrophin is to act as an actin-binding protein which mediates a link between the extracellular matrix component laminin and the sub-sarcolemmal membrane cytoskeleton. Integral or surface-associated proteins that are relatively tightly connected with dystrophin are represented by α-,β-, γ-, and δ-sarcoglycan, α- and β-dystroglycan, sarcospan, α-, β1-, and β2-syntrophin, α- and β-dystrobrevin, laminin-2 and cortical actin. The backbone of this sarcolemma-spanning protein assembly is formed by the dystroglycans. The extreme carboxy-terminus of 43 kDa β-dystroglycan contains a binding site for the second half of the hinge-4 region and the cysteine-rich domain of Dp427, thereby indirectly connecti ng the actin membrane cytoskeleton via the amino-terminus of the dystrophin molecule to the surface membrane. Since β-dystroglycan is also tightly associated with the peripheral merosin-binding protein α-dystroglycan, this complex provides a stable linkage to the laminin α2-chain in the basal lamina. Deficiency in dystrophin triggers the disintegration of complexes normally formed by the above listed sarcolemmal components and thereby renders muscle fibres from patients afflicted with Duchenne muscular dystrophy (DMD) more susceptible to necrosis. In analogy to the pathobiochemical findings in DMD, the dystrophic animal model mdx mouse also exhibits a drastic reduction in all dystrophin-associated glycoproteins in bulk skeletal muscle. This might explain at least partially the decreased osmotic stability and higher vulnerability of stretch-induced injury in dystrophin-deficient muscle fibres. An abnormal increase in cytosolic Ca2+- levels might trigger a drastic inc rease in net protein degradation and might be one of the initial steps in the molecular pathogenesis of inherited muscular dystrophy. That the other members of the dystrophin -glycoprotein complex, besides dystrophin, play a role in the DMD pathology, is demonstrated by the fact that primary abnormalities in sarcoglycans and laminin are responsible for certain forms of limb-girdle muscular dystrophy and congenital muscular dystrophy, respectively. In contrast to muscle, much less is known about the molecular mechanisms underlying brain abnormalities in the most frequent neuromuscular disease in humans. One factor which probably makes pathophysiological studies of the dystrophic central nervous system more difficult is the greater complexity of dystrophin and utrophin isoforms present in the brain. Seven promoters drive the tissue-specific expression of various dystrophin protein (Dp) isoforms from the human DMD gene, i.e.

Dp427-M in skeletal and cardiac muscle, Dp427-B in brain, Dp427-P in Purkinje neurons, Dp-260 R in retina, Dp -140 - B/K in brain and kidney, Dp -116-S in Schwann cells, Dp-71-B/U in brain and many non-muscle tissues. In addition, dystrophin-related proteins are represented by brain DRP-2 and the autosomally-encoded dystrophin homologue utrophin, which forms a full-length 395 kDa isoform (Up395) and two truncated molecular species named Up116 and Up71, also referred to as G-and U-utrophin. Besides full-length brain Dp427 and a relatively low-abundance, carboxy-terminal isoform termed brain Dp140, in the central nervous system the major dystrophin isoform is represented by Dp71. While Dp427 was shown to be present in cortical neurons, hippocampal neurons and cerebellar Purkinje cells, probably mostly associated in these cell types with the postsynaptic density, the two smaller dystrophin brain isoforms were described to be associated with microvasular glial cells. A developmental study suggests that dystrophin expression in perivascular astrocytes coincides with the formation of the blood-brain barrier. Dystroglycans are also present in brain and a subpopulation localizes to the glial-vascular interface. Recently, Blake et al. showed that different dystrobrevin isoforms are present in neuronal versus glial dystrophin complexes. With respect to dystrophin-related proteins, full-length utrophin is more widely distributed in the central nervous system and is possibly involved in the maintenance of regional specialization of the brain. To complement these neurobiological studies and in order to determine the fate of dystroglycans in dystrophin-deficient forebrain, we employed two established genetic animal models. The mdx mouse is missing Dp427 due to a point mutation in exon 23, while a mutation in exon 65 in the mdx-3cv mouse affects the splicing of both the 4.8 and 14 kb dystrophin mRNAs resulting in the additional loss of the Dp71 isoform. Neurobehavioral studies have shown that the dystrophic animal models used in this study exhibit moderate alterations in associative learning and deficits in long-term consolidation memory. Our analysis of these mutant strains indicates that β-dystroglycan appears to be located at the endothelial-glial interface in the forebrain and that not all dystroglycans are reduced in dystrophic brain, making it different from dystrophic muscle fibres. Possibly an impaired oligomerization of the major brain Dp71 isoform plays a role in the molecular pathogenesis in the dystrophic central nervous system.

CONCLUSION

Conclusions In conclusion, this report demonstrates that β-dystroglycan is not present at high concentrations in central neurons of the forebrain region, but seems to be mostly located at the interface between endothelial cells and glia. These structures possibly represent endfeet on astrocytes at the blood-brain barrier. In dystrophic forebrain, β-dystroglycan expression is not drastically affected, possibly due to the up-regulation of utrophin isoforms which partially compensate for the deficiency in brain dystrophins. Chemical crosslinking analysis showed that Dp71 exists in contrast to its normally oligomeric form in mdx brain as a monomeric protein. Thus, the lack in brain dystrophins does not necessarily lead to a loss in all associated glycoproteins and possibly abnormal oligomerization of the brain dystrophin might play a role in the molecular pathogenesis of abnormal brain functions in muscular dystrophy.