Distribution of Dystrophin and Dystrophin-Associated Protein 43DAG (β-dystroglycan) in the Central Nervous System of Normal Controls and Patients with Duchenne Muscular Dystrophy


In skeletal muscles of patients with Duchenne muscular dystrophy (DMD), the absence of dystrophin was thought to lead to the large reduction in all of the dystrophin-associated proteins (DAPs). Of the seven types of DAPs identified in skeletal muscle, only the 43-kDa glycoprotein (β-dystroglycan) has recently been found in the monkey brain. To clarify the distribution and characterization of dystrophin and β-dystroglycan in the brain of humans, we carried out immunostaining and immunoblotting studies on tissues from three DMD patients with intellectual disturbances (ages 17, 22, and 26 year) and in five controls (age range, 42–74 year). An antidystrophin antibody revealed dystrophin to be localized in neuronal cells and in the vascular wall in control brains, but it was absent from these tissues in DMD patients. In contrast, β-dystroglycan was distributed throughout neuronal cells and in the vascular wall of control brains, and was well preserved in the brain of patients with DMD.


Key words: brain type dystrophin, immunostaining, immunoblotting, neuronal cells, dystrophin-associated protein

Introduction

The gene for Duchenne muscular dystrophy (DMD) encodes dystrophin, a large membrane cytoskeletal protein found in skeletal muscle (1–4). This protein is localized on the inner surface of the sarcolemma in normal skeletal muscle, but is absent from the skeletal muscle of patients with DMD (5–9). Since the discovery of dystrophin, dystrophin-associated proteins (DAPs) (10–15), utrophin (16, 17), and several dystrophin isoforms such as Dp71 (18, 19), 75-kDa protein (20), apodystrophin-1 and -3 (21, 22), and Dp116 (23) have been described. Campbell and Kahl (10) demonstrated that dystrophin is tightly linked to a large oligomeric complex of sarcolemma glycoproteins. Ervasti et al (11) and Yoshida and Ozawa (15) showed that the glycoproteins are comprised of a 156-kDa glycoprotein (α-dystroglycan), a 94-kDa protein (A0), three kinds of 59-kDa protein (α-, β1-, β2-syntrophins), a 50-kDa glycoprotein (α-sarcoglycan), two 43-kDa glycoproteins (β-

dystro-glycan and β-sarcoglycan), a 35-kDa glycoprotein (γ-sarcoglycan), and a 25-kDa protein (25DAP). Yoshida et al (24) showed that there is an additional 43 kDa glycoprotein β-sarcoglycan which is differentiated from β-dystroglycan, on the basis of several criteria. It has been proposed that α-dystroglycan and β-dystroglycan span the sarcolemma to provide a link between the subsarcolemmal cytoskeleton and the extracellular matrix component, laminin (12, 25, 26).

Campbell’s group reported that, in the skeletal muscle of patients with DMD, the absence of dystrophin led to a large reduction in all DAPs (11, 25, 27). Conversely, Yoshida et al (28) and Mizuno et al (29) demonstrated that β-dystroglycan is rather well retained in DMD muscle, as compared with adhalin. β-dystroglycan and adhalin each belong to different groups of DAP (24). We reported earlier on the localization of dystrophin and utrophin in humans (30) and in murine brains (31). We describe here the localization and characterization of dystrophin and β-dystroglycan in the human brain.
Subjects and Methods

The brains and skeletal muscles were obtained at autopsy from three male patients (ages 17, 22, and 26 year) with DMD and from five controls (four men and one woman aged 42–74 year) who died from causes other than neurological or psychiatric disease. Autopsy was done within 4 hours of death. All of the subjects were Japanese. The recorded IQ scores of the three DMD patients were 61, 68, and below 50, respectively (tested with the Wechsler Intelligence Scale for Children-Revised or the Wechsler Adult Intelligence Scale). Tissues were divided into frontal, parietal, and occipital lobes, cerebellum, and skeletal muscle and were immediately frozen in liquid nitrogen-cooled isopentane and stored in a deep freezer (−85°C) until the start of the experiments.

For immunostaining, frozen sections were cut into 10 μm sections and stained by the avidin-biotinylated horseradish peroxidase method, as described previously (30, 31). For primary incubation, two types of region-specific antidystrophin antibodies were used: rabbit antidystrophin antibody (antibody 6-10) and mouse antidystrophin antibody (Dys-2; Novocastra Laboratories, Newcastle upon Tyne, UK). Antibody 6-10 is a polyclonal antibody that is produced in a rabbit immunized with a dystrophin polypeptide expressed in E. coli from dystrophin cDNA residues 6,181–9,544; it is excellent for the detection of brain-type dystrophin. Dys-2 is a monoclonal antibody raised

![Figure 1. Immunoblot of the brain and skeletal muscle from a normal control and a patient with DMD. Normal control, lanes 1, 3, 5, 7; DMD, lanes 2, 4, 6, 8. Lanes 1 and 2, skeletal muscle; lanes 3 and 4, frontal lobe; lanes 5 and 6, parietal lobe; lanes 7 and 8, cerebellum. Blots were stained with antibody 6-10 (Panel A) and antibody PA3a (Panel B). The dystrophin band is clear in the normal control brain (lanes 1, 3, 5, 7), but it is not evident in the DMD brain (lanes 2, 4, 6, 8). With antibody PA3a, the 43 kDa band (β-dystroglycan) was clearly evident in control skeletal muscle and brain. In DMD, the 43 kDa band was very faint in skeletal muscle, but was clearly noted in brain tissue.](image-url)
Dystrophin and β-Dystroglycan in DMD Brain

against the 3,669–3,685 (C terminus). For the detection of 43DAG, we used antibody PA3a, a polyclonal anti-43DAG antibody raised in a rabbit against synthetic polypeptide, that corresponds to the internal amino acid sequence determined by microsequence analysis (28). For immunoblot studies, tissues were homogenized in a buffer containing 2% SDS, 5% β-mercaptoethanol, 4 mM EDTA, 1 mM PMSF, 40 mM Tris, 0.24 M glycine, 40% glycerine, and 0.001% bromphenol blue (pH

Figure 2. Immunohistochemical localization of dystrophin in the cerebral cortex of a normal control (A, C, E) and a DMD patient (B, D, F), labeled with preimmune serum (A, B) and antidystrophin antibody (antibody 6-10) (C–F). Punctate immunoreactivity was evident along the surface membranes of cell bodies and dendrites of the control cerebral cortical neurons (arrows), but immunoreactivity was absent in DMD (arrowheads) (A–D, ×330; E, F, ×825).
Figure 3. Immunohistochemical localization of β-dystroglycan in skeletal muscle (control, A; DMD, B), cerebral cortex (control, C, E, and G; DMD, D, F, and H), labeled with preimmune serum (C, D) and antibody PA3a (E–H). Positive labeling was evident along the sarcolemma (A, B; arrows), the surface membrane of neurons (E–H; arrows) and the vascular wall (E–H; arrowheads) (A, B, ×83; C, D, ×330; E, F, ×250; G, H, ×550).
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8.5). Ten micrograms of protein were electrophoresed through 3.5 to 12% SDS polyacrylamide gradient gels (1 mm × 10 cm × 9 cm) at 15 mA for 2.5 hour. After transfer onto a membrane (Immuno-Blot, Millipore Corporation, Bedford, MA, USA), the specimen was blocked with 0.1% casein-0.1% gelatin in PBS overnight at 4°C; blots were stained with antidystrophin and anti-β-dystroglycan antibodies at room temperature for 2 hour. Dystrophin-antidystrophin and β-dystroglycan-anti-β-dystroglycan immune complexes were detected with affinity-purified secondary antibodies conjugated to avidin-biotinylated horseradish peroxidase (Vectastain ABC kit and Elite ABC kit, Vector Laboratories, Burlingame, CA, USA). For statistical analysis, the β-dystroglycan band was measured using a densitometer (Cosmo F808, Cosmo Co. Tokyo, Japan).

Results

Immunoblots

Following immunoblots of the control cerebrum (frontal, parietal, and occipital lobes), cerebellum, and skeletal muscle, antibody 6-10 revealed a clear dystrophin band at 427 kDa and this band was absent in brain and skeletal muscles from DMD patients (Fig. 1A). With Dys-2, no dystrophin band was noted in either control or DMD brains (data not shown). With antibody PA3a, the β-dystroglycan band was clearly noted at 43 kDa in all brains examined and there was no significant difference in the density of the 43DAG band between DMD and control brains (Fig. 1B). The β-dystroglycan band of skeletal muscle was clear in controls, but very faint in advanced DMD.

Immunostain

With immunostaining, antibody 6-10 was localized in a punctate manner along the cell bodies and dendrites of cerebral cortical neurons and Purkinje cells of control brains, but was absent in the brains of DMD (Fig. 2). With Dys-2, the vascular wall was positively labeled in control brains, but cerebral cortical neurons and Purkinje cells did not stain positively in either control or DMD brains. With antibody PA3a, the surface membranes of cerebral cortical neurons, Purkinje cells, pia mater, and the vascular wall were positively stained in all of the brains examined (Fig. 3).

Discussion

The absence of dystrophin dramatically reduced all the DAPs in the sarcolemma of patients with DMD and of mdx mice (11, 25, 27). Yoshida et al (28) and Mizuno et al (32) demonstrated that β-dystroglycan is retained in DMD muscles, as determined using anti-β-dystroglycan antibody (PA3a). In the present study, sarcolemma of the DMD muscle was fairly well stained with PA3a but the amount of β-dystroglycan was reduced on immunoblots, probably due to the severe decrease in the total number of muscle fibers. In the DAPs, β-dystroglycan was present in monkey brain (32). We investigated the distribution and characterization of dystrophin and β-dystroglycan in the human brain. Although the localization of dystrophin in the CNS has been clarified in an animal model and in humans (33–35), its role in the CNS is not clear. Mental retardation and decreased intellectual function are frequently noted in DMD patients (36–42). However, intellectual disturbance in DMD is not progressive, and there is no necrosis and/or degeneration of cortical neurons in the brain of such patients which is in contrast to findings in their muscles. Using antibody 6-10, we found that dystrophin was localized in neuronal cells of the control brains but was absent from neuronal cells in the brains of DMD patients with intellectual disturbances. In contrast, β-dystroglycan was well distributed in the control brains and was well preserved in DMD brains. Obviously, a deficiency of dystrophin does not always lead to a marked reduction of DAP in the DMD brain. Retention of DAP 43DAG may possibly be related to the preservation of neurons in the DMD brain.

In DMD patients, we confirmed that there is a lowering of the IQ and also an abnormality of event-related brain potentials (P-300) (unpublished data). Pillers et al (43) reported evidence of dystrophin in the outer plexiform layer of the human retina, and found that five patients with Becker’s dystrophy and six with Duchenne dystrophy had abnormal electroretinograms. These data suggest that dystrophin is related to the diversified physiological functions in tissues.

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