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β-dystrobrevin, a member of the dystrophin-related protein family

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ABSTRACT **The importance of dystrophin and its associated proteins in normal muscle function is now well established. Many of these proteins are expressed in nonmuscle tissues, particularly the brain. Here we describe the characterization of β-dystrobrevin, a dystrophin-related protein that is abundantly expressed in brain and other tissues, but is not found in muscle. β-dystrobrevin is encoded by a 2.5-kb alternatively spliced transcript that is found throughout the brain. In common with dystrophin, β-dystrobrevin is found in neurons of the cortex and hippocampal formation but is not found in the brain microvasculature. In the brain, β-dystrobrevin coimmunoprecipitates with the dystrophin isoforms Dp71 and Dp140. These data provide evidence that the composition of the dystrophin-associated protein complex in the brain differs from that in muscle. This finding may be relevant to the cognitive dysfunction affecting many patients with Duchenne muscular dystrophy.**

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy are allelic muscle-wasting disorders that affect approximately 1 in 3,500 and 1 in 30,000 boys, respectively. Both diseases are caused by mutations in the DMD gene, which encodes the cytoskeletal protein dystrophin (1). Although skeletal and cardiac muscle are the predominant sites of dystrophin expression, dystrophin also is found in the brain. Cognitive defects and brain abnormalities have been described in a large number of DMD patients and are a consistent feature of the disease (2). These defects vary in severity from mild verbal difficulties to profound mental handicap. Cognitive disabilities also have been described in some patients with Becker muscular dystrophy and can present before the onset of muscle disease (3). Consistent with a role in cognition, dystrophin is concentrated at the postsynaptic regions of neurons in the cortex and hippocampus (4, 5) and is enriched in the postsynaptic density fraction of forebrain homogenates (6). Furthermore, neurons in the hippocampus of the dystrophin-deficient *mdx* mouse are more susceptible to hypoxia-induced loss of synaptic activity than those from normal mice (7). In the brain dystrophin is transcribed from two promoters. The C-promoter is active in the cortex and hippocampus (8–10) whereas the P-promoter controls the transcription of dystrophin in cerebellar Purkinje cells (11). In addition to "full-length" dystrophin, the dystrophin isoforms Dp71 and Dp140 are found in the brain (12). Dp140 is enriched in the brain microvasculature (13) whereas the Dp71 transcript is found throughout the brain but is particularly abundant in the dentate gyrus and the olfactory bulb (14).

Dystrophin binds to a complex of proteins and glycoproteins, the dystrophin-associated protein complex (DPC), which effectively forms a transmembrane link between the extracellular matrix and the cytoskeleton of the muscle fiber (15, 16). The DPC can be divided into three subcomplexes: the dystroglycan complex, the sarcoglycan complex, and the cytoplasmic complex (17). The dystroglycan complex consists of two proteins, α- and β-dystroglycan, that bind to laminin and dystrophin, respectively (18). The sarcoglycan complex is composed of four transmembrane glycoproteins: α-, β-, γ- and δ-sarcoglycan, and a 25-kDa protein 25DAP. Mutations in all four sarcoglycan genes have been found in patients with different types of limb-girdle muscular dystrophy (19). The cytoplasmic component of the DPC is composed of the syntrophin family of related proteins and the dystrophin-related protein, dystrobrevin (87K or A0). The mammalian dystrobrevin gene encodes several protein isoforms that are expressed in different tissues, including brain and muscle (20, 21). Two of these isoforms, dystrobrevin 1 and dystrobrevin 2, copurify with the syntrophins (20). The syntrophin family of proteins are thought to bind directly to both dystrophin or utrophin, a homologue of dystrophin and dystrobrevin (22, 23). In nonmuscle tissues, Dp71 and utrophin appear to replace dystrophin and copurify with the syntrophins and other proteins, suggesting that a dystrophin-associated protein complex exists, albeit in a modified form, outside the muscle (22).

To understand the role of dystrophin in the central nervous system, we have determined which components of the DPC are found in the brain. In particular we have examined the dystrobrevin family of proteins. Here we describe the characterization of a member of the dystrophin-related protein family, which we have called β-dystrobrevin. We show that β-dystrobrevin is expressed in neurons, but not muscle, and can coimmunoprecipitate with Dp71 and Dp140. Our findings are not only relevant to the role of the dystrophin family of proteins in the brain but are clinically important because they may contribute to our understanding of the molecular events underpinning the cognitive disabilities that affect patients with DMD and Becker muscular dystrophy.

MATERIALS AND METHODS

Molecular Biology. cDNA libraries were prepared from adult mouse brain and kidney mRNA as described previously (20). *Eco*RI/*Bst*XI-adapted cDNA was ligated in to the *Bst*XI sites of pcDNA2 (Invitrogen) and used to transform *Escherichia coli* XL-1Blue competent cells. Approximately 5×10^5 colonies were plated onto nylon filters and screened by hybridization with radiolabeled cDNA probes. Hybridizing clones were purified and sequenced by using standard protocols. RNA isolation, Northern blots, and reverse transcriptase–PCR have been described previously (20).

Antibody Production. The C terminus of β-dystrobrevin (βCT-FP) was produced as a thioredoxin fusion protein by cloning the 1.2-kb insert from clone m13c1 (see below) into the *Eco*RI site of pET32(b) (Novagen). The C terminus of α-dystrobrevin 1 (α1CT-FP) was produced as a glutathione *S*-transferase fusion protein by cloning the 1.1-kb *Eco*RI restriction fragment of clone m24 (20) into the *Eco*RI site of pGEX4T-3 (Pharmacia). Affinity-purified fusion proteins were used to immunize New Zealand White rabbits. A synthetic peptide, β521, (NH_2-CATG-

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Abbreviations: DMD, Duchenne muscular dystrophy; DPC, dystrophin-associated protein complex; TBST, 150 mM NaCl/50 mM Tris·HCl, pH 7.5/0.1% Tween-20.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AJ003007).

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SPHTSPTHGGGRPM), corresponding to a unique region in the C terminus of β-dystrobrevin was coupled to keyhole limpet hemocyanin and also used to immunize rabbits. The β521 antiserum was affinity-purified by column chromatography using the immunizing peptide coupled to Affigel-10 (Bio-Rad).

Western Blotting. Proteins were prepared from mouse tissues by homogenization in treatment buffer [4 M urea/3.8% SDS/20% (vol/vol) glycerol/75 mM Tris·HCl, pH 6.8/5% (vol/vol) 2-mercaptoethanol]. One hundred micrograms of protein from each tissue were separated on 8% SDS/polyacrylamide gels and transferred to nitrocellulose membranes in transfer buffer (20% methanol/0.1% SDS/192 mM glycine/25 mM Tris·HCl, pH 8.5) for 90 min at 80 V. Membranes were blocked in 5% milk powder in TBST (150 mM NaCl/50 mM Tris·HCl, pH 7.5/0.1% Tween-20) for 1 hr. Primary antibodies were applied in milk powder/TBST solution at the following dilution: βCT-FP, 1:1,000, α1CT-FP 1:2,000, anti-utrophin C terminus, MANCHO3 1:100 (24), anti-dystrophin rod domain, MANDYS1 1:100 (25), for 1 hr at room temperature. Blots were washed twice in TBST for 5 min and twice in 5% milk powder in TBST for 5 min. Horseradish peroxidase-conjugated secondary antibodies, donkey anti-rabbit, and donkey anti-mouse (Jackson ImmunoResearch) were applied at a dilution of 1:5,000 for 1 hr at room temperature and then washed extensively in TBST. Proteins were detected by using a BM chemiluminescence detection kit (Boehringer Mannheim).

***In Situ* Hybridization.** Antisense oligonucleotides were synthesized from the sequences of α- and β-dystrobrevin: βAS-1 5′-TGAAGAGCTGCCTCTTCTCCGCCATGGTCTTCCGCTTGTTCCCGC; αAS-1 5′-GAGAGCACCGCCTCCAGGCGGGCCACGTTGAGTTCTATGTTTGGG; αAS-2 5′-TGCAATGGGACCGGCGCCTGTGTCACCCACTCGCCAAACTCTTAG.

The oligonucleotides were labeled and hybridized to sections of mouse brain as described previously (26).

Immunohistochemistry. Mouse brains were embedded in OCT and frozen in liquid nitrogen-cooled isopentane. Ten-micrometer sections were cut on a cryostat cooled to −18°C. Sections were collected onto SuperFrost Plus (BDH) slides, air dried, and stored at −70°C. For immunofluorescence, the sections were blocked for 30 min in 10% fetal calf serum in Tris-buffered saline (TBS) (150 mM NaCl/50 mM Tris·HCl, pH 7.5). Slides were incubated with the following dilutions of primary antibodies: β521 peptide, 1:10; α1FP-CT, 1:1,000; ab308 1:50 (20), anti-agrin IIA, 1:200; antidystrophin C terminus 1583, 1:1,000 for 1 hr at room temperature and then washed twice for 5 min in TBS. Fluorescein isothiocyanate-conjugated donkey anti-rabbit or donkey anti-mouse (Jackson ImmunoResearch) were applied in TBS at a dilution of 1:100.

Coimmunoprecipitation. A single mouse brain was homogenized in 10 ml of PBS plus protease inhibitors (Sigma). Crude membranes prepared by centrifugation at 100,000 g were homogenized in PBS containing 1% Triton X-100 plus protease inhibitors and incubated for 1 hr at 4°C. The Triton-soluble supernatant was obtained after centrifugation of the homogenate at 100,000 g for 30 min. Dystrophin and utrophin immunoreactive proteins were immunoprecipitated from the Triton-solublized membranes by incubating 0.5 ml of supernatant with 25 μl of MANDRA1, raised against the C terminus of dystrophin (25) or MANCHO3 for 4 hr at 4°C. Immune complexes were magnetically captured after overnight incubation with 20 μl of goat anti-mouse IgG Dynabeads (Dynal, Oslo) at 4°C. The immune complexes were washed thoroughly in PBS and 0.1% Triton X-100 and eluted in 40 μl of 2× sample buffer [125 mm Tris·HCl, pH 6.8/4% (wt/vol) SDS/20% (vol/vol) glycerol/5% (vol/vol) 2-mercaptoethanol]. Precipitated proteins were separated on SDS/polyacrylamide gels and processed as described above.

RESULTS

Cloning and Characterization of the Mouse β-Dystrobrevin cDNA. During the isolation of the dystrobrevin clones from an adult mouse brain cDNA library (20), one cDNA clone (*m13*) was found that had 77% nucleotide sequence identity to the C terminal-encoding region of the dystrobrevin-1 cDNA. The *m13* cDNA clone was used to screen mouse brain and kidney cDNA libraries. Overlapping clones that generated the full-length cDNA were sequenced. Because the sequence of this transcript is similar to dystrobrevin 1, we have named the product of this gene β-dystrobrevin and renamed dystrobrevin, α-dystrobrevin. Fig. 1*A* shows the nucleotide sequence and deduced amino acid sequence of mouse β-dystrobrevin. The β-dystrobrevin cDNA encodes a protein with a calculated molecular mass of 69 kDa. All dystrophin-related proteins have four EF-hand-like motifs, a ZZ domain, and a C-terminal coiled-coil domain (26, 27) (Fig. 1*B*). These domains are believed to be involved in protein–protein interactions.

The expression pattern of β-dystrobrevin was determined by using Northern blots of mouse RNAs. A single 2.5-kb transcript was detected predominantly in the brain and kidney and to a lesser extent in liver and lung (Fig. 2*A*). No β-dystrobrevin hybridizing transcripts were detected in skeletal and cardiac muscle even after long exposures suggesting that the β-dystrobrevin transcript was weakly expressed or absent in muscle. The same Northern blot was stripped and rehybridized with a probe derived form the α-dystrobrevin cDNA to demonstrate the integrity of the RNAs in the skeletal and cardiac muscle lanes. Multiple transcripts were detected in each lane (data not shown).

Sequence analysis of the cDNA clones isolated from brain and kidney cDNA libraries and of reverse transcriptase–PCR products prepared from various tissues revealed that the β-dystrobrevin transcript is alternatively spliced at four sites designated A-D (Fig. 2*B*). Alternative splicing at site A removes amino acids 361–390. This site has homology to the muscle-expressed vr3 exons in the α-dystrobrevin gene that has been previously described (20). This splice variant is found only in brain. Alternative splicing at site B results in the insertion of seven charged amino acids (EEEQKQA) at position 518 of the primary sequence. This splice variant is found predominantly in the brain and is homologous to the vr2 sequence of α-dystrobrevin 1 and 2 (20). Transcripts lacking one or both of the last two coding exons (ΔC, ΔD) also are found predominantly in the brain. Neither of these exons are known to be alternatively spliced in the α-dystrobrevin 1 transcript. Although full-length β-dystrobrevin is present in brain, the major isoform lacks exons A, C, and D but has the seven amino acid insertion at site B. This β-dystrobrevin isoform, which we have called ΔACD, has a calculated molecular mass of 62 kDa. The two major β-dystrobrevin isoforms in kidney lack alternatively spliced exon B (ΔB), or exons B and D (ΔBD). These proteins have a predicted molecular mass of approximately 68 kDa.

Tissue Distribution of the Dystrobrevin Proteins in the Mouse. To determine the tissue distribution of β-dystrobrevin relative to α-dystrobrevin, dystrophin, and utrophin, antibodies were raised to the β- and α-dystrobrevin sequences. The choice of potential immunogen was limited because of the extensive sequence similarity between β- and α-dystrobrevin. The antipeptide antibody β521 was raised against amino acids 521–537 of β-dystrobrevin (Fig. 1*A*). This antibody should detect only β-dystrobrevin because only seven of 18 amino acids are identical between the two sequences. The α1CT-FP antibody was raised to the unique C terminus of α-dystrobrevin 1. This antibody detects only α-dystrobrevin 1. A third antibody, βCT-FP, was raised against the last 308 amino acids of β-dystrobrevin. This antibody detects α-dystrobrevin 1 and 2 in addition to β-dystrobrevin. The differences in molecular weight and relative mobility on SDS/polyacrylamide gels of β-dystrobrevin and α-dystrobrevin 1 and 2 allow each protein to be clearly resolved (see below).

β521 detected a protein with a molecular mass of 6l kDa on Western blots of brain microsomes. This band was not detected after preincubation of β521 with the immunizing peptide (data not shown). In common with the antibodies ab308 and ab433

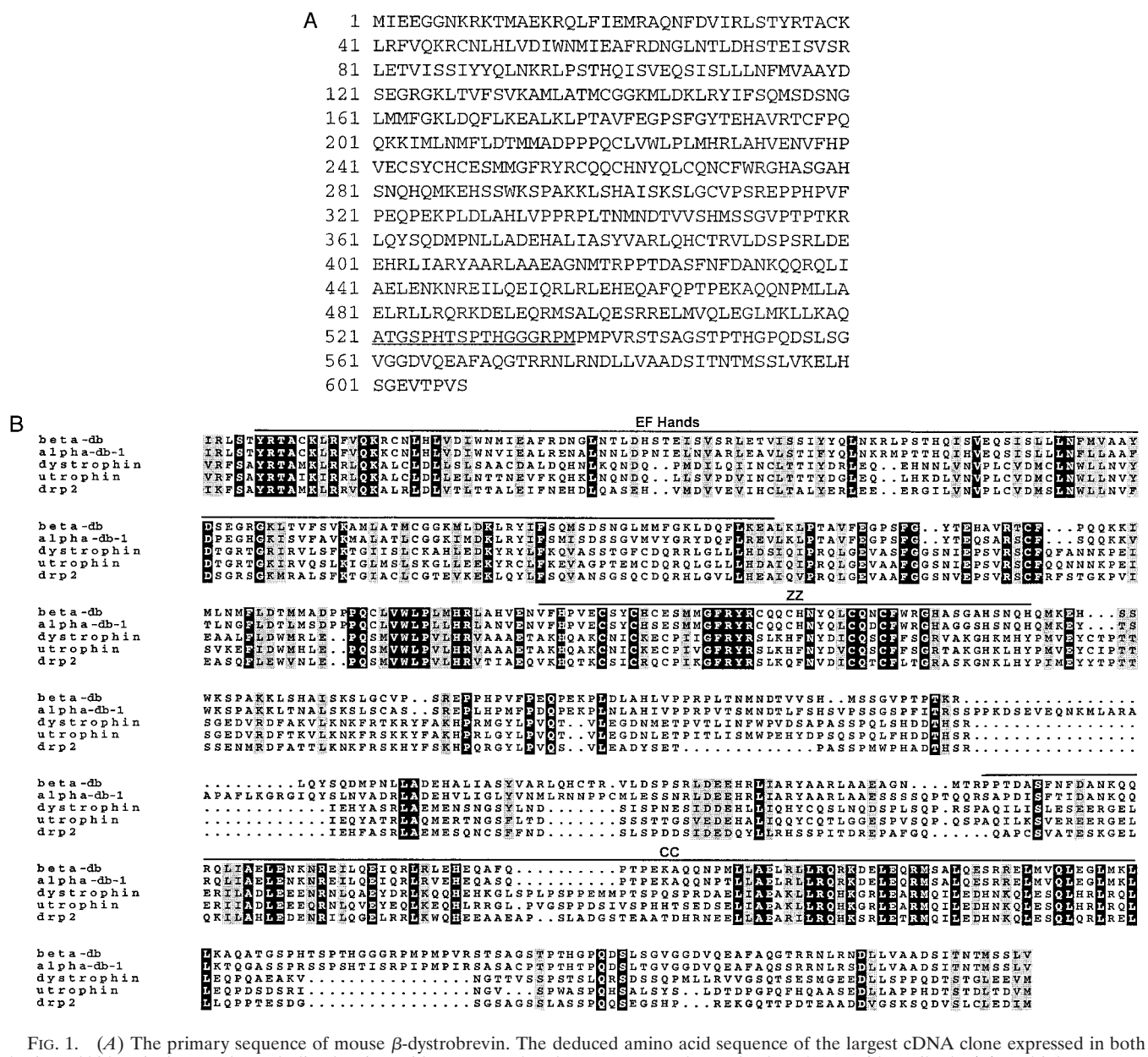

FIG. 1. (*A*) The primary sequence of mouse β-dystrobrevin. The deduced amino acid sequence of the largest cDNA clone expressed in both brain and kidney is shown. The underlined amino acids correspond to the sequence used to make the β521 peptide antibody. (*B*) Multiple sequence alignment of the dystrophin-related protein family. Sequences of murine β-dystrobrevin (amino acids 30–596), the muscle-expressed isoform of α-dystrobrevin 1 (amino acids 30–629), dystrophin (amino acids 3094–3653), G-utrophin (amino acids 413–966), and human DRP2 (amino acids 395–931) were aligned by using the program PILEUP (GCG version 8.0). The sequences were shaded by using the program BOXSHADE. The dark shading represents amino acid identity between all five proteins whereas the lightly shaded sequences represent amino acid similarities at a given residue. The positions of the EF hands, ZZ domain, and CC domains are indicated by lines.

raised against β-dystrobrevin peptides, β521 only worked on enriched material such as crude membrane preparations (20). The antibody βCT-FP detected a complex pattern of proteins on Western blots prepared from mouse tissues (Fig. 3). The protein with the highest molecular mass is α-dystrobrevin 1. The insertion of 57 amino acids by alternative splicing accounts for the increase in molecular mass of α-dystrobrevin 1 in skeletal and cardiac muscle compared with brain and lung (20). This increase can be clearly seen with the α1CT-FP antibody that detects only α-dystrobrevin 1 (Fig. 3). β-dystrobrevin is detected as a 61-kDa protein in brain, kidney, liver, and lung. β-dystrobrevin can be distinguished from α-dystrobrevin 2 because it has a lower relative mobility. As with α-dystrobrevin 1, α-dystrobrevin 2 in heart and skeletal muscle has a higher molecular mass because of tissue-specific alternative splicing of the vr3 exons (20). Slight differences in size of the β-dystrobrevin in brain compared with kidney, liver, and lung also can be observed (Fig. 3). No correlation can be made between the relative abundance of the dystrobrevins in different tissues compared with their potential binding partners dystrophin and utrophin (Fig. 3). Dystrophin levels are highest in muscle and heart whereas utrophin is abundantly expressed in the lung. Full-length dystrophin and utrophin are weakly expressed in the brain (Fig. 3).

***In Situ* Hybridization Studies.** To determine the location of β- and α-dystrobrevin in mouse brain, antisense oligonucleotides were designed that would detect each of the dystrobrevins uniquely. The hybridization pattern of oligonucleotide probes detecting the α- and β-dystrobrevin transcripts in mouse brain were very similar (Fig. 4.) The signal is clearly detected in the dentate gyrus and in the CA regions of the hippocampal formation, especially in the CA3 (Fig. 4 *A*, *C*, and *D*). The olfactory bulb is labeled by both probes with the majority of the signal being confined to the granule cells (Fig. 4 *B* and *C*). Both β- and α-dystrobrevin are expressed predominantly in the granule cell

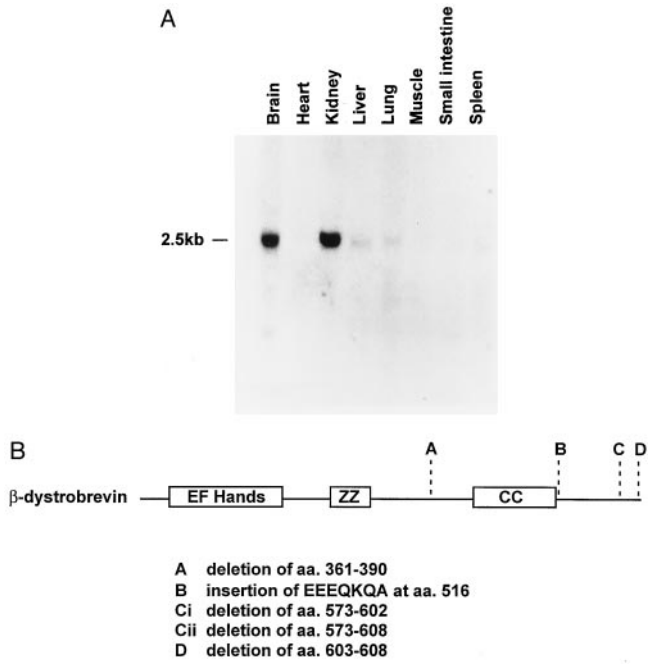

FIG. 2. (*A*) Expression of the β-dystrobrevin mRNA in mouse tissue. Northern blot of total RNA prepared from different mouse tissues hybridized with a 1.2-kb partial cDNA probe (m13c1) encoding the last 306 amino acids and part of the 3′ untranslated region of β-dystrobrevin. This probe detects a 2.5-kb transcript and does not detect α-dystrobrevin encoding transcripts under the conditions used for hybridization and washing. (*B*) Alternative splicing of the β-dystrobrevin transcript. Diagrammatic representation of the β-dystrobrevin protein and the sites that are alternatively spliced. Alternative splicing at sites C and D generates proteins with different C termini. The majority of clones isolated from the kidney cDNA library kidney contain exons C and D whereas in brain most cDNAs lack one or both of these regions.

layer of the cerebellum (Fig. 4 *A-C*). The weak labeling of the cortex with both probes seems to be because of expression of β- and α-dystrobrevins in a subpopulation of cells in all cortical layers. There is also some labeling of the subcortical structures in particular the caudate putamen with both probes (Fig. 4 *B* and *C*).

Several precautions were used to ensure that the hybridization pattern was specific. First, probes designed from two regions of the α-dystrobrevin sequence, αAS-1 and -2, gave an identical labeling pattern in several independent experiments (data not shown). Additionally, αAS-2 recognizes the 5′ untranslated region of α-dystrobrevin, which has no obvious similarity to the β-dystrobrevin sequence. Second, hybridization with unrelated probes always resulted in a different labeling pattern from that seen with the dystrobrevin probes. Third, incubation with a 25- to 50-fold molar excess of unlabeled oligonucleotide combined with the normal concentration of labeled probe prevented specific binding.

Immunocytochemical Localization of β-Dystrobrevin in the Brain. To determine location of the dystrobrevins in the brain, coronal sections of mouse brain were stained with a panel of polyclonal antibodies. The anti-β-dystrobrevin specific antisera, β521, stained the cell bodies of hippocampal pyramidal neurons and dentate gyrus strongly (Fig. 5 *A* and *B*) and also a subset of pyramidal neurons in cortical layers *II–V* (data not shown). Preincubation of control sections with the β521 antibody and an excess of immunizing peptide (100 μM) completely abolished antibody labeling (data not shown). The α1CT-FP antibody predominantly strongly stained the brain microvasculature (Fig. 5*C*) and some neurons in the hippocampus and cortex. The βDB-FP antibody that detects β-dystrobrevin and α-dystrobrevin 1 and 2 also strongly stained the hippocampus and dentate gyrus but also stained the microvasculature presumably because of cross-reactivity with α-dystrobrevin (data not shown). The staining pattern of ab308 (Fig. 5*D*) that detects α-dystrobrevin 1 and 2, but also weakly cross-reacts with β-dystrobrevin, was essentially identical to the α-dystrobrevin 1 staining pattern (Fig. 5*C*). This vascular staining pattern was confirmed with an antibody raised against the extracellular matrix glycoprotein, agrin (Fig. 5*E*), which is known to stain the brain microvasculature (29). Agrin also labels neurons in the hippocampus and cortex (data not shown). Interestingly, the antibody 1583, raised against the C terminus of dystrophin, strongly labels the brain microvasculature (Fig. 5*F*). In addition to the microvasculature staining this antibody detects dystrophin proteins in the dentate gyrus, hippocampus, and cortex (data not shown). This staining pattern represents the combined regional distribution of full-length dystrophin, Dp71, and Dp140 in the brain. Anti-utrophin and anti-α-syntrophin antisera also strongly labeled the microvasculature, choroid plexus, and ependymal lining of the ventricles (data not shown).

Coimmunoprecipitation of the β- and α-Dystrobrevin 1 with Dystrophin Isoforms. Potential associations between β-dystrobrevin and dystrophin, utrophin, and the dystrophin isoforms were tested by coimmunoprecipitation. Proteins from Triton X-100 solublized crude membranes were prepared from normal C57, *mdx*, and mdx^{3Cv} mouse brains. The mdx^{3Cv} mouse was used in this experiment because it has a splicing mutation in intron 64 of the DMD gene that essentially abolishes the production of dystrophin and the distally encoded isoforms (30) whereas the *mdx* mutation only abolishes production of full-length dystrophin. Triton X-100-solublized proteins were incubated with the anti-dystrophin mAb MANDRA1 or the anti-utrophin mAb MANCHO3. Precipitated protein complexes were resolved on denaturing polyacrylamide gels, Western blotted, and detected with the βCT-FP antibody. MANDRA1 precipitated β-dystrobrevin and α-dystrobrevin 1 from the normal C57 strain and dystrophin-deficient *mdx* mouse brain extracts but not from mdx^{3Cv} mouse brain (Fig. 6*A*). MANCHO3 failed to precipitate any dystrobrevin-immunoreactive proteins (data not shown). These results were confirmed with other anti-dystrophin and anti-utrophin mAbs. Because the epitope for MANDRA1 is in the C terminus of dystrophin, these data suggest that β- and α-dystrobrevin 1 coimmunoprecipitate with the C-terminal dystrophin isoforms. To confirm this hypothesis, Western blots of the immunoprecipitates were probed with the 1583 polyclonal antibody. This antibody detected Dp71 and Dp140 in the immunoprecipitates from C57 and *mdx* brains but not in the mdx^{3Cv} brain (Fig. 6*B*). These data indicate that β-dystrobrevin can form a complex with α-dystrobrevin 1 and Dp71 or Dp140 in the brain. A direct association between full-length dystrophin and β-dystrobrevin could not be demonstrated. Additional analysis showed that the majority of full-length dystrophin is insoluble in 1% Triton X-100 but was soluble in 2% SDS (D.J.B., unpublished results).

DISCUSSION

In this paper we describe the characterization of a member of the dystrophin-related protein family, β-dystrobrevin. β-dystrobrevin is expressed in brain, kidney, liver, and lung but was not detected in muscle. Furthermore, β-dystrobrevin coimmunoprecipitates with the dystrophin isoforms Dp140 and Dp71 from the brain. These data provide evidence that the composition of the DPC in the brain differs from that in muscle and demonstrates that novel proteins can be associated with the dystrophin isoforms in addition to dystrophin itself.

The dystrophin-related protein family now has been extended to include five proteins; dystrophin, utrophin, DRP2, α-dystrobrevin, and β-dystrobrevin (Fig. 1*B*). All of these proteins possess sequence features that are typical of the dystrophin family, namely the cysteine-rich ZZ domain (27) and a pair of predicted α-helical coiled-coils in the C terminus, the CC domain (28). The sequence homology between the members of the dystrophin

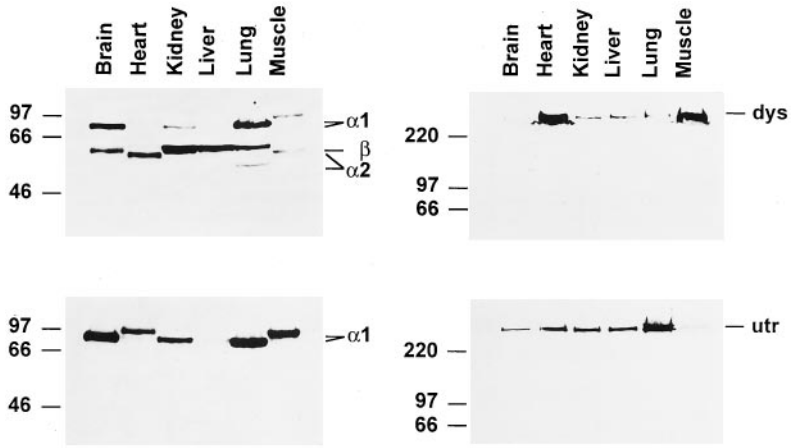

FIG. 3. Expression of the dystrobrevins, dystrophin, and utrophin in mouse tissue. Identical Western blots of mouse tissues were probed with the following antibodies: (*Upper left*) βCT-FP; (*Lower left*) α1CT-FP; (*Upper right*) MANDYS1 raised against the dystrophin rod domain; and (*Lower right*) MANCHO3 raised against the C terminus of utrophin. The complex pattern of proteins detected by βCT-FP correspond to muscle α-dystrobrevin 1 (94 kDa), nonmuscle α-dystrobrevin 1 (78 kDa), β-dystrobrevin (61 kDa in brain and 63 kDa in kidney), muscle α-dystrobrevin 2, and nonmuscle α-dystrobrevin 2. Dystrophin is expressed at high levels in heart and skeletal muscle whereas utrophin is ubiquitously expressed but is most abundant in the lung. Note the low level of dystrophin in brain homogenates.

family of related proteins allows certain structural and functional predictions to be made. The CC domain of dystrophin has been shown to bind syntrophin and dystrobrevin (31). In a model recently proposed by Peters *et al*. (23), syntrophin binds to the CC domain of dystrophin and dystrobrevin, whereas dystrobrevin binds directly to dystrophin. Thus a complex of dystrophin, syntrophin, and dystrobrevin is formed with a stoichiometry of 1:2:1. Because of the extensive sequence similarity between α- and β-dystrobrevin and our finding that dystrobrevin is associated with Dp71/Dp140 in brain, this model also could be extended to include interactions with β-dystrobrevin and the dystrophin isoforms. We have not determined whether both β- and α-dystrobrevin 1 bind to Dp71/Dp140 independently or as a complex or whether this complex contains syntrophin.

In situ hybridization studies have shown that the Dp71 mRNA is expressed predominantly in the dentate gyrus and olfactory bulb whereas dystrophin is expressed in the pyramidal cell layer of the hippocampus and in the cortex (7, 14). To date no studies localizing the Dp71 protein within the brain have been described. By contrast, Dp140 has been shown to stain the brain microvasculature (13). Dp71 is the most abundant product of the DMD gene in brain (31). Its abundance is unlikely to be caused by expression in the dentate gyrus and olfactory bulb alone but may be caused by expression in other regions of the brain such as the microvasculature. Because β-dystrobrevin is not detected in the microvasculature, it is possible that β-dystrobrevin binds to Dp71 in the dentate gyrus, olfactory neurons, and perhaps in other, as yet undefined, populations of neurons or glia.

It is noteworthy that β-dystrobrevin is not found in the microvasculature. Antibodies raised against α-dystrobrevin, agrin, syntrophin, utrophin, and even the C terminus of dystrophin all stain the microvasculature (Fig. 5 *C-F*). Utrophin is known to be

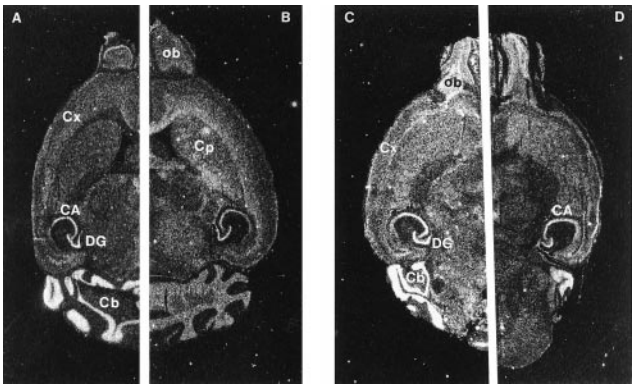

FIG. 4. *In situ* localization of dystrobrevin transcripts in mouse brain. Dark-field autoradiograms showing horizontal brain sections hybridized with ^{35}S-labeled oligonucleotides detecting the α-dystrobrevin transcripts (*A* and *C*) or the β-dystrobrevin transcript (*B* and *D*). α- and β-dystrobrevin are expressed in similar regions of the brain. Labeling of the hippocampal formation, both CA regions and over the dentate gyrus (DG) is shown. In addition, signal in the olfactory bulb (ob), cerebral cortex (Cx), and cerebellum (Cb) is detected. Some subcortical regions, especially the caudate putamen (Cp), also are labeled.

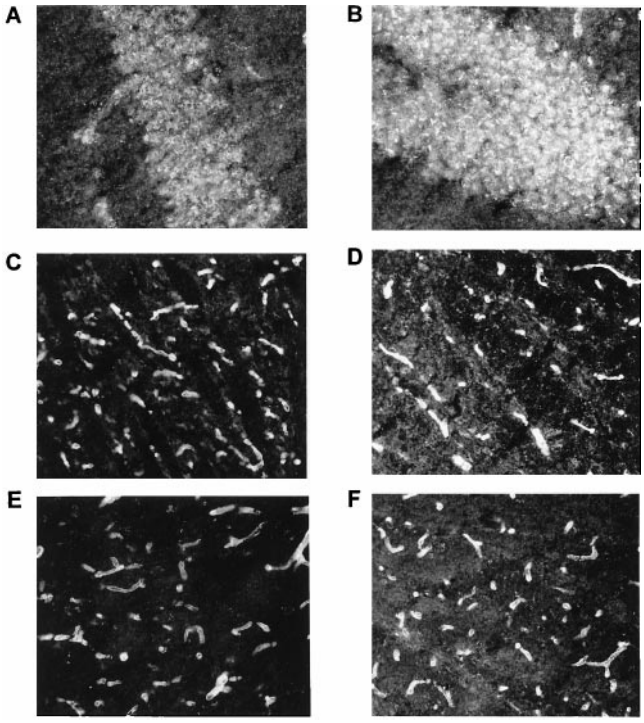

FIG. 5. Immunolocalization of β-dystrobrevin in the brain. Coronal mouse brain sections were stained with the following antibodies: anti-β-dystrobrevin, β521 (*A* and *B*), anti-α-dystrobrevin 1, α1CT-FP (*C*), anti-dystrobrevin, ab308 (*D*), agrin IIA (*E*), and anti-dystrophin 1538 (*F*). β521 stains neurons in the pyramidal cell layer of the hippocampus and the dentate gyrus (*A* and *B*). The other antibodies label the microvasculature. Magnification: ×400 (*A* and *B*) and ×200 (*C-F*).

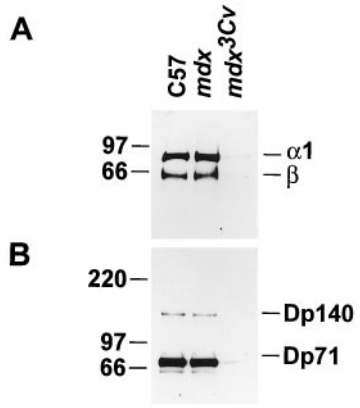

FIG. 6. Immunoprecipitation of the dystrobrevins with Dp71 and Dp140. Triton X-100 solublized proteins from C57B10, *mdx*, and *mdx*^{3Cv} mouse brains were immunoprecipitated with MANDRA1. Coimmunoprecipitated proteins were detected with the βCT-FP antisera (*A*) or the antidystrophin antisera 1538 (*B*). Precipitated proteins, α-dystrobrevin1 and β-dystrobrevin and Dp71 and Dp140 (*B*) are indicated.

enriched at the foot processes of astrocytes surrounding capillaries in the brain (32). Detailed investigations of the location of other components of the DPC in the brain have generated very similar results. Laminin-α2 chain and dystroglycan both are found at the capillary basement membrane, where they localize to the glial/vascular interface (33, 34). Thus, β-dystrobrevin and dystrophin are novel among members of the DPC in brain because they are found only in neurons.

Because β-dystrobrevin and dystrophin are expressed in similar populations of neurons in the hippocampus and cortex it is possible that β-dystrobrevin may interact directly with dystrophin (Fig. 5 *A* and *B*). If this were the case then β-dystrobrevin levels may be reduced in DMD patients similar to the reduction in sarcolemmal staining seen with other components of the DPC in dystrophic muscle. Reduced levels of β-dystroglycan have been described in the brains of *mdx*^{3Cv} mice (35). However, this reduction is likely to reflect the disruption of β-dystroglycan binding to Dp71 because β-dystroglycan is strongly expressed in the brain microvasculature and glia. Similar changes in the β-dystroglycan levels were not observed in *mdx* mice, possibly reflecting the paucity of dystrophin relative to Dp71 in the mouse brain (35).

In this study we were unable to coimmunoprecipitate dystrophin and β-dystrobrevin from detergent-solublized brain membranes. Dystrophin and a significant proportion of β- and α-dystrobrevin 1 are resistant to detergent extraction (D.J.B., unpublished results). It is well documented that postsynaptic density proteins such as dystrophin are resistant to detergent extraction (36). Thus, it remains to be established whether β-dystrobrevin directly interacts with dystrophin and is also a component of the postsynaptic density.

The data presented in this paper provide characterization of a novel dystrophin-associated protein, β-dystrobrevin. β-dystrobrevin is expressed in brain but was not detected in muscle and is therefore unlikely to be a component of the sarcolemmal DPC. Our data raise the possibility that other novel dystrophin-associated proteins could exist in the brain and other tissues where they may form protein complexes with dystrophin or the C-terminal dystrophin isoforms.

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