Dystrophin and dystrophin-associated protein in muscles and nerves from monkey

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Since all organs (i.e. skeletal, cardiac, smooth muscles and sciatic nerve) are never only taken from a single patient, all these tissues were obtained from one cynomolgus monkey, a model closely resembling humans. This work describes an up-to-date reinvestigation of the dystrophin-glycoprotein complex and related molecules in various monkey tissues such those cited above. We used monoclonal and polyclonal antibodies produced in our laboratory, which are directed against dystrophin, utrophin, short-dystrophin products, α-dystrobrevin, β-dystroglycan, α-syntrophin, α-, β-, γ-, δ-, ε-sarcoglycan, and sarcospan. For each molecule, we determined their molecular weight and tissue localization. Regardless of the tissue analyzed, at least one dystrophin or utrophin as full-length molecule and one short-dystrophin product or dystrobrevin as proteins belonging to the dystrophin superfamily were found. β-dystroglycan, β and δ sarcoglycans were always detected, while other sarcoglycans varied from all to only three components. ε sarcoglycan appears to be specific to smooth muscle, which is devoid of α sarcoglycan. Sarcospan is only absent from sciatic nerve structures. Among the different muscles investigated in this study, short dystrophin products are only present in cardiac muscle. All of these findings are summarized in one table, which highlight in one single animal the variability of the dystrophin-glycoprotein complex components in relation with the organ studied. This statement is important because any attempt to estimate protein restoration needs in each study the knowledge of the expected components that should be considered normal.

Key words: dystrophin, glycoproteins, muscle, nerve, monkey.

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Dystrophin is a large cytoskeletal protein (427 kDa) associated with the cell membrane of cardiac, smooth and skeletal muscle (Burghes et al., 1987) and it has also been described in other non-muscle tissues (Gorecki et al., 1992). The N- and C- terminal parts of this protein bind to the actin network and the dystrophin-glycoprotein complex (DGC), respectively (Ervasti and Campbell, 1991; Rybakova et al., 2000). The major DGC proteins are dystroglycans (α and β), that bind the dystrophin carboxy-terminus, span the sarcolemma and interact with laminin in the extracellular matrix (Ibragimov-Beskrovnaya, et al., 1992). Other major components of the DGC are sarcoglycans, composed of five subunits, α, β, γ, δ, ε (Hack et al., 2000; McNally et al., 1998). This complex is associated with sarcospan, a 25 kDa protein that contains four transmembrane-spanning helices with both N- and C-terminal domains localized intracellularly (Crosbie et al., 1997). Different syntrophins (all Mr 59 kDa) have been identified and correspond to α-, β-1 and β-2 in skeletal muscle (Adams et al., 2001) and also recently γ-1 and γ-2 isoforms (Piluso et al., 2000).

Since the discovery of dystrophin (Hoffman et al., 1997), three other dystrophin-related proteins, i.e. DRP1 (dystrophin related protein type 1 = utrophin), DRP2 (dystrophin related protein type 2) and dystrobrevins have also been identified as products of different genes (Enigk and Maimone, 2000; Ahn et al., 1996; Blake et al., 1996). Various dystrophin transcripts (short dystrophin products) have been detected in recent years (see reviews: Fabbrizio et al., 1997; Winder, 1997).

Duchenne and Becker muscular dystrophy are caused by mutations or deletions in the dystrophin gene. Defects in dystrophin have been shown to disrupt the normal expression and localization of other DGC proteins. These proteins are clearly critical with respect to the various forms of muscular dystrophy, since primary defect in any DGC compo-

nents result in muscle tissue degeneration often attended with secondary defects in other DGC components (Repetto et al., 1999; Straub and Campbell, 1995). However, removing the C-terminal part of dystrophin results in normal dystrophin associated protein assembly at the muscle membrane, as recently highlighted with the presence of dystrobrevin (Crawford et al. 2000).

In this paper, we carried out an updated investigation on the presence and distribution of dystrophin-family products and some dystrophin-associated proteins in various cynomolgus monkey tissues (skeletal, cardiac and smooth muscles, and sciatic nerve) using immunoblot and immunofluorescence analyses. We focused on the nature of the dystrophin family products found in each tissue and also detected integral and peripheral components. We used a large battery of specific monoclonal and polyclonal antibodies produced in our laboratory. Their specificity was already described in previous studies related to dystrophin, utrophin and associated proteins (Pons et al., 1994; Rivier et al., 1996; 1999; 2000). We used Western blot technique to determine the presence and size of the molecules detected. Then, in a second step, we analyzed the histoimmuno-localisation of these molecules in various cell membranes. Several findings are summarized in this work and highlight the variability of the dystrophin complex in relationship with the organ studied.

Materials and Methods

Tissues

Tissues from skeletal, smooth and cardiac muscles, and sciatic nerve from cynomolgus monkey (*Macaca fasciularis*) were quickly dissected and flash-frozen in isopentane, cooled in liquid nitrogen, then stored at –80 °C. All tissues were submitted to cryostat sections used for indirect histochemical detection with fluorescent microscope and for crude protein extraction analyzed by Western blot method.

Antibodies

Monoclonal antibodies (5G5, 12G9) were directed against dystrophin molecules, (5F3) was directed against the last C-terminal 31 amino acid initially reported to belong to the dystrophin short product named Dp 71. The specificity of each monoclonal antibody was previously confirmed in muscle and nerve tissues of rabbit (Fabbrizio et al., 1995), Torpedo (Rivier et al., 1996), and adult bovine heart (Rivier et al., 1999). We also produced new polyclonal antibodies directed against dystrophin, utrophin, α-dystrobrevin, epsilon-sarcoglycan and sarcospan that were obtained by injecting synthetic peptides corresponding to their C-terminal sequence (SRGNIPGKPMREDTM), (CPNVPSRPQAM), (GVSYVPYCRS), (PQQQTTGKWYP) and (SLTAESEGPQQKI) as antigens, respectively, according to a previously described protocol (Rivier et al., 1996). In this study, we also used other polyclonal antibodies directed specifically against each sarcoglycan, as follows α (residues PLILDQH), β (residues AGYIPIDEDRHL), γ (residues VREQYTTAEGI), δ (recombinant protein, residues 84-290), and β-dystroglycan (residues PPPYVPP). The antibody specific for α-syntrophin, (residues 191 to 206) was only working in immunofluorescente detection. All of these polyclonal antibodies were previously characterized in adult bovine heart (Rivier et al., 1999) and in a Becker patient (Rivier et al., 2000). To analyze in western blot the presence of α or β syntrophins, a specific polyclonal antibody was developed, corresponding to the 8 last C-terminal residues (VTRLGLLA), but unfortunately it was not able in our hand to give clear immunofluorescente images. Monoclonal antibodies directed against sarcoglycans (α, β, γ, and δ), and β-dystroglycan were also obtained from Novocastra. These antibodies, which are commercially available (Novocastra), were also used to confirm the results presented in this work (this was our general strategy when antibodies were available). All of the results presented here were obtained with polyclonal antibodies that we developed in our laboratory since in some cases, as indicated in the results, no labeling was detected with commercial antibodies.

Western blot analysis

Crude protein extracts were obtained from 100 cryostat sections of each tissue with the extraction buffer (0.05M Tris-HCl, pH 8) complemented with a cocktail of protease inhibitors (100 mM iodoacetamide, 0.1 mM phenylmethyl sulfonic fluoride, 0.01 mg/mL of soybean trypsin inhibitor, 1 mL/µL leupeptine) in the presence of 1% Triton X-100. Homogenates were centrifuged for 10 min at 10000 rpm. Protein solutions were mixed with an equivalent volume of SDS buffer (10% SDS in Tris/HCl

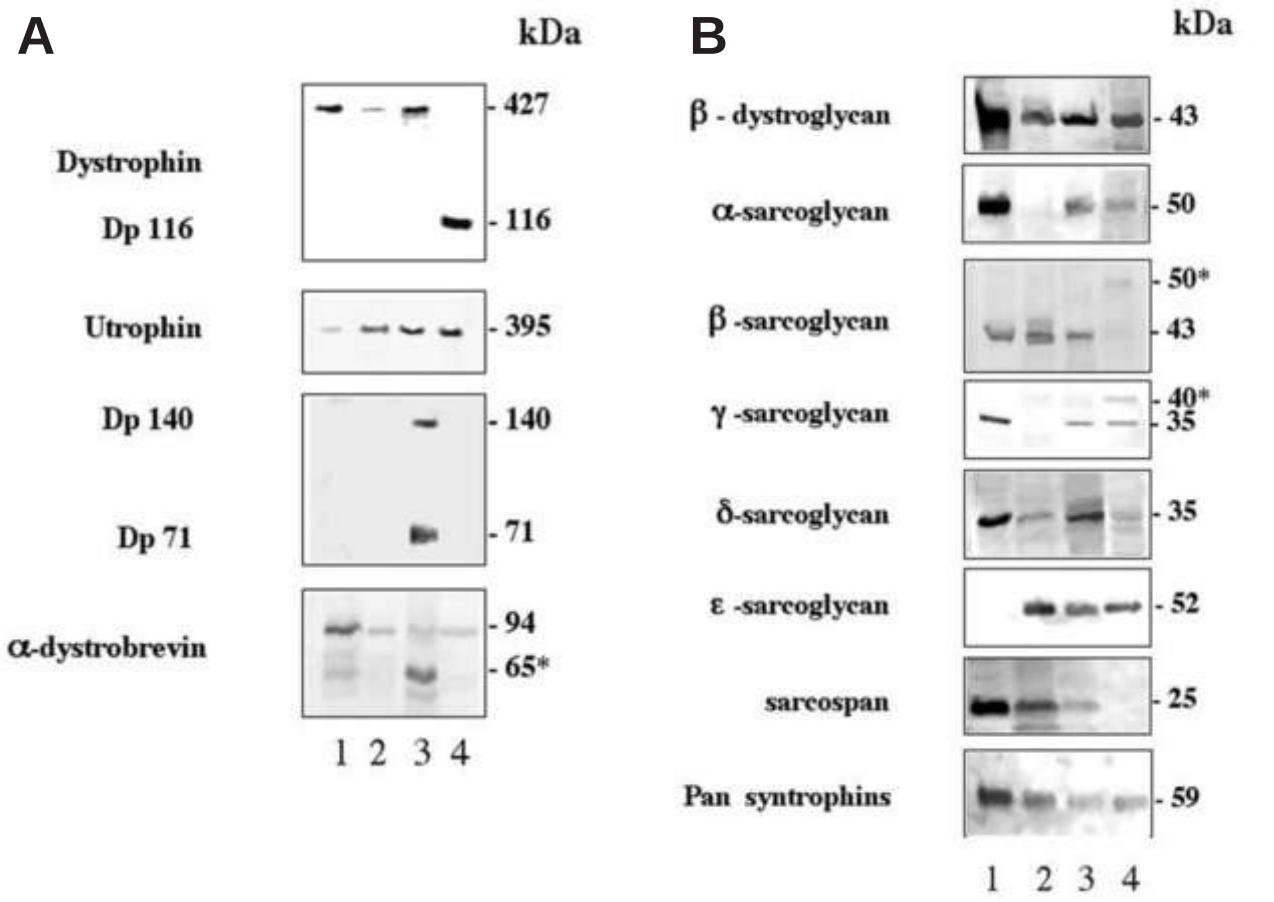

Figure 1. Western blot revelation of some dystrophin superfamily components and associated proteins in various monkey tissues. Crude tissue extracts corresponding to lane 1 = skeletal muscle, lane 2 = smooth muscle, lane 3 = cardiac muscle, and lane 4 = sciatic nerve were treated after transfer by incubation with specific primary antibodies. Part A: Antibodies directed against: various dystrophin superfamily components, as indicated on the left side of each nitrocellulose sheet. Dystrophin (12G9 monoclonal antibody). Utrophin (CUT specific polyclonal antibody directed against the C-terminal end of this molecule). Short dystrophin products containing the 31 amino acids specific to Dp 71 (5F3 monoclonal antibody). α dystrobrevin (specific D1 polyclonal antibody). Figure 1B. Specific polyclonal antibodies directed against: beta-dystroglycan (JAF), each sarcoglycans, sarcospan and pan syntrophins have been used as primary antibodies in similar monkey tissues. The name of the protein detected is indicated on the left side and the molecular weights of the major protein bands are indicated in kDa on the right side of each Western blot. When another protein band was also detected with an unexpected molecular weight, this is indicated by a star (*) following the molecular weight.

pH 8.0, containing 50% glycerol, 0.1 mM 2-β-mercaptoethanol and 0.1% bromophenol blue). The mixture was denatured for 5 min at 100°C, and 10 µL homogenate aliquots were separated in SDS-polyacrylamide slab minigels (3-8 or 5-15% gradient gels). Separated proteins were transferred overnight (30V, 100 mA) in the transfer buffer (25 mM Tris-HCl, 192 mM glycine, pH 8.3, 0.1% SDS and 20% methanol). Nitrocellulose membranes (0.2 µm) were blocked with 3% BSA dissolved in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH 8) for 1 h with specific antibodies, and then incubated with a phosphatase-labeled second antibody (1:3000 dilution, SIGMA BIO-Sciences Laboratory). The protein band was visualized with p-nitroblue tetrazolium, and 5-bromo-4-chloro-3-indoylphosphate substrate as previously described (Fabbrizio et al., 1995).

Scanning densitometry

Western blots were digitized with a 256 gray scale and images were quantitatively analyzed comparatively using the NIH 1.62 image program. Each lane was treated independently and corrected by estimation of the relative optical density found in the Coomassie blue gel, corresponding to the amount of actin present in each tissue extract. Each blot was obtained in triplicate to avoid error due to differences in signal intensities. Values were standardized according to averages of arbitrary values obtained in three assays for each protein analyzed and increasing intensity levels were recorded

according to arbitrary units. An absence of any protein band was noted as = 0.

Immunofluorescence light microscopy

Cryostat sections (10 µm) of unfixed muscle were labeled with hematoxilin or with different specific antibodies. Immunoreactions were detected with Cy3-conjugated sheep anti-mouse Ig (monoclonal antibodies) or Cy3-conjugated sheep anti-rabbit Ig (polyclonal antibodies).

The specificity of the immunochemical procedures was checked by incubation of sections with non-immune serum instead of the primary antibody. As positive controls for this study, skeletal muscle sections of mouse (polyclonal antibodies) or rabbit (monoclonal antibodies) were treated at the same time as the monkey tissues.

Results

Western blot analyses

As shown in Figure 1A, western blot analysis revealed the size of the protein identified by each antibody used in this study. A monoclonal antibody directed against dystrophin (5G5 or 12G9) detected 427 kDa protein bands in skeletal, smooth and cardiac muscle extracts (Lanes 1 to 3 respectively). According to previous studies (Ervasti and Campbell, 1991; Imamura et al., 2000), no full length dystrophin was detected in sciatic nerve (Lane 4) but the short product named Dp 116 was revealed (only when using 12G9 monoclonal antibody). A specific utrophin antibody allowed detection of a 395 kDa protein in all protein extracts. Utrophin full length was more clearly present in sciatic nerve, (see scanning measurements in Table 1). The Western blot pattern obtained with monoclonal antibody 5F3 revealed, only in cardiac extract, two protein bands of Mr 140 kDa and 71 kDa, corresponding to dystrophin-short products Dp 140 and Dp 71 (Lane 3). α dystrobrevin was detected in all tissue extracts. While the α-1 isoform (Mr 94 kDa) was found everywhere but found to be richer in skeletal muscle extract (Lane 1), the α-2 isoform (Mr 65 kDa) appeared to be present in striated muscles and at relatively high abundance in cardiac muscle (Lane 3).

The results of the analysis of some dystrophin associated components are presented in Figure 1B. All tissues studied were found to contain β-dystroglycan (43 kDa). The 50-kDa protein band corresponding to α-sarcoglycan was revealed in all tissues, but it was not present as expected in smooth muscle extract (Lane 2). β-sarcoglycan (43 kDa) was also detected everywhere, but (Lane 4) a more intense 50 kDa protein band was revealed in sciatic nerve extract. Absent from smooth muscle (Lane 2), γ-sarcoglycan (35 kDa) was revealed in all other extracts but a slight reactivity was also found for a 40 kDa protein band in sciatic nerve (Figure 1B, lane 2). Delta-sarcoglycan (about 35 kDa) was found at different intensities in all extracts. ε-sarcoglycan (about 52 kDa) was clearly present in smooth, cardiac and nerve protein extracts (Lanes 2-4). Absent from sciatic nerve extracts (Lane 4), sarcospan was detected in all other tissues (25 kDa). In addition, no result was obtained in monkey tissue in western blots with the specific α-syntrophin that was used in this study. However a pan-syntrophin antibody directed against a peptide region present in all syntrophin molecules (α and β products) revealed a protein band of about 59 kDa in all extracts with variable intensities. All of these data are summarized in Table 1, along with an esti-

Table 1. Distribution of the dystrophin-associated protein complex in different monkey organs. Crude protein extracts were analysed by western blot, and the proteins detected are indicated. Relative intensities of the protein band are noted by values in arbitrary units, after scanning the corresponding Western blot. When another protein band was detected with an unexpected molecular weight, this is indicated by a star (*), following this number and values in arbitrary units are in parenthesis. Otherwise the size of the protein band detected corresponded to the expected molecular weight of the molecule in reference to skeletal muscle. When no protein band was detected, this is indicated by a zero = 0.

Detected Protein	*Mr*	*Muscles Skeletal*	*Smooth*	*Cardiac*	*Sciatic Nerve*
Dystrophin	400	172.34	43.93	189.77	0
Utrophin	395	22.95	82.89	106.94	134.58
Dp 140	140	0	0	130.30	0
Dp 116	116	0	0	0	245.44
Dp 71	71	0	0	153.85	0
α-dystrobrevin	94	94.60	27.41	20.07	31.03
α-dystrobrevin	65*	–	–	(80.01)	–
β-dystroglycan	43	78.73	131.41	188.57	96.94
α-sarcoglycan	50	197.11	0	70.87	37.31
β-sarcoglycan	43	65.70	74.17	50.72	6.76
β-sarcoglycan	50*	–	–	–	(7.52)
γ-sarcoglycan	35	147.91	0	60.51	68.33
γ-sarcoglycan	40*	–	–	–	(39.00)
δ-sarcoglycan	35	166.92	34.73	106.10	30.83
ε-sarcoglycan	52	0	165.51	119.21	119.55
Sarcospan	25	176.10	98.97	39.92	0
Pan-syntrophins	59	139.40	105.78	54.74	66.78

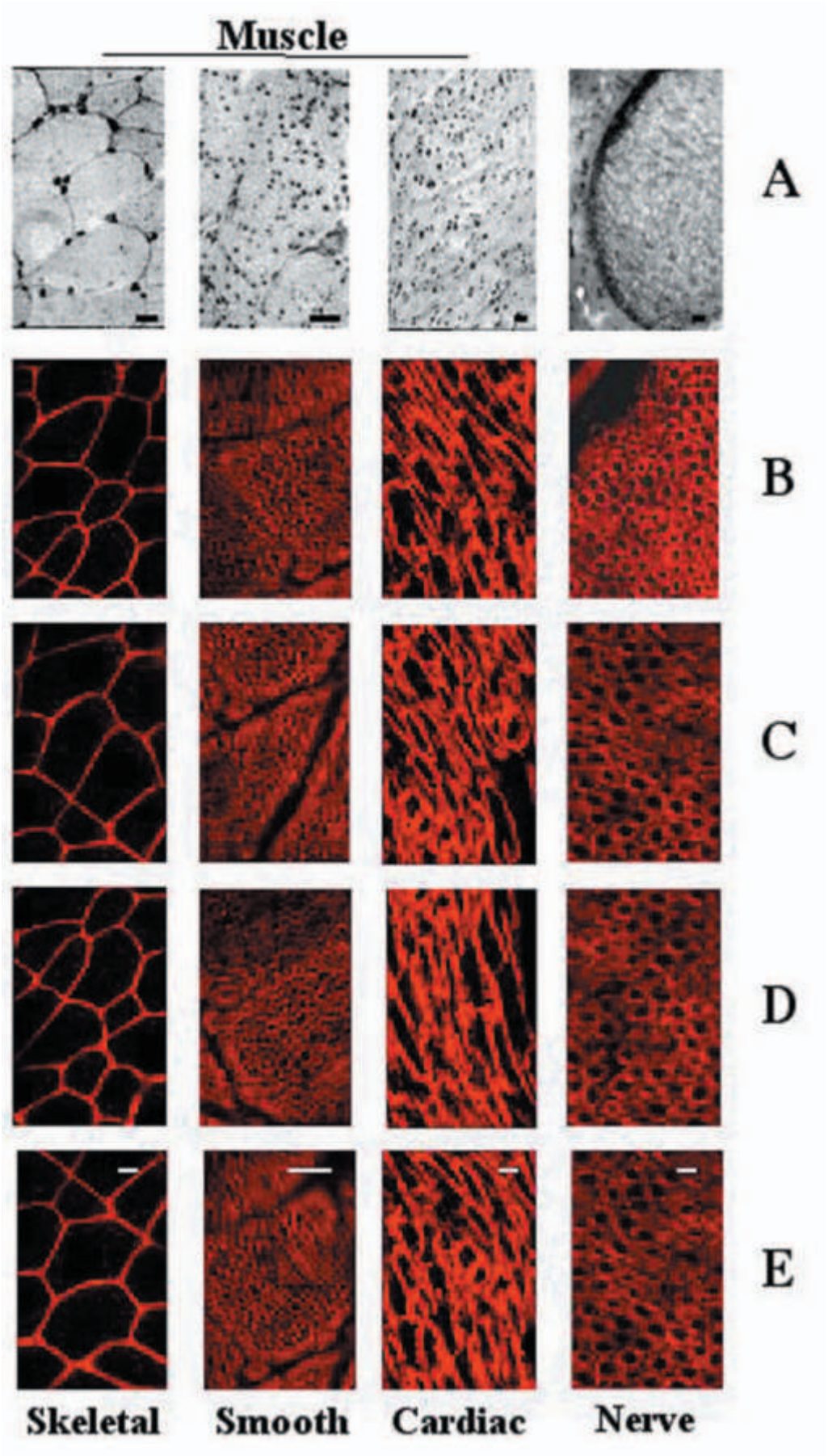

Figure 2. Comparative immunofluorescence in various monkey tissues. As indicated on the top of the panels. The comparative distribution of either dystrophin or the dystrophin glycoprotein complex (DGC) was revealed in skeletal, smooth and cardiac muscles and sciatic nerves, respectively. Here is shown the image obtained after hematoxylin treatment (part A), or using specific antibodies directed against dystrophin (part B); β-dystroglycan (part C), β-sarcoglycan (part D) and δ-sarcoglycan (part E). Bar = 20 μm.

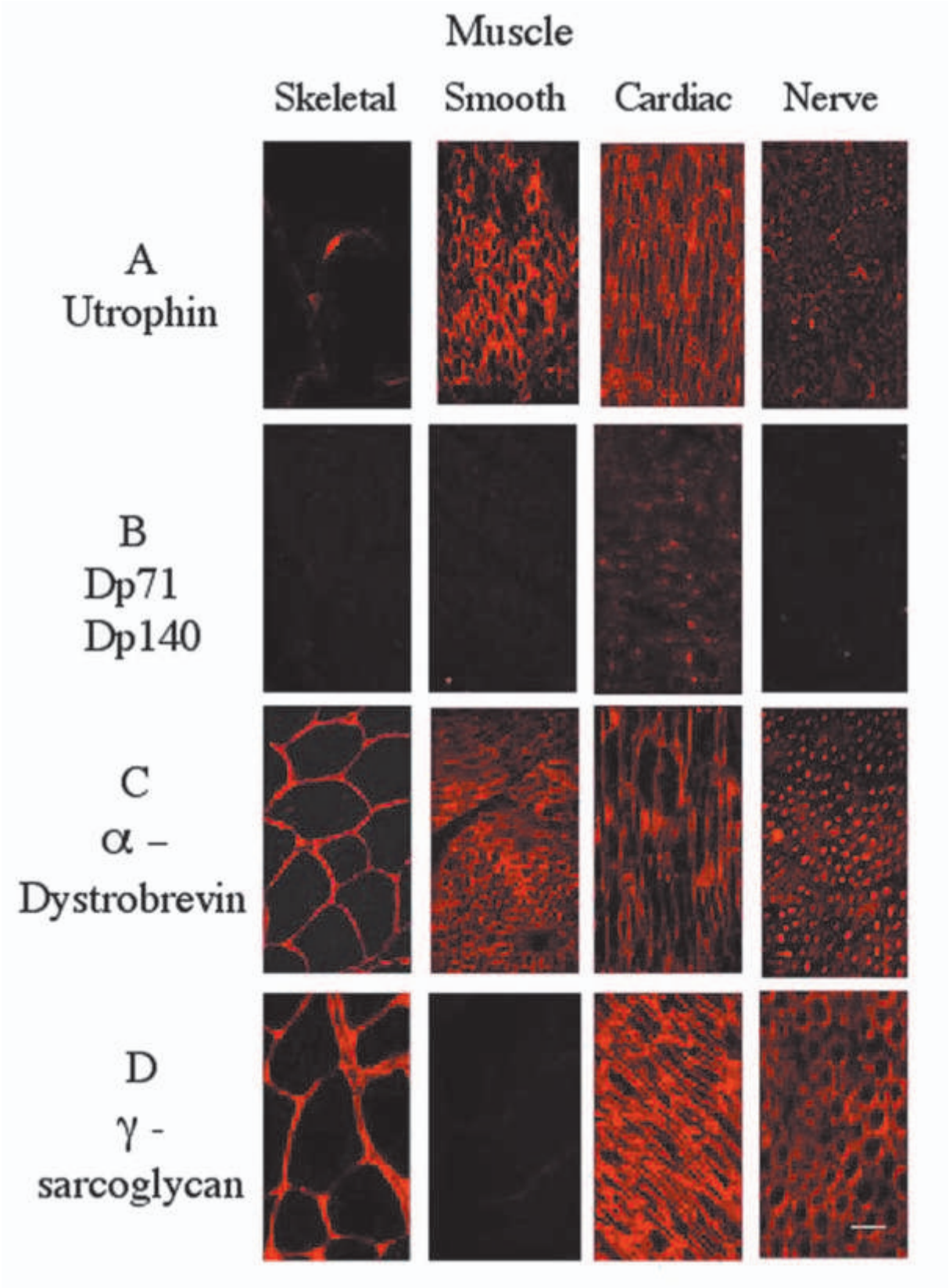

Figure 3. Differences in immunofluorescence images obtained in various monkey tissues. Similarly to previous figure, skeletal muscle, smooth muscle and cardiac muscles as well as sciatic nerves were treated with four different antibodies for specific detection of (A) utrophin, (B) 5F3, short dystrophin products, (C) α-dystrobrevin and (D) γ-sarcoglycan. Bar = 20 μm.

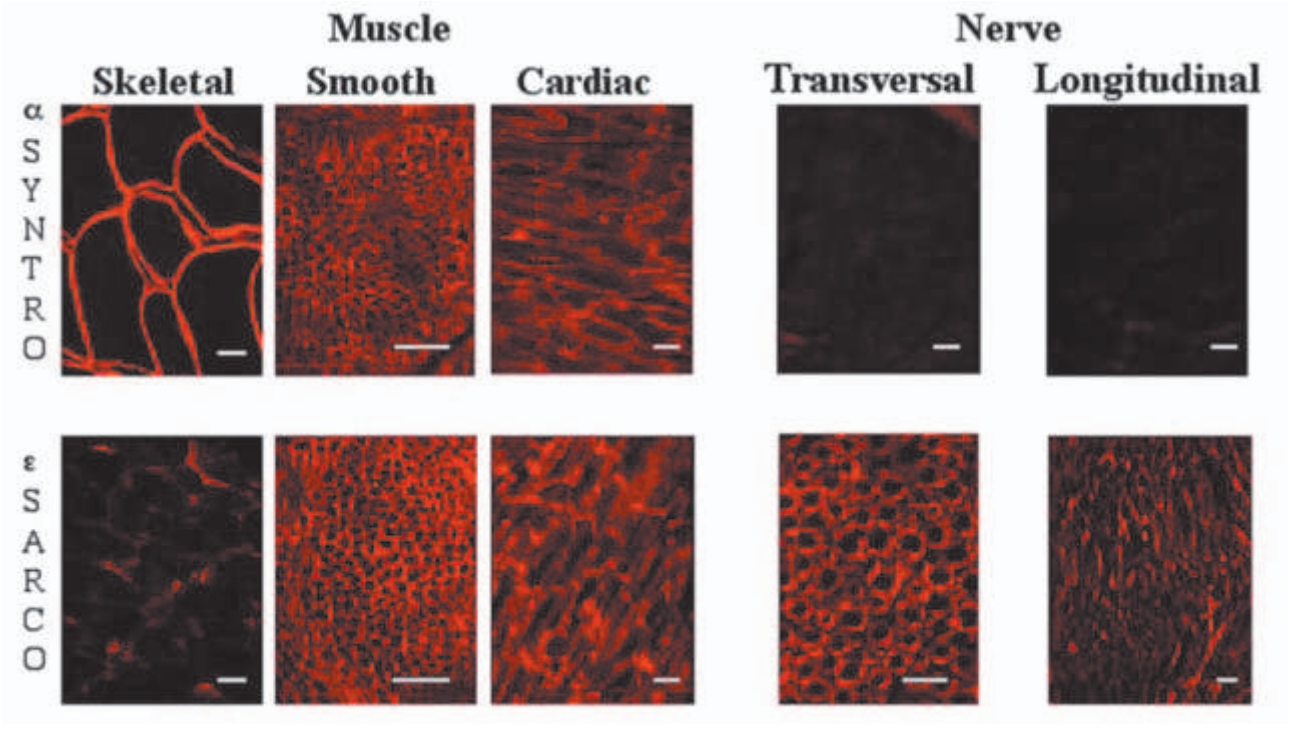

Figure 4. Comparative immunofluorescence in various monkey tissues. According to the same presentation as in Figure 2, skeletal, smooth and cardiac muscles and sciatic nerves were treated to illustrate the corresponding detection for α-syntrophin (top panel); and epsilon sarcoglycan (bottom panel). Bar = 20 µm.

mate of the comparative relative amounts of each protein as deduced from scanning analyses.

Immunofluorescence analysis

Each specific tissue was investigated as follows: skeletal muscle, smooth muscle, cardiac muscle, and sciatic nerve. As presented in Figure 2 (top panel A), staining with hematoxylin indicated good preservation of muscle and sciatic nerve structures and architectures. The periphery of all muscle cell membranes was clearly detected, and fluorescent labelling was also observed in the sheath surrounding each Schwann cell in sciatic nerves when using specific antibodies directed against dystrophin (B), β-dystroglycan (C), β- (D) and δ-sarcoglycan (E) as shown in Figure 2 (panels B to E). In contrast, specific images were obtained when using the following antibodies. Utrophin antibody (Figure 3A) clearly reacted only at the NMJ in skeletal muscle as expected from previous work (Pons et al., 1991), faintly at the cell periphery in smooth muscle, intensively at intercalated disks in cardiac muscle and at Schwann cell membrane and perinerve in sciatic nerve. There was also labelling in microvessels when present in any tissue section analyzed. Monoclonal 5F3, specific to short dystrophin products, gave fluorescent detection only in cardiac muscle (Figure 3B). It was found to detect T-tubules in bovine heart as shown previously (Fabbrizio et al., 1994), but here in monkey cardiac muscle the labelling was discrete and appeared to be restricted to the nuclear and microvessel levels. The α-dystrobrevin specific antibody was clearly detected in all muscles but also in Schwann cell membrane, including axons from sciatic nerve (Figure 3C). This was only obtained with polyclonal antibody that we produced, and was not observed with the corresponding commercial antibodies. The γ-sarcoglycan specific antibody stained cell membranes from skeletal and cardiac muscle but no membrane cell labelling was detected in smooth muscle, while Schwann cell membranes were labelled (Figure 3D). In addition a similar labelling than γ sarcoglycan was obtained with α sarcoglycan (*not shown*), i.e. absence, as expected, from smooth muscle and normal presence in other tissues. In Figure 4 (top view), absence of any significant labelling with α-syntrophin antibody was recorded from either longitudinal or transversal sections of sciatic nerve but present in microvessels and at the periphery of all muscle cells. No

clear western blot was obtained with α-syntrophin polyclonal antibody, however the pan-syntrophin polyclonal antibody has allowed us to detect by western blot either α or β syntrophins in all tissues. Sarcospan was also absent from sciatic nerve (*not shown*), but was clearly present at the periphery of all muscle cells. Epsilon sarcoglycan was restricted to the NMJ in skeletal muscle (Figure 4 bottom view). Smooth muscle membrane and Schwann cells from peripheral nerves were clearly labelled, as well as intercalated disks and microvascularisation in cardiac muscle.

Discussion

More than 10 years after its discovery, the function and localization of the dystrophin complex with dystrophin-associated proteins has not yet been fully elucidated in all tissues. In all muscle cells, dystrophin is thought to play a mechanical function (with the stabilization of membrane integrity during repeated contraction-relaxation cycles), but also a regulatory function (control of elevated free intracellular $Ca2^{+}$ and water concentration, (Durbeej and Campbell, 2002)). In peripheral nerves the role of the dystrophin system is not fully understood.

In a previous immunoblot's study, the distribution of some dystrophin isoforms and some dystrophin-associated proteins was investigated in various monkey tissues (Mizumo et al., 1993). However, due to the discovery of many new dystrophin-associated proteins (10 to 14 now known), it is important to fully review the dystrophin complex in terms of the tissue studied. This is of particular interest for potential therapeutic assays because of the genetic closeness of monkeys and humans which was the focus of the present study.

In our work, the dystrophin superfamily products were clearly identified in different tissue using specific antibodies that we produced and characterized. There was a 400-kDa protein band detected in all muscle tissues studied. This protein band corresponded to full length dystrophin, while it was clear that in sciatic nerve it was present as a short-dystrophin product referred to as Dp 116. The 140-kDa and 70 kDa protein bands were detected and postulated to be short-dystrophin products (corresponding to Dp 140 and Dp 71) only in cardiac muscle. The 70-kDa protein band (identified as Dp71) was controlled to be the most abundant dystrophin product in brain (not shown) and the 5F3 antibody used in this study was previously revealed to be specific to the 31 C-terminal end of this molecule (Imamura et al., 2000). The above result highlighted three points: (i) cardiac muscle contains Dp 71, as previously reported (Fabbrizio et al., 1994), but also Dp 140; (ii) both dystrophin short products may have similar exon splicing (exon 78) to be detected by 5F3 specific antibody, (iii) the distribution of these short dystrophin products mainly appeared at the plasma membrane, rather than in T-tubules or intercalated disks.

All other polyclonal antibodies used in this work were able to detect a protein band that was closely correlated with the expected size according to its specificity (at least in protein extracts from monkey skeletal muscle). We thus considered each of them to be specifically directed against the proteins indicated in Table 1.

All of these findings are illustrated and confirmed by immunofluorescence approaches.

In skeletal muscle, no dystrophin short product was present. Other dystrophin-associated proteins were clearly located at the plasma membrane. However, epsilon sarcoglycan was not detectable in western blots but appeared to be mainly distributed at the neuromuscular junction (NMJ), as illustrated in Figure 4, bottom side. We thus assumed that epsilon sarcoglycan is not a major component of the dystrophin associated protein complex in skeletal muscle cell, but could provide to the dystrophin complex a specific role at the NMJ. However, this result could be a consequence of the specificity of the antibody produced. Nevertheless, in agreement with this data and contrary to other sarcoglycans, the recently reported pathological pattern involving epsilon sarcoglycan defects is related to the myoclonus-dystonia syndrome (Zimprich et al., 2001). Dystonia, which involves movement disorders rather than muscle weakness (Zimprich et al., 2001), could thus be in related more with the nervous system (i.e; neuromuscular junction) than with the contractile apparatus and maintenance of membrane stability. In smooth muscle, all dystrophin short products and clearly α-sarcoglycan and gamma sarcoglycan are proteins that were found to be absent.

These two types of muscle analyzed above revealed the presence of the full length dystrophin molecule together with α-dystrobrevin (a dystrophin family-related product), but with a clear

difference in the composition of the sarcoglycan complex. This feature may reveal some functional specificities with regards to the required role in each tissue.

The data in cardiac muscle indicate the presence of all sarcoglycans. There is probably formation of multiple dystrophin-like complexes by analogy to recent reports in brain (Moukhles and Carbonetto, 2001). First, both dystrophin and utrophin full length molecules are present along with two dystrophin short product with Mrs 140 and 70 kDa. In addition to these dystrophin family products, the α-dystrobrevin was also present. Immunofluorescence images revealed that some labelling was distributed in the plasma membrane of cardiac cells, in T-tubules and intercalated disks but also distributed to the nuclear and microvessel levels. Thus we propose that in cardiac muscle, unlike skeletal muscle, there is an association of some components of the sarcoglycan complex (β and ε sarcoglycans) in both structures cited above. We assume that these complexes may play a particular role in cell to cell communications in regards to cardiac muscle self-contractile stimulation (i.e. cardiac and nodal tissues).

In sciatic nerve, two dystrophin family products were found in the sheath surrounding each Schwann cell (Dp 116 and utrophin), but dystrobrevin appeared to be located in the axon itself. Sarcospan was absent and gamma sarcoglycan appeared to be distributed in Schwann cell membrane. α, β, δ and ε sarcoglycans are found in the outer Schwann cell membrane of peripheral nerve in accordance with results of Imamura et al. (Imamura et al., 2000). In addition, among syntrophin molecules, there was no presence of α-syntrophin but β-syntrophin must be present in accordance with the western blot detection. There was γ-1 isoform that appeared to be distributed in the axon (*personal observation*).

The comparison between these different monkey tissues demonstrated similarities but also clear differences between dystrophin-glycoprotein complexes. There is always at least one full-length dystrophin family product (about 400 kDa), or utrophin, and one short dystrophin family product (referred to as Dp140, Dp 116, Dp71 or dystrobrevin). Dystroglycans, i.e. β-dystroglycan detected in the present work, but also α-dystroglycan, were present in all tissues as well as β-sarcoglycan. All other sarcoglycans were present with at least one representative of the largest isoform (i.e. α- or ε-sarcoglycans with an Mr of about 50 kDa) and at least one representative of the smallest isoform (i.e. γ- or δ-sarcoglycans of about 35 kDa). In detail, epsilon sarcoglycan was relatively absent from skeletal muscle and appeared to be limited to the NMJs or intercalated disks and T-tubules in cardiac muscle. α and γ-sarcoglycan were missing in the sarcoglycans complex in monkey smooth muscles. In smooth muscles there is presence of δ, γ and ε sarcoglycans but immunodetection was unsuccessful for γ-sarcoglycans (Barresi et al., 2000; Straub et al., 1999). Here in smooth muscle from cynomolgus monkey we show that there is complete absence of γ-sarcoglycan. Then it will be of interest to clarify this point with other antibodies directed specifically against γ-sarcoglycan, due to sequence homologies with δ-sarcoglycan and possible crossreactivity. The sarcospan distribution was determined in each tissue studied, and appeared to be only absent in monkey sciatic nerves. This is in agreement with recent reports on the absence of sarcospan in rabbit sciatic nerves (Imamura et al., 2000). Syntrophins were present with a stoechiometry of two molecules, corresponding to various isoforms, and were each present with the full-length or the short-product of the dystrophin family molecules present in the tissue studied. This was in agreement with our immunodetection studies. All tissues appeared to contain syntrophins, proteins that are clearly involved in nNOS and aquaporin distribution within the plasma membrane (Adams et al., 2001).

Then we clearly identified full length and short dystrophin superfamily products (Dps) in each tissue studied. We assumed that this complex could be present under an heterodimeric form in all tissues. β-dystroglycan anchors full-length dystrophin superfamily products to various cell membranes. Short dystrophin superfamily products were always present (Dps and/or α dystrobrevin) with sarcoglycans specific to each tissue studied, in line with published results on skeletal muscle (Blake et al., 1996; Crawford et al., 2000).

Research on human mutations related to the dystrophin system has revealed variability in certain muscular pathologies (Repetto et al., 1999; Straub and Campbell, 1995). Specific antibodies are highly useful tools for clinical diagnosis and identification of deficient proteins in pathological tissue, and thus it will soon be time to begin restoring these deficient elements. Then in future therapeutic strategy to restore any dystrophin or dystrophin-associ-

ated protein it should be focused on gaining further insight into the nature of dystrophin complex components in each organ. This was the thrust of the present study but it would also be important to define the exact role of the dystrophin complex in each organ. This information is essential because any attempt to assess the potential restoration requires a clear knowledge on the normal presence and role of dystrophin complex components in each muscle and non-muscle tissue.

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