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The dystrophin protein family: localization and physiological role

Thesis of the doctoral dissertation

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2007

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Introduction

Duchenne muscular dystrophy (DMD) is one of the most common, severe, life-threatening X-linked human disease. Mutations in the DMD gene are responsible for the disorder. Due to the mutations, dystrophins, the protein products of the DMD gene, are not expressed or expressed in functionally impaired form. The dystrophin proteins are members of the dystrophin-associated protein complex (**D**ystrophin-**A**ssociated **P**rotein **C**omplex or: DAPC) located in the sarcolemma in the striated muscle. The DAPC links the extracellular matrix components to the actin-based cytoskeleton and anchors various signalling molecules. The autosomal homolog of dystrophin, the utrophin protein has got very similar structure to dystrophin, and they can partly substitute each other in the DAPC. The main manifestation of the DMD is the muscular atrophy, nevertheless, cognitive functions are also affected in about one-third of the patients. Verbal IQ, reading capacities, context comprehension and learning abilities are affected most frequently, and memory problems also occur. These phenomena suggest that the central nervous system, more pecisely certain brain regions might be also affected in Duchenne muscular dystrophy.

Numerous data confirm that several members of the DAPC (the DAPC proteins) are localized in neurons and glia cells within the central nervous system and they interact with each other building up different types of DAPCs. The most adundant dystrophin in the brain is Dp71. The dystrophin-related and dystrophin-associated DAPC proteins, the dystrobrevins (DBs) are physiologically important members of DAPC, providing a molecular bridge to syntrophins, the adapter proteins involved in intracellular signalling processes. DBs can connect other members of the DAPC only through dystrophin or utrophin proteins.

The localization, colocalization of the DAPC proteins and the construction of the DAPC itself within the brain is not fully understood thus we also wanted to find out what kind of changes might occur with DAPC proteins without dystrophin in dystrophin-deficient mutant *mdx*$^{\beta geo}$ mice. There are several DAPC protein variants located around the capillaries of the brain which might play a role in the blood-brain barrier. We supposed that in other organs constituting barriers (like lung and testis) there are also DAPCs.

Aims of the research

I. Ultrastructural localization of one splice variant (Dp71f) of the Dp71 in the brain of the rat.

II. Detecting the dystrophin-related and dystrophin-associated α-dystrobrevin (α-DB) protein in the brain microvasculature of wild type and dystrophin-deficient *mdx*$^{\beta geo}$ mice. Search for further DAPC members like β-DB and utrophin in glial cells and reveal their possible colocalization with α-DB in the astroglial endfeet around brain capillaries of the wild type mice.

III. As we revealed the localization of α-DB proteins in neurons in the lateral hypothalamus, we wanted to clarify which isoforms of α-DB are expressed in neurons of wild type and *mdx*$^{\beta geo}$ mice brain.

IV. Search for the colocalization of α-DB with utrophin, β-DB, hypothalamic neuropeptides, like galanin, neuropeptide Y (NPY), melanin concentrating hormone (MCH) and the neuronal nitric oxide synthase (nNOS) in neurons in the hypothalamus of the wild type and dystrophin-deficient *mdx*$^{\beta geo}$ mice.

IV. Localization of the α-DB protein in some barrier making organs like testis and lung.

Materials and method

Animals

Adult male Wistar rats and adult, male C57BL/6 wild type and *mdx*$^{\beta geo}$ mutant mice were used.

Methods

Immunohistochemical and immunocytochemical methods were applied for light microscope, immunofluorescence microscope and transmission electron microscope studies.

Results

- Morphological evidence was provided for the presence of Dp71f dystrophin splice variant in the stratum lucidum of the CA3 region of the rat hippocampus. We localized Dp71f in the postsynaptic densities of axo-dendritic and spine-synapses. Labelled and unlabelled synapses were both present in the examined area. Synapses given by mossy-fiber axons were consistently negative for Dp71f. Besides its synaptic localization we found Dp71f in some myelinated and non-myelinated axons. Immunprecipitate appeared on the axonal membrane of the non-myelinated mossy-fiber segments. Myelinated axons were also heterogeneous as there were morphologically similar labelled and unlabelled axons. In immunopositive axons the protein was attached to the cytoskeletal elements of the axoplasm.

  Our results provided ultrastructural proof for the presence of Dp71f in postsynaptic densities for the first time. Furthermore we detected the protein in a cell compartment (in axons) where dystrophins were not found before.
- Localization and colocalization of some DAPC protein (α-DB and β-DB, utrophin) were examined in the brain microvasculature of wild type and *mdx*$^{\beta geo}$ mice. α-DB protein was found in pericapillary astrocyte endfeet around brain capillaries. The α-DB and the utrophin proteins, the α-DB and β-DB proteins colocalized around the capillary walls in all examined brain regions at the light microscopic level. Comparison of the wild type and the *mdx*$^{\beta geo}$ mice showed that much less veins were immunopositive in the dystrophin-deficient

animals. Ultrastructural examinations revealed that immunolabelling decreased considerably in the pericapillary astrocyte endfeet of the mutant mice. We suggest that -taking into account that dystrophin is needed to attach α-DB to the DAPC- the absence of dystrophin protein can lead to the decreased level of α-DB. The remaining low level of α-DB expression is probably due to utrophin partially replacing the dystrophin.

Our results concerning the localization and colocalization of several DAPC proteins in wild type and dystrophin-deficient mice widen the knowledge of the structure of DAPC in the microvasculature of the brain.

- The α-DB protein was detected also in neurons in the lateral hypothalamus, between Bregma -1.22 mm and -1.94 mm in wild type and *mdx*$^{\beta geo}$ mice. Further characterization uncovered that out of α-DB protein isoforms α-DB1 is not expressed in these cells but α-DB2 and/or α-DB4 can be found in the neurons of the lateral hypothalamus. α-DB2/α-DB4 protein was located in the perikaryon, in axon processes and in synapses. The α-DB2/α-DB4 protein was attached to the luminal side of the membranes of the dilated cisternae of the endoplasmatic reticulum. In the vicinity of labelled perikaryons, immunopositive and immunonegative axons and postsynaptic densities were also found. α-DB2/α-DB4-containing axons and postsynaptic densities very probably belong to the immunopositive cell-bodies. α-DB2/α-DB4 colocalized with β-DB in a group of neurons, however we could detect β-DB in neurons which did not consist α-DB2/α-DB4. The β-DB was found in the cytoplasm and nucleus of the neurons. We proved that the

hypothalamical neurons expressing α-DB2/α-DB4 did not contain utrophin, galanin, NPY and nNOS, but there was perfect colocalization between α-DB2/α-DB4 and MCH in wild type and *mdx*$^{\beta geo}$ mice.
Our results showing the expression pattern of α-DB2/α-DB4 in neurons of the lateral hypothalamus represent the first demonstration of the neuronal localization of α-DB proteins in adult mice.

- We examined the distribution of the α-DB protein in the testis and lung of wild type mice at ultrastuctural level. α-DB was found in structures which are parts of the blood-testis and blood-gas barrier. We detected α-DB in the testis where the plasma membrane of the Sertoli cells is facing the basement membrane and where it is facing the germ cells. In the lung we found the protein in the cytoplasm of the type-1 alveolar epithel cells.
  Our findings in the testis and lung provide new data which lay the foundation of further studies on the localization and the role of the DAPC in the blood-testis and blood-gas barrier.

The localisation and colocalization of DAPC proteins will help to clarify the construction of DAPC within the central nervous system, testis and lung. The increase of our knowledge on dystrophin proteins and the DAPC will help to improve our understanding of the physiological and pathological mechanisms of the DMD.

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Acknowledgement

First of all I would like to thank my supervisor, Dr. Veronika Jancsik for her support, advice, important and useful critical remarks. I thank Professor Peter Sótonyi, head of the Department of Anatomy and Histology (SZIE) for making my dissertation possible. Special thanks to Dr. Katalin Halasy for her useful advice and great help during the laboratory work. I thank Dr. László Németh for his support, Dr.Tibor Baranyai and Dr.Csaba Fekete for their assistance in making the light microscopic pictures. I thank the Department of Pathology and Forensic Veterinary Medicine (SZIE) for the possibility for using the electron microscope. I thank Klára Pető and Olga Szász assistants for their support throught the whole work. I thank my family and friends for their patience and support.