SUPPORT FROM THE COMMITTEE FOR AID AND EDUCATION IN NEUROCHEMISTRY (CAEN)

Category 1B: Research supplies for use in the applicant's home laboratory

FINAL REPORT

Support awarded: **October 2012**

Contact details of the applicant:

Name: Kristina Mlinac Jerković
Title: PhD
Institution: Laboratory for Molecular Neurobiology and Neurochemistry, Croatian Institute for Brain Research, School of Medicine, University of Zagreb
Address: Šalata 12, 10 000 Zagreb, Croatia
Telephone: +3851 4596 853
E-mail address: kristina.mlinac@mef.hr
Current position: Postdoctoral researcher – senior teaching assistant

Na^+/K^+-ATPase expression and activity in brain tissue of mice lacking complex gangliosides

Background:

Na^+/K^+-ATPase, or sodium pump, catalyzes active transport of cations through hydrolysis of ATP across the cell membrane maintaining steep electrochemical gradients and is therefore vital to all eukaryotes. This pump is an oligomer consisting of alpha (catalytic) and beta subunit which is required for biogenesis and correct folding and positioning of the alpha subunit in the membrane (McDonough et al 1990). In our laboratory, we previously detected changes in gene expression of beta3 subunit of Na^+/K^+-ATPase (*Atp1b3*) in brain tissue of mice lacking complex gangliosides. There is an already observed effect of gangliosides, sialic acid-bearing membrane glycosphingolipids, on the activity of Na^+/K^+-ATPase, GM1 ganglioside in particular (Leon *et al* 1981; Fighera *et al* 2006). However, the exact relationship between gangliosides and Na^+/K^+-ATPase remains to be elucidated.

In our laboratory we used mice with a disrupted *B4galnt1* gene (*B4galnt1 null* mice) which lack the enzyme GM2/GD2 synthase and consequently do not synthesize the major gangliosides common to vertebrate brain but instead synthesize comparable amounts of simpler gangliosides GM3 and GD3 (Schnaar 2010). These mice display progressive motor neuropathies, axon degeneration and dysmyelination, even tonic-clonic seizures (Chiavegatto *et al* 2000). Recently discovered human mutation in the same ganglioside biosynthetic enzyme results in various degree of intellectual disability and susceptibility to seizures (Boukhris *et al* 2013; Harlalka *et al* 2013) so *B4galnt1 null* mouse model can be considered as a phenocopy of the human disease. Keeping this in mind, the clarification of the precise nature of ganglioside effect on the activation of sodium pump could give insight into molecular mechanisms of the pathology associated with this human disease.

Hypothesis:

The expression of isoforms other than beta3 subunit of Na^+/K^+-ATPase, as well as the function of the whole pump is altered in mice lacking complex gangliosides due to changed ganglioside composition of the plasma membrane. Since the activity of Na^+/K^+-ATPase depends on proper positioning within the membrane, we suggest that specific ganglioside membrane composition could lead to changes in assembly of the whole pump resulting in decrease or increase of its catalytic activity.

Aims:

I) To analyze the expression and distribution of different isoforms of Na^+/K^+-ATPase beta subunit in the brain of *B4galnt1 null* mice in comparison to wild-type (wt) animals using Western blotting and immunohistochemistry.

II) To measure the activity of Na^+/K^+-ATPase in the brain tissue of *B4galnt1 null* mice in comparison to wt animals.

Results:

I) Immunohistochemical and Western blotting analyses were performed using brain tissue of *B4galnt1 null* mice compared to wt animals. Several different primary antibodies recognizing beta subunits of Na^+/K^+-ATPase (or whole Na^+/K^+-ATPase) were used:

a) mouse polyclonal anti-ATP1B3 antibody (Abcam, #ab67409);

b) rabbit polyclonal anti-ATP1B3 antibody (Proteintech, # 11142-1-AP);

c) rabbit monoclonal anti-sodium potassium ATPase antibody (Abcam, # ab76020);

d) goat polyclonal anti-ATP1B4 antibody (Santa Cruz, # sc-168697).

The visualization was performed with either diaminobenzidine (DAB), or Cy3-conjugated secondary antibodies were used. The results of immunohistochemical staining were inconclusive since extremely low overall expression of beta 3 subunit of Na^+/K^+-ATPase was found in brain tissue slices of *B4galnt1 null* compared to wt mice. Example of immunohistochemical staining is given in Figure 1. Since different beta subunits are expressed in specific cellular subpopulations of brain tissue, eg. beta1 in neurons, beta2 in astrocytes, beta3 in oligodendrocytes (Benarroch *et al* 2011), poor immunoreactivity could just be the result of low abundance of the antigen.

Example of Western blotting results is shown in Figure 2. Again, for beta3 subunits the expression was practically below detection limits, probably for the same reason as in immunohistochemical staining. When antibody recognizing the whole sodium pump was used, unspecific bands were observed (data not shown).

Figure 1. Example of immunohistochemical staining for beta3 subunit of Na^+/K^+-ATPase. Visualization was performed with diaminobenzidine (DAB). Whole brain slices are shown. NC=negative control (primary antibody omitted), WT=wild type, *Null=B4galnt1 null* mice which lack complex gangliosides.

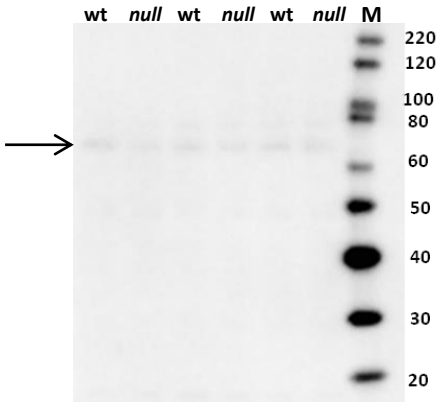

Figure 2. Western blotting results: the expression of beta3 subunit of Na^+/K^+-ATPase in hippocampal homogenates of wild-type (wt) and *B4galnt1 null* (*null*) mice is shown. M=MagicMark Xp Western Standard (Invitrogen). Molecular weights are indicated. One faint band (indicated with an arrow) corresponding to ~ 64 kDa (in agreement with manufacturer's datasheet, Proteintech # 11142-1-AP)

II) The activity of Na^+/K^+-ATPase in brain tissue of *B4galnt1 null* mice in comparison to wt animals was measured according to published protocol (Mršić-Pelčić *et al* 2004). The results are shown in Figure 3. When whole brain homogenates were analyzed, *B4galnt1 null* mice show higher activity of Na^+/K^+-ATPase than wt animals (3.69 compared to 2.31 µmol P_i/(h mg) protein in wt animals). Statistical analysis of obtained quantitative data was not performed due to the fact that only 2 animals in each group were used. However, this preliminary finding is highly encouraging and we plan to investigate this further.

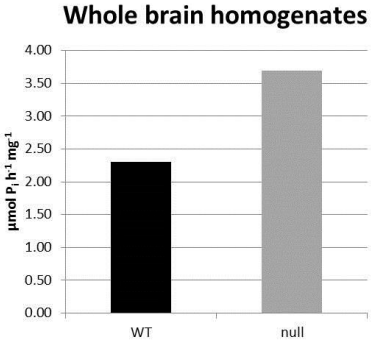

Figure 3. Activity of Na^+/K^+-ATPase expressed as µmol P_i/(h mg) protein is shown. Whole brain homogenates of wild-type (wt) and *B4galnt1 null* (*null*) mice were analyzed.

Results communications:

The results were in part presented at 9th FENS Forum in Milan, Italy held from 5th to 9th July 2014. as a poster presentation titled "Effects of altered ganglioside environment on the expression and localization of selected mebrane proteins in mammalian nervous tissue" (Mlinac, Viljetić, Jovanov Milošević, Schnaar, Smalla, Heffer, Kalanj Bognar).

Purchases:

The funds from this support were spent on acquiring antibodies and chemicals, as well as smaller equipment and laboratory consumables necessary for the completion of immunohistochemical and Western blotting analyses:

- Primary antibodies
- Secondary antibodies (both HRP- and Cy3-conjugated)
- DAB Peroxidase substrate kit (DAB Tablets with Metal Enhancer)

- Albumin from bovine serum (BSA)
- Histological mounting medium
- Slide staining trays, jars and racks
- Western blotting equipment: mini gel tank and Bolt mini blot module (transfer module).

In conclusion, I will gratefully like to thank The committee for aid and education in neurochemistry (CAEN) for the opportunity given to me. With this support I was able to pursue a research idea I wouldn't be able to investigate otherwise and very promising preliminary results were obtained which will be explored further. Finally, the support to me as a postdoctoral researcher was of immense importance as a starting point and an important first step towards future independent career.