Annual report on project grant: The role of *N*-arachidonoyl phosphatidylethanolamine phospholipase D in regulating the activity of primary sensory neurons in naive and inflammatory conditions

Our aim in this project is to characterise the expression pattern, and the effect, of N-arachidonoyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression on spontaneous and noxious stimulus-evoked activity of primary sensory neurons (PSN) in naive conditions and during the inflammation of peripheral tissues.

We have started the project on 1st February 2009.

Objective No 1.

To describe the expression pattern of NAPE-PLD in the perikarya as well as in the central and peripheral processes of the two major sub-populations of PSN.

We have characterised two NAPE-PLD antibodies (from Professors Ken Mackie and Maurice Elphick) on wild type (WT) and NAPE-PLD knock out (KO) mice cerebellum and dorsal root ganglia (DRG). We found that similarly to that reported recently (Cristini et al., 2008) the antibodies produced staining in Purkinje cells and in the neuropil in the molecular layer from WT mice. No such staining was observed in tissues from KO animals (Figure 1 A and B). Next, we studied staining in DRG of WT and KO mice (Figure 1 C and D). While the antibodies produced staining in a sub-population of neurons in DRG of WT, they did not label any structure in DRG of KO, animals.

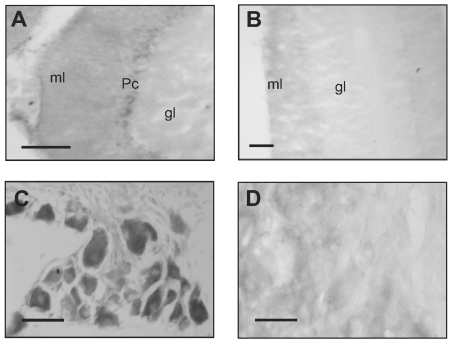

Figure 1. Cerebellum (A and B) and DRG (C and D) sections from WT (A and C) and NAPE-PLD KO (B and D) mice immunoreacted with Professor Ken Mackie's NAPE-PLD antibody. Note that the antibody produced staining in tissues taken from WT but not from KO mice.

Since Professor Mackie's antibody works in a higher dilution than Professor Elphick's antibody, we used the former first to analyse NAPE-PLD expression in rat DRG (Figure 2). We found that 57.6±4.7% (353 of 581; n=3) of the neurons showed positivity for NAPE-PLD. Although, the NAPE-PLD immunolabelled DRG neurons appeared smaller than those of the immunonegative cells the difference was not significant (1383±122µm^2 and 1616±135µm^2 for the NAPE-PLD immunopositive and immunonegative,. respectively). Next, we studied the co-expression between NAPE-PLD and the transient receptor potential vanilloid type 1 ion channel (TRPV1), which is one of the targets of anandamide in DRG neurons (Zygmunt et al., 1999). We found that 71.7±2.4%; (n=3) of the TRPV1 immunopositive cells were NAPE-PLD immunolabelled and that 44±0.8%; (n=3) of NAPE-PLD immunolabelled cells were TRPV1 immunopositive.

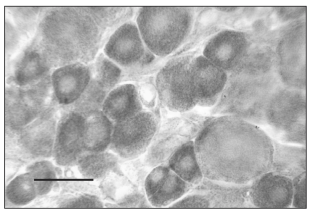

Figure 2. Immunolabelled small-medium size DRG neurons in a rat DRG section.

We have prepared NAPE-PLD/calcitonin gene-related peptide/IB4 labelling on rat DRG sections. Analysis of these data is in progress. Initial findings indicate that NAPE-PLD does not show preference for peptidergic or non-peptidergic cells.

In spinal cord sections from rats, the antibody revealed NAPE-PLD expression in the neuropil of the superficial dorsal horn. In addition small cellular structures, probably glia cells were also visible in the dorsal horn. This finding requires further studies.

At peripheral tissues (skin and urinary bladder) the antibody produced staining both in neuronal fibres and various other non neuronal structures. Currently, we are preparing sections with double immunostaining using the anti-NAPE-PLD antibody and an anti b-tubulin antibody to facilitate recognition of nerve fibres.

In conclusion, we found in these experiments that NAPE-PLD, as indicated by our recent findings (Nagy et al., 2009) is expressed by a sub-population of small-medium size DRG neurons and their peripheral and central terminals. A larger proportion of the NAPE-PLD neurons also express TRPV1. We also found that NAPE-PLD, in addition to primary afferent fibres, is also expressed by other structures, which should requires further studies.

Objective 2.

To characterise changes in NAPE-PLD mRNA and protein expression induced by inflammation of the peripheral tissues.

Due to the rapid developments of the field, we modified our original objective. In addition to NAPE-PLD, anandamide-synthesising enzymes with known molecular identity were also included in studies designed to find inflammation-induced changes. First we studied the mRNA expression of glycerophosphodiester

phosphodiesterase 1 (GDE1), α/β-hydrolase 4 (ABH4), protein tyrosine phosphatase, non-receptor type 22 (PTPN22), group 1B soluble phospholipase A_2 (IBsPLA2), inositol 5' phosphatase SHIP1 (SHIP1) in intact rat DRG and cultured DRG neurons (Figure 3) grown in control medium and in the presence of capsaicin (Figure 4).

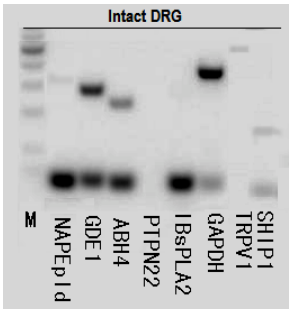

Figure 3. mRNA expression of anandamide-synthesising enzymes with known molecular identity in intact rat DRG.

We found in these experiments that all the known enzymes except PTPN22 is expressed both by intact and cultured DRG neurons and that 2 day culturing does not produce apparent changes in the expression. Semi-quantitative PCR analysis (Figure 5) revealed that in addition to a significant reduction in TRPV1 and NAPE-PLD expression (Nagy et al., 2009) the expression of GDE1, ABH4, and SHIP1 is also reduced by capsaicin treatment. These data suggest that all enzymes, except PLA2 (and PTPN22) are expressed by capsaicin-sensitive cells. However, the non-calcium-sensitive enzymes (GDE1, ABH4, and SHIP1), in contrast to NAPE-PLD, are expressed by a major proportion of the non-capsaicin-sensitive neurons, too. The sensitivity of NAPE-PLD expression to capsaicin is in agreement with our recent findings on mouse DRG neurons (Nagy et al., 2009).

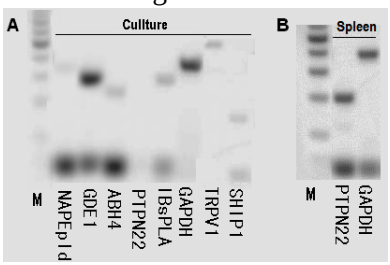

Figure 4. mRNA expression of anandamide-synthesising enzymes with known molecular identity in control rat DRG cultures.

We also studied the effect of capsaicin on enzymes expression by real-time quantitative PCR (qPCR). We used primers used in RT-PCR with SYBR-green. These data revealed that PTPN22 is also expressed by DRG cultures, though its expression is very low as it is detectable only after ~32 cycles. Further, PTPN22 expression is significantly increased by capsaicin treatment indicating that it might not be expressed by cells which degenerate during long-term capsaicin exposure. qPCR did not confirm reduction in NAPE-PLD, ABH4 and SHIP1 expression. However, these data should be interpreted with caution since dimer-primer pairs could give false fluorescent reading. Clearly these experiments should be repeated.

The mRNA expression of the various enzymes was also studied in mouse intact DRGs. We found that the expression pattern could show species differences: in the mouse PTPN22 showed a weak expression while no expression for PLA2 was detected.

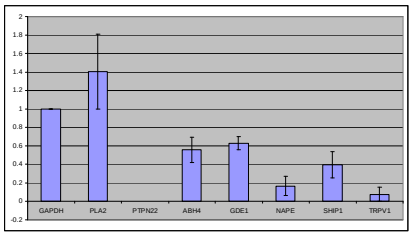

Figure 5. Semi-quantitative analysis of mRNA expression of various anandamide-synthesising enzymes.

We injected complete Freund's adjuvant and carrageenan into the right hind paw of both rats and mice. We have already isolated RNA. Following re-running the qPCR experiments with cultured rat DRG samples and making sure that no false positivity distorts our data, samples from control, saline-injected and the inflamed animals will be studied.

We have also collected DRGs for sectioning and subsequent analysis for NAPE-PLD expression by immunohistochemistry.

Objective 3.

To find the effect of siRNA-mediated knock-down of NAPE-PLD expression in DRG on spontaneous and mildly noxious heat- and mechanical stimulation-evoked activity of PSN after evoking inflammation of peripheral tissues.

We have started pilot experiments and collected tissue samples following intrathecal cannulation and saline injection. Since we are quickly approaching the tenure of this project , we decided to suspend experiments for this objective until we finish studies for objective 1 and objective 2.

References

Cristino L, Starowicz K, De Petrocellis L, Morishita J, Ueda N, Guglielmotti V, Di Marzo V. Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. Neuroscience. 2008 Feb 19;151(4):955-68. Epub 2007 Dec 8.

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