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DISSERTATION

"Pathological pain processing in the mouse model of multiple sclerosis and spinal cord injury: contribution of Plasma Calcium ATPase 2 (PMCA2)"

by

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Cancer Center, G-1196

ABSTRACT

Neuropathic pain is often observed in individuals with multiple sclerosis (MS) and spinal cord injury (SCI) and is not adequately alleviated by current pharmacotherapies. A better understanding of underlying mechanisms could facilitate the discovery of novel targets for therapeutic interventions. We previously reported that decreased PMCA2 expression in the dorsal horn (DH) of healthy PMCA2$^{+/-}$ mice is paralleled by increased sensitivity to evoked nociceptive pain. These studies suggested that PMCA2, a calcium extrusion pump expressed in spinal cord (SC) neurons, plays a role in pain mechanisms. However, the contribution of PMCA2 to neuropathic pain mechanisms in the DH remains undefined. The present studies investigated the role of PMCA2 in neuropathic pain processing in the DH of wild–type mice by utilizing three different models of disease or injury: experimental autoimmune encephalomyelitis (EAE), an animal model of MS and SC inflammation, SCI, and chronic constriction injury (CCI) of the sciatic nerve, a model of peripheral nerve injury.

EAE was induced in female and male C57Bl/6N mice via inoculation with myelin oligodendrocyte glycoprotein fragment 35-55 (MOG_{35-55}) emulsified in Complete Freund's Adjuvant (CFA). CFA-inoculated mice were used as controls. A severe SC contusion injury was induced at thoracic (T8) level in female C57Bl/6N mice. Pain was evaluated by the Hargreaves' and von Frey filament tests. PMCA2 levels in the lumbar DH were analyzed by western blotting and qRT-PCR. The effectors that decrease PMCA2 expression were analyzed in pure SC neuronal cultures.

Increased pain in mice with EAE was paralleled by a significant and selective decrease in PMCA2 levels in the DH, whereas the expression of other PMCA isoforms was not changed. In contrast, PMCA2 levels remained unaltered in the DH of mice with EAE that manifested motor deficits but not increased pain, supporting the notion that a decrease in PMCA2 coincides with pain. Astrocyte and microglia activation were comparable in mice affected by EAE with and without pain symptoms. However, Interleukin-1β (IL-1β), Tumor Necrosis Factor α (TNFα) and IL-6 expression were robustly increased in the DH of mice with EAE demonstrating pain, whereas these cytokines showed a modest increase or no change in mice with EAE in the absence of pain. This suggested that cytokines could be triggers that reduce PMCA2 expression in the DH, through direct or indirect actions on neurons. Although IL-1β, TNFα and IL-6 receptors were expressed in pure SC neuronal cultures, only IL-1β decreased PMCA2 levels through direct actions on neurons, *in vitro*.

In mice sustaining a SCI, there was a selective decrease in PMCA2 in the lumbar DH which coincided with increased below level neuropathic pain. In contrast, increased pain sensitivity was not paralleled by a reduction in PMCA2 in the lumbar DH following CCI.

Taken together, these results support the notion that PMCA2 is a contributor to neuropathic pain mechanisms in EAE and SCI and could be a potential novel therapeutic target. We propose that a decrease in PMCA2 in DH neurons is paralleled by increased pain sensitivity, most likely through increased intracellular Ca^{2+} which leads to perturbations in Ca^{2+} signaling, Ca^{2+}-dependent transcription factor activation and pro-nociceptive gene expression, and excitability of DH neurons.