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Direct functional coupling between the $Ca_V3.2$ T-type calcium channel and nitric oxide synthase contributes to nociception

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As described for many membrane proteins, ion channels are often parts of multiprotein complexes. This concept is well described for voltage gated calcium channels of the Ca_V1 and Ca_V2 families. Such concept is less documented to date for members of the CaV3 family coding for low voltage gated T-type calcium channels. In the recent years, a particular notion emerged on the implication of the $Ca_V3.2$ subtype in pain perception and transmission by primary afferent nociceptors. In this context, we investigated if the $Ca_V3.2$ subunit is able to selectively interact with intracellular partners. Our results show that the $Ca_V3.2$ C-terminus ends up with a class 3 PDZ ligand motif that interacts with the neuronal Nitric Oxide Synthase (nNOS). This enzyme is responsible for the production of the gaseous transmitter Nitric Oxide (NO) implicated in a plethora of modulations including in the tuning of sensory neuron excitability. We show that nNOS is targeted to the plasma membrane as function of $Ca_V3.2$ coexpression. The close vicinity of nNOS and $Ca_V3.2$ optimizes the nNOS calcium dependent enzymatic activity, and leads to an increased NO production. Inversely, the nNOS-$Ca_V3.2$ interaction leads to a reduction of $Ca_V3.2$ current density in heterologous expression systems. This decrease is not due to a reduction of channel trafficking at the plasma membrane but rather to a tonic reduction of channel activity. The molecular determinant of this feedback $Ca_V3.2$ inhibition implicates an extracellular histidine residue previously showed to participate in the binding site of metal ions. Since both nNOS and $Ca_V3.2$ has demonstrated pronociceptive roles, we investigated whether uncoupling this interaction *in vivo* would modulate nociception. Intrathecal injection of the of cell-penetrating decoy peptides composed of the TAT sequence fused to the last $Ca_V3.2$ amino acids showed that hampering the nNOS-$Ca_V3.2$ interaction in sensory neurons has a profound analgesic effect. Control experiments using mutated decoy peptide lacking the PDZ ligand sequence, and the use of $Ca_V3.2$ knock out animals showed the selectivity of this pharmacological modulation of the nNOS-$Ca_V3.2$ interaction.

All in all, the perspectives of these findings are interesting in the understanding of the pathophysiology of pain since both nNOS and $Ca_V3.2$ are considered as validated targets for the discovery of innovative analgesics.