Painless NGF (pNGF) and the mechanisms of pain sensitization in DRG neurons

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Nerve Growth Factor (NGF) is being considered as a therapeutic candidate for Alzheimer's disease (AD) treatment, however, the development of a NGF-based therapy is limited by the partial access of NGF to the brain and to its potent nociceptive activity in animals and humans. Indeed, NGF is involved in pain transmission and perception. Recently, mutated forms of NGF at residue R100 were discovered, allowing to increase the dose of NGF without triggering pain. The 'painless' hNGF hNGFP61S/R100E (pNGF) displays full neurotrophic and anti-amyloidogenic functions in neuronal cultures but a reduced pain perception *in vivo.* However, the molecular mechanism through which pNGF exerts a reduced nociceptive activity has never been investigated.

Aim of the present study was to assess, in dorsal root ganglia cultured neurons (DRG), the molecular properties of pNGF, with respect to NGF, first analyzing the different gene profiles by microarray analysis. We therefore studied the expression and release of Substance P (SP) and Calcitonin gene-related peptide (CGRP) as main neuromediators involved in peripheral NGF response, characterizing the related mechanism of action.

From the microarray profile we conclude that pNGF regulates in DRG neurons a small number of genes, differentially with respect to NGF. Altogether, this gene set identifies a pain network differentially modulated by pNGF versus NGF, that could subserve the reduced pain sensitization ability of pNGF.

Moreover, we demonstrated that NGF and pNGF up-regulate in the same way SP and CGRP expression at both mRNA and protein level, however, upon bradykinin (BK) stimulation, pNGF treated DRGs release a reduced amount of SP and CGRP, as compared to NGF pre-treated neurons. We therefore established, by Western blot and immunofluorescence analysis, a slight up-regulation of BK receptor 2 (B2-R) expression by pNGF, unlike the strong increase induced by NGF treatment. As a consequence of B2-R up-regulation by NGF, we observed a robust phosphorylation of the transient receptor channel subfamily V member 1 (TRPV1) following BK application in NGF-treated neurons, differentially with respect to pNGF.

At last, Fluo-3 traces demonstrated that BK treatment induces a higher and more sustained cytoplasmic Ca^{2+} signal in NGF-, as compared to pNGF-treated DRGs.

Altogether these results indicate that in DRG neurons pNGF induces a reduced up-regulation of B2-R and consequently a lower phosphorylation of TRPV1 receptors by BK.

The reduced TRPV1 receptor activation induces a lower intracellular Ca^{2+} mobilization responsible for the minor release of noxious mediators.