

ABSTRACT OF DISSERTATION

Title	CGRP Induces Differential Regulation of Cytokines from Satellite Glial Cells in Trigeminal Ganglia and Orofacial Nociception
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<p>Background</p> <p>In the peripheral nervous system (PNS), inflammatory pain may depend on the phenomena occurring inside the sensory ganglia. Neurons in sensory ganglia are pseudo-unipolar, as their axons bifurcate and project into the brainstem or spinal cord and the periphery. The non-synaptic transmission occurs by released diffusible chemical messengers, such as cytokines. A distinct type of glial cells called as satellite glial cells (SGC) surrounds neuron soma. These features, which are specific to the PNS, allow the occurrence of bidirectional communication through the axon to the periphery and center of both ends, chemical transmission due to neuroinflammatory substances and neuron-glia interaction leading to cross-excitation.</p> <p>Intra-ganglionic (IG) secretion of calcitonin gene related peptide (CGRP) modulates the neuronal transmission of pain signals. CGRP contributes to the development of peripheral and central sensitization in orofacial inflammatory and neuropathic pain and migraine. In trigeminal ganglion (TG), CGRP is primarily found in the small- medium- diameter neurons whereas CGRP receptors are found on large diameter neurons and SGCs. Thus, the CGRP released can locally affect SGCs and sensitize the primary afferent neurons, which in turn can activate positive feed-forward circuitries that can initiate and or sustain a painful event. Therefore, blocking this loop may exert a therapeutic effect. The aim of the present study was to investigate the role of the SGCs in TG on cytokine-related nociception in response to IG administration of CGRP.</p> <p>Methodology</p> <p><i>IG drug administration.</i> CGRP alone (10 μl of 10^{-5} M), Minocycline (Min, 5 μl containing 10 μg) followed by CGRP with one hour gap (Min + CGRP), or normal saline (10 μl) were administered directly inside the TG in independent experiments.</p> <p><i>Behavioral assessment.</i> Rats (n = 7) were evaluated for thermal hyperalgesia at three temperatures (37, 45 and 10 ° C) at 6 and 24 hours post-injection. The reward-licking</p>	

events/stimulus-contact events ratio (L/F) and stimulus duration/stimulus-contact events were evaluated.

Quantitative RT-PCR. For seven genes (n = 5) was performed to evaluate the expression of Tbp (TATA box binding protein), IL-1 β , IL-6, TNF- α , IL-1RA, NaV 1.7 and GFAP mRNA.

Immunohistochemistry. To measure the expression of glial fibrillary acidic protein (GFAP) following IG drug administration in the TG sections (n = 3).

Cytokine measurement. 29 cytokines released in culture media from glial rich cultures (n = 3) was evaluated under control conditions and following overnight exposure to 1 μ M CGRP using the Proteome Profiler Rat Cytokine Array Panel A.

Statistical analysis. Within-group differences were examined using the paired t-test and between group differences using t-test. mRNA expression was compared using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test. Sample size was not calculated due to unavailability of data from previous similar studies.

Results

IG CGRP significantly reduced L/F ratio at 6 hours ($41.5\% \pm 9.0$) and 24 hours ($57.8\% \pm 13.7$) post injection. Stimulus duration/stimulus-contact events were significantly reduced at 6 hours ($61.9\% \pm 12.4$). Injecting Min 1 hour before CGRP significantly increased L/F ratio ($165.145\% \pm 44.74$) compared to the only CGRP injection (41.5 ± 9.0) and stimulus duration/stimulus-contact events (181.9 ± 44.1) to only CGRP (61.9 ± 12.4) at 6 hours. There is a significant increase in GFAP mRNA and protein expression between 1 and 6 hours that was accompanied with a significant increase in expression of cytokine IL-1 β and IL-1RA post CGRP injection and injecting Min 1 hour before CGRP significantly down regulated the GFAP mRNA and protein expression and mRNA expression of cytokines IL-1 β and IL-6. Overnight stimulation of glial rich culture with 1 μ M CGRP led to a more than 1.5-fold increase in the protein expression of 20 cytokines (including IL-1 β , IL-6 and IL-1RA), no change in 6 cytokines (between 1-1.5-fold change) and down-regulation of 3 cytokines, as compared to control conditions.

Conclusion

IG CGRP induces thermal hyperalgesia, which may be related to the secretion of various cytokines within the ganglion. IG administration of Min 1 hour before CGRP alleviated thermal hyperalgesia and downregulated cytokine expression. These findings support the notion that increased glial activity contributes to hyperalgesia and that glial inhibition can be effectively used to alleviate it.