TRPV1 RECEPTORS ARE UPREGULATED IN NODOSE GANGLIA BUT NOT DORSAL ROOT GANGLIA FOLLOWING *NIPPOSTRONGYLUS BRASILIENSIS* (Nb) INFECTION IN MICE

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Introduction

TRPV1 (VR1) receptors are predominantly expressed on peripheral sensory neurons and have been implicated in various acute models of sensitization. VR1 has been found in subpopulations of neurons in both nodose ganglia (NG) and dorsal root ganglia (DRG). The aim of this study was to determine if there are long term differences in expression of TRPV1 receptors in visceral sensory afferent nerves following *Nippostrongylus brasiliensis* (Nb) infection in mice.

Methods

Balb/c mice were infected with 500 L3 larvae of Nb or sham-infected before being sacrificed 21 days later. Three to four days prior to sacrifice, animals were given an intraperitoneal (IP) injection of Cholera Toxin B - Alexa Fluor 488 ® (CTB488, Molecular Probes, OR) to label visceral nerves. All procedures were approved by the Animal Care Committee. Neutral buffered formalin (NBF) fixed, paraffin-embedded NG and DRG were sectioned and stained using a polyclonal IgG antiserum (Oncogene, MA) to immunolocalize VR1. Detection was by a biotinylated 2° Ab and a HRP streptavidin 3° Ab followed by 3-amino-9-ethylcarbozole chromogen. The VR1-immunoreactivity (IR) was quantified in sections by determining the integrated optical densities of specific and background labelling, using QWin image analysis software (Leica, ON). CTB488-labelled neurons were also laser microdissected and mRNA was hybridized to murine Affymetrix arrays to assess relative levels of RNA expression. Quantitative PCR (RTq-PCR) was also employed to measure of mRNA expression levels. Histology data are expressed as mean ± SEM and were analyzed using ANOVA and Tukey's tests. RTq-PCR data are expressed as mean ± SEM and were analyzed with a two-tailed t-test.

Results

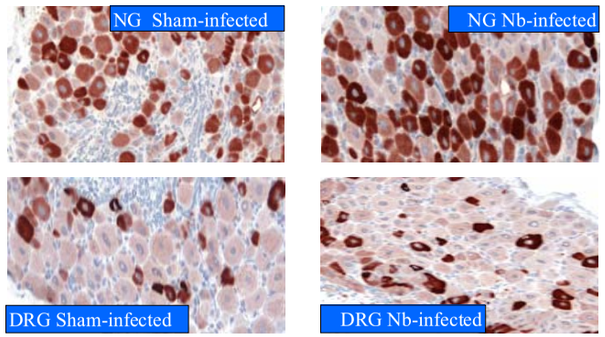

Figure 1: VR1-immunoreactivity in preparations of sham and Nb-infected ganglia Nodose and Dorsal Root Ganglia
VR1-IR appeared to be greater in NG, compared with DRG; and increased in NG following Nb infection. Sham and post-Nb DRG appeared similar.

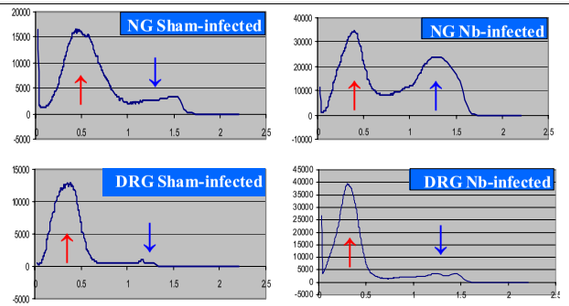

Figure 2: Sample VR1-IR Optical Density (OD) Measurements
Specific VR1-IR (blue arrows) was separated from background staining (red arrows) with a cut-off of 0.75 OD.

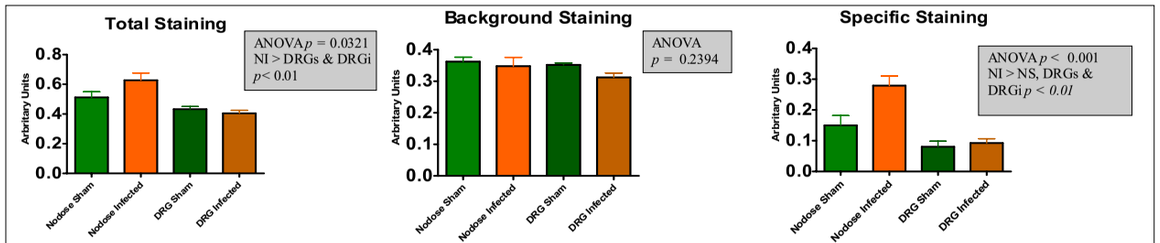

Figure 3: Integrated Optical Densities of VR1-IR
VR1-IR was greater in NG, compared to DRG; and increased in NG following Nb infection (Sham 0.15 ± 0.03, Nb = 0.28 ± 0.03 units, p<0.05) . However, VR1-IR was not altered in DRG (Sham = 0.08 ± 0.02 vs Nb 0.09 ± 0.01 units, NS).

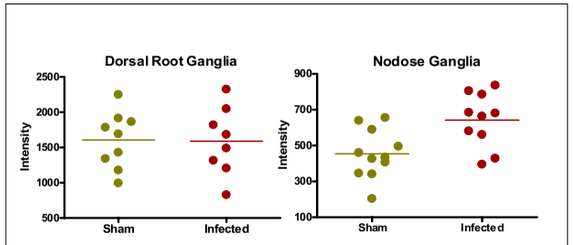

Figure 4: VR1 expression - microarray data
Baseline VR1 mRNA expression detected on microarrays was greater in DRG (1605 ± 133 units) than NG (446 ± 41 units). VR1 was upregulated following Nb infection only in NG (1.4 fold difference, FD) and not in DRG (0.99 FD).

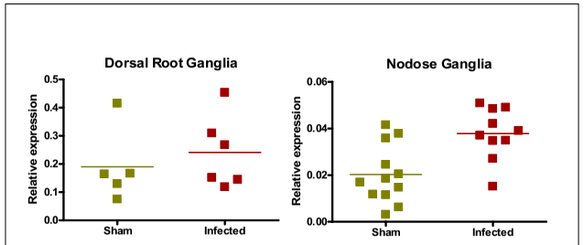

Figure 5: VR1 expression - RTq-PCR data
The changes determined following Nb infection using microarrays were confirmed with RTq-PCR. There was a significant increase in VR1 mRNA in NG (1.85 FD, p<0.01) but not in DRG (1.23 FD, p=0.36).

Conclusions

- VR1-IR and mRNA expression are increased in NG but not DRG, 21 days after Nb infection in mice, at a time-point when the acute inflammation has subsided.
- This suggests modulation of visceral sensitivity mediated by VR-1 receptors on the vagus nerve.
- Additional experiments are required to determine any functional consequences of the altered TRPV1 expression.