Genetic and Pharmacological Modulation of Trigeminal Pain Molecules in a Model of Traumatic Brain Injury

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Introduction

Headache following traumatic brain injury (TBI) is highly prevalent, and it is also the most common and persistent symptom of post-concussion syndrome [1,2]. It could arise de novo or as a pre-existing headache disorder, in which migraine was the most frequent headache phenotype in both civilian and military patients with mild TBI [1,2]. Most post-traumatic headaches will resolve within a couple of weeks following injury; however, headache in a substantial subset of patients may last more than three months and contribute to a prolonged recovery from injury, disability, and a poor quality of living [2]. Headaches can persist well beyond the acute inflammatory period, necessitating intervention and preventative strategies for chronic post-traumatic headache as a time-sensitive problem to address.

Key pain signaling molecules, calcitonin gene-related peptide (CGRP) and nitric oxide synthase (NOS), play a role in post-traumatic headache pathophysiology as they do for migraine. CGRP and NO/NOS are proposed to have reciprocal feedback mechanisms in the trigeminovascular system. CGRP, a well-studied nociceptive neuropeptide in the fields od pain and migraine, increases in the trigeminal pain system after TBI [3]. A recent study by our laboratory noted significant iNOS (inducible nitric oxide synthase) positive cells from a microglial and/or macrophage source in the cortical area proximal to the injury [4]. Typically, iNOS is characterized locally at the injury site making the trigeminal pain circuit, an area remote from the injury site, a novel site to investigate. Nitric oxide triggers headache in migraineurs and animal models via abnormal activation of the trigeminovascular system. Nitric oxide is produced by different isoforms of the nitric oxide synthase (NOS) enzymes. Of the NOS isoforms, the inducible isoform was investigated as inflammation is a predominant feature of TBI and post-traumatic headache. Therefore, the goals of this research were threefold: (1) to investigate if iNOS gene and protein expression in the trigeminal ganglia and trigeminal nucleus caudalis (TNC) are altered in a murine model of controlled cortical impact (CCI) injury, (2) to identify the cellular source for changes in the expression of iNOS, and (3) to examine the proposed synergism between CGRP and iNOS in an in vivo model of TBI.

Methods

Model of Traumatic Brain Injury

Traumatic brain injury (TBI) was induced in mice using a CCI injury model as described previously by our laboratory [3,4,5]. Animals were anesthetized with isoflurane (3% induction; 2-2.5% maintenance). A right-sided 4 mm craniotomy was performed. CCI was induced using an electromagnetic stereotaxic impactor (Leica Biosystems) at 1.0 mm depth, 3.0 m/sec (100 ms contact time) at a 90° impact angle to the cortical surface.

Experimental Design and Outcomes:

Pharmacotherapies known to alter the actions of CGRP, a CGRP antagonist (MK8825; 100 mg/kg) and sumatriptan (1 mg/kg), were administered i.p. for two consecutive days during week two post-injury (on day 13 and 14) and compared to saline controls. All treatment group's endpoints were at two weeks after injury. The effects of treatment on iNOS mRNA and protein were determined in the trigeminal ganglia and TNC using quantitative RT-PCR and immunohistochemistry. The cellular source and distribution of iNOS was assessed. In separate experiments, the effects of CCI in iNOS knockouts (KO) were compared to wild-type mice with CCI on CGRP levels in the TNC using an ELISA. There were two endpoints examined for iNOS KO groups: 3 days and 2 weeks.

Sensory behavior indicative of headache, trigeminal allodynia and photophobia were also measured in all groups. Trigeminal allodynia testing was performed as previously described using von Frey thresholds (Macolino et al., 2014). Photophobia, an aversion to light, has been shown in mouse models overexpressing CGRP to study migraine [6]. Mice are placed in the white Plexiglas illuminated compartment with either ambient ceiling lighting (approx. 400 lux) or under bright light (approx. 4000 lux; Utilitech). For treated groups, testing was performed under bright light conditions. The time spent in the light compared to the darkened black compartment is recorded along with exploratory behaviors using the ANYmaze software (Stoelting).

Results

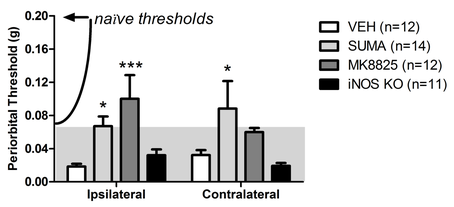

Figure 1: Trigeminal allodynia at 2 weeks measured by von Frey filaments on the sides ipsilateral and contralateral to controlled cortical impact (CCI) injury. Wild-type CCI mice treated with sumatriptan and MK8825 compared to vehicle, *p<0.05, ***p<0.001. iNOS knockout (KO) compare to vehicle-treated CCI wild-type mice were not statistically significant.

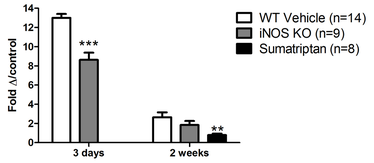

Figure 3: CGRP levels in the TNC at 3 days after CCI in wild-type-vehicle (n=6) and iNOS KO (n=4) mice, and at 2 weeks in wild-type-vehicle (n=8), iNOS KO mice (n=5) and sumatriptan mice (n=8) *** p<0.001, **p<0.01.

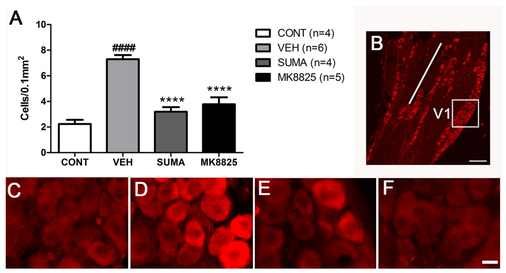

Figure 4: Immunofluorescent iNOS labeled trigeminal ganglia cells (A) The number of iNOS positive cells at two weeks post-operatively in incision control, CCI treated with vehicle (VEH), sumatriptan (SUMA), and MK8825, ####p<0.0001 compared to control, and ****p<0.0001 compared to vehicle. (B-F) Images showing iNOS immunofluorescently labeled ganglia cells. (B) low power image showing a trigeminal ganglia section with the opthalamic V1 region of interest (box) indicated, scale bar = 200 µm. High power images of the ganglia from (C) control, and CCI groups treated with (D) vehicle, (E) sumatriptan and (F) MK8825, scale bar = 10 µm.

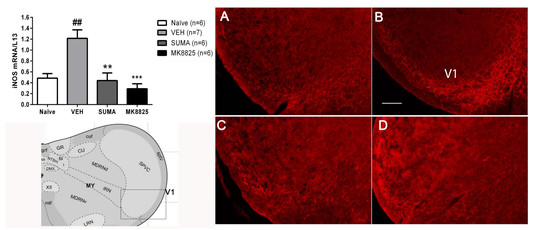

Figure 5 ***LEFT:*** iNOS mRNA levels in the TNC were increased after CCI compared to control, ##p<0.01, whereas treatment with SUMA and MK8825 reduced iNOS mRNA compared to vehicle, **p<0.01, ***p<0.001. BOTTOM LEFT: Atlas of mouse medulla showing the TNC/SPVC region outlined in light grey and the ophthalamic V1 RO1 (BOX); source: Allen Institute for Brain Science. ***RIGHT***: Images showing iNOS immunoreactivity in the TNC V1 region (A) in the uninjured control is negligible, (B) increased in the CCI mice treated with vehicle, (C) reduced in sumatriptan treated mice, and (D) reduced in MK8825 treated mice. Scale bar = 100 µm.

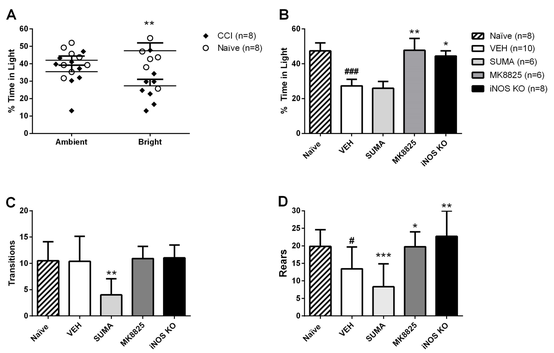

Figure 2: Photophobia assessed as a an aversion to bright light (~4000 lux) compared to ambient light (~400 lux) conditions. (A) Percentage of time spent in the bright light compartment during was reduced during bright light exposure for wild-type CCI mice treated with vehicle compared to naïve mice, **p<0.01. There were no group differences under ambient light conditions. (B) % time spent in the bright light compartment for CCI groups treated with SUMA, MK8825 and iNOS KO compared to VEH *p<0.05 and ** p<0.01, and naïve ### p<0.001. (C) Transitions compared to VEH, **p<0.01. (D) Number of rears in the light compartment compared to VEH, *p<0.05, **p<0.01, **p<0.001, and to naïve mice, p<0.05.

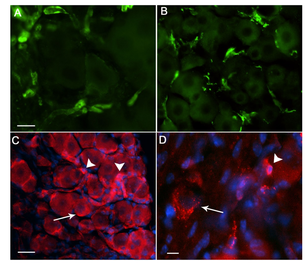

Figure 6: Trigeminal ganglia cells labeled for ionized calcium binding adapter molecule 1 (IBA-1) positive microglia, glial fibrillary acidic protein (GFAP) positive satellite, and iNOS markers. GFAP satellite cells (A) IBA-1 microglia cells (B) sit juxtaposed between neurons in the trigeminal ganglia and have distinct cellular morphologies. After injury, iNOS is expressed by cells with a neuronal morphology indicated by arrows and by non-neuronal cells indicated by arrow heads (C and D), scale bars = 10 µm.

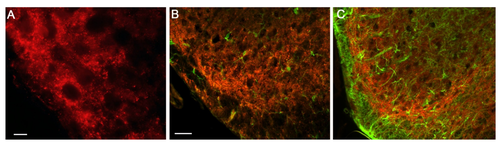

Figure 7: iNOS immunoreactivity in the TNC. A) iNOS appears granular overapping cell processes but not within the soma. iNOS (red) double labeling with B) IBA-1$^+$ microglia (green) and C) GFAP$^+$ astrocytes do not show a clear cellular co-localization.

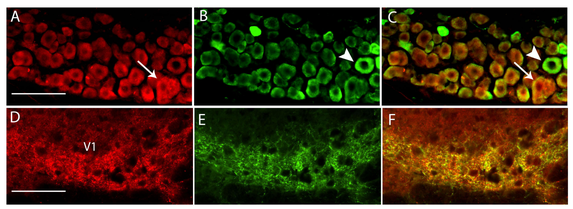

Figure 8: iNOS (red), CGRP (green) and co-localization (yellow) in the (A-C) trigeminal ganglia and trigeminal nucleus caudalis (D-F). iNOS and CGRP (yellow) co-localize in the TNC (F). In trigeminal ganglia cells, co-localization is shown; some CGRP positive cells (arrowheads) do not co-localize with iNOS (arrows) . Bar = 100 µm.

Conclusions

- Synergism between iNOS and CGRP in the trigeminal pain pathway was demonstrated in an in vivo model of TBI. Findings indicate the significance of this relationship contributes to the exacerbation of trigeminal allodynia following injury.
- Although photophobia may be mediated via CGRP and iNOS mechanisms, these pathways may participate independently in the activation of photo-sensitive neurons.
- At the level of the trigeminal ganglia, iNOS is expressed by both neuronal and non-neuronal cell types.
- Blockade of CGRP has therapeutic potential in the management of post-traumatic headache, especially in which there may be a delayed therapeutic window.

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References: 1)Theeler et al, 2013 Headache; 2) Lucas et al., 2014 Cephalalgia; 3) Elliott MB et al. Headache. 2012; 4) Amenta et al., J Neuroinflammation 2014; 5) Macolino et al., J Neuroscience Methods, 2014; 6) Russo et al., 2009 Mol Cell Pharmacol

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