Headache is a highly prevalent symptom in all severities of traumatic brain injury (TBI), and it is one of the most common symptoms of post-traumatic headache. Post-traumatic headache (PTH) remains the most common chronic pain syndrome within the TBI patient population. Mild and moderate forms of TBI are more prevalent than severe forms, but it is currently believed that PTH occurrence is not related to the severity of TBI. Despite being a common symptom following injury, little is known about the pathogenesis of post-traumatic headache. This is partly due to a lack in preclinical animal models studying PTH. A large proportion of mild head injuries are blunt head traumas (i.e., closed head injury) caused by vehicular crashes, falls, or recreational activities, and military training regardless of deployment setting. Mild TBI (mTBI) can be a diffuse injury (closed head injury or concussion) or a focal-diffuse injury (e.g., blunt trauma with a lesion).

In many patients, PTH resolves within 3 months of injury; however, in others it can continue for much longer. The International Classification of Headache Disorders defines PTH as headache secondary to head trauma developing within seven days of injury. Headache pain involves abnormal activation of the trigeminovascular system. This can cause the release of calcitonin gene-related peptide (CGRP), a neuropeptide with a known role in migraine. CGRP was also shown to play a role in PTH. CGRP is released in the trigeminal pain pathway by the trigeminal ganglia of cranial nerve V. The release of CGRP has been shown to increase inducible nitric oxide synthase (iNOS) release from trigeminal ganglial cells, and a reciprocal relationship between the two is said to exist. The release of iNOS can cause the excessive release of nitric oxide (NO), a damaging free radical. Increases in CGRP have been reversed with sumatriptan, as well as other drugs. Previously, our lab has shown an increase in CGRP in the caudal brainstem after a focal TBI, indicating sensitization of the trigeminal ganglia neurons. The objective of this study was to determine the role of CGRP in iNOS production by employing pharmacological blockade. This study determined the effects of a 5-HT1 receptor agonist (sumatriptan) and a CGRP antagonist (MK8825) on levels of iNOS as these drugs inhibit CGRP release and binding, respectively, on iNOS levels in the ganglia and trigeminal nucleus caudalis.

Methods

**CCI:*** Mild/moderate CCI (1 mm depth, 3 m/s, 0-100 sec dwell time) or incision control procedures were induced in C57BL6 male, 8-week old mice (n=33). Tissue samples (trigeminal ganglia and caudal brainstem) were collected post-mortem and processed for either immunohistochemistry, qRT-PCR, or ELISA.

**Treatments:** MK8825 (100 mg/kg), sumatriptan (1 mg/kg) or a vehicle saline were given post-operatively on days 13 and 14.

**Mechanical Allodynia Testing:** Periorbital von Frey testing using graded forces (0.008-0.4 g) after treatment was provided. There were no group differences in baseline thresholds (mean 0.3 g ±0.2 g) (data not shown).

**ELISA:** CGRP ELISA was performed according to manufacturer instructions (Cayman Chemical, Ann Arbor, MI).

**PCR:** RNA from brainstem tissue was extracted and reverse transcribed to yield cDNA product for each sample. The cDNA was then used for RT-PCR to quantify levels of iNOS mRNA.

**Immunohistochemistry:** CGRP (Sigma, C8198, 1:200) and iNOS (Enzo, ADI-905-431-1, 1:100) primary antibodies were used in immunofluorescence. Secondary (Jackson, 1:200) antibodies in either red (111-165-144) or green (111-545-144) were used. DAPI (Life Technologies, P36931) was used to coverslip ganglia sections and provided a nuclear stain. For TNC sections, glycerol was used to coverslip.

**Cell Counting:** Positive cells within a grid of 100 μm² were counted and averaged to determine how many positive cells each sample had per mm².

Conclusions

- Inhibition of CGRP release and binding using sumatriptan and MK8825, respectively, attenuated periorbital allodynic thresholds and levels of iNOS mRNA and protein in the trigeminal pathway.
- These findings, in combination with the co-localization of iNOS and CGRP, confirms that CGRP influences the production of iNOS.
- Blockade of CGRP reduces iNOS expression and is associated with improvement in headache-like behavior (periorbital allodynia).
- Findings indicate the interactions between CGRP and iNOS after head injury may contribute to the pathology underlying post-traumatic headache. Blockade of either CGRP and/or iNOS has therapeutic potential in the management of headache following head injury.