Hmgb1 silencing in the amygdala reduces pain-related behaviors in a sex-specific manner in a chronic neuropathic pain model

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Introduction

Chronic pain is a prevalent nationwide healthcare issue, yet many knowledge gaps exist with regard to brain mechanisms of pain, particularly involving potential sexual differences. An intricate interplay between sensory, cognitive, and emotional-affective dimensions forms the complex experience of pain, presenting a challenge to identifying effective treatment options. Maladaptive neuroplasticity is a key contributor to the transition from acute protective to chronic pathological pain. Mechanistic investigation of this transition is of particular interest for the potential discovery of new therapeutic strategies. This transition may be associated with neuroimmune mechanisms in the brain, which have not yet been elucidated. The amygdala is a limbic brain center that is a key player in the emotional-affective dimensions of pain and pain modulation. The role of neuroimmune signaling in the amygdala in chronic pain states is an important knowledge gap. High mobility group box 1 (HMGB1) is a proinflammatory signaling molecule involved in pain-related crosstalk between neurons and glial cells in the spinal cord, yet its role in the amygdala in pain states has yet to be explored. Here we provide new evidence that Hmgb1 is involved in pain-related amygdala plasticity and that inhibition of this molecule can reduce neuropathic pain behaviors, potentially through sexually dimorphic mechanisms. Identification of brain mechanisms of pain will aid in the development of sex-specific therapeutic strategies for chronic neuropathic pain relief.

Methods

Animals

Male and female Sprague-Dawley rats (n=150–200g) were housed in a temperature-controlled vivarium and exposed to a 12:12 hour light-dark cycle with unrestricted access to food and water. Chronic constriction injury (CCI) rat model was used to induce neuropathic pain. Male rats were briefly anesthetized with isoflurane and perfused with phosphate-buffered saline (PBS). Brains were extracted and the central nucleus of the amygdala (CeA) was dissected using the Neuron Tissue Dissection Kit with Pipem (Miltenyi Biotec). RNA was isolated using the MagMAX-96 Total RNA isolation Kit (Thermo Fisher Scientific) or TRIzol (Invitrogen), and extracted on a QIAshredder (Qiagen) and cleaned using the RNaseasy Mini Kit (Qiagen). Gene expression was measured using the TaqMan Gene Expression Assay (Applied Biosystems) and primers for genes of interest (Thermo Fisher Scientific). Results were analyzed using R software and GraphPad Software (GraphPad). The levels of gene expression were normalized to the geometric mean of beta-actin (Abcam), monocyte-induced TNF-α (R&D), and ribosomal protein L13 (R&D), and delta-CT value was calculated.

Experimental protocol

Male and female rats were anesthetized with isoflurane (3%) and a small unibladder hindpaw incision was made two weeks before surgery. Four weeks after surgery in the post-treatment group. Using a stereotaxic apparatus (David Kopf Instruments), Hmgb1-siRNA (scrambled siRNA vector or scrambled siRNA vector with 5%) was delivered into the right CeA using a 5μl Hamilton syringe (23g) with the following coordinates: 2.3–2.8 mm caudal to销售人员根据身体的实际对冲方向，7.8–8.2 mm lateral to the midline, −8.6 mm depth. Behavioral assays were performed 4 weeks after surgery. Sensory withdrawal thresholds and emotional affective responses were measured. The brain was then extracted and the Ca2+-dependent for RNA isolation. Pain-related behavioral test

atLabeled behavioral thresholds were measured using a plantar electronic von Frey filament in the originally validated von Frey technique. Animal behavioral responses to von Frey stimuli were measured using a pressure-sensitive plastic dome on the left hind paw until a withdrawal reflex was produced.

Statistics

Significance was assessed at the level P < 0.05. All values represent means ± SEM.

Results

Neuroimmune signaling in the CeA in neuropathic pain

CII rats were randomized into four groups: (A) sham surgery and saline injection, (B) sham surgery and Hmgb1 siRNA injection, (C) CCI surgery and saline injection, (D) CCI surgery and Hmgb1 siRNA injection. Groups were compared using two-way ANOVA and Dunnett’s test (n=6–10 per group). Tukey’s test, P < 0.05 was considered significant compared to the sham group. One-way ANOVA with Bonferroni post hoc tests were used for comparisons between the groups. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Means with different letters are significantly different.

Neuropathic pain behaviors and mRNA expression effects of Hmgb1 siRNA post-treatment

Conclusions

Neuroimmune signaling mechanisms in the CeA in neuropathic pain

Many genes related to neuroimmune signaling were differentially expressed in the right CeA of male rats following CII and sham surgery. Hmgb1 may be involved in the pain-related crosstalk between neurons and glial cells in the spinal cord, facilitating maladaptive CeA CeA CeA neuropathic pain. Hmgb1 was differentially expressed in the right CeA of females and sham rats. Hmgb1 may be released by CeA neurons to trigger a proinflammatory signaling cascade in glial cells that leads to maladaptive CeA CeA CeA neuropathic pain.

Effects of Hmgb1 siRNA as a pre-treatment to SNL surgery

Pre-treatment with Hmgb1 siRNA did not significantly affect Hmgb1 mRNA levels in the right CeA, sensory withdrawal thresholds, or emotional affective responses in either sex at the chronic stage of neuropathic pain.

Future Directions

Cell-type-specific (astrocytic, microglial, and neuronal) bulk and single cell RNA sequencing from neuropathic male and female rats will be used to determine the source of Hmgb1 release. Differential expression of these genes in the amygdala of both sexes will help determine additional molecular targets that may impact both neuronal and non-neuronal signaling in a chronic neuropathic pain state. These molecular targets may differ between the sexes and across different stages of neuropathic pain.