Acid Sensing Ion Channel 3 of Knee Joint Afferents and Its Role in Arthritic Pain

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Introduction

Arthritis is associated with decreases in local pH, and acidosis induces joint pain. Protons activate nociceptors by opening proton-gated cation channels, such as acid sensing ion channels (ASICs) and capsaicin sensitive TRPV1. Of these, ASIC3 is abundantly expressed in dorsal root ganglion (DRG) and critical for the development of inflammatory pain. To clarify the role of ASIC3 in the development of arthritic pain, we examined the difference of pain behavior in carrageenan-induced knee arthritis between ASIC3 knockout mice (ASIC3-/-) and wildtype mice (ASIC3+/+). We also examined ASIC3 expression in DRG neurons innervating the knee joint.

Methods

Congenic ASIC3 -/- and ASIC3 +/+ mice on a C57Bl/6J background were used in this study. Arthritis was induced by intrarticular injection of 20µl 3% carrageenan. All experiments were approved by the University of Iowa Animal Care and Use Committee.

Pain behavior experiments

Mechanical sensitivity of the paw was tested bilaterally by assessing the number of responses to repeated application of a 0.4 mN von Frey filament. An increase in the number of responses was interpreted as secondary mechanical hyperalgesia, i.e. increased nociceptive response to noxious stimuli outside the joint.

Mechanical sensitivity of the knee joint was tested bilaterally by squeezing the knee joint with a calibrated pair of tweezers until the mouse withdrew from the stimulus. A decrease in threshold was interpreted as primary mechanical hyperalgesia.

ASIC3 expression experiments

Six days before the carrageenan injection, left knee joint was injected with 10µl at 1% Fast Blue (FB) for retrograde labeling. At 24h after carrageenan injection, ipsilateral L3-5 DRGs were removed, placed in 2% paraformaldehyde and 15% sucrose overnight, embedded, and frozen. 10µm sections were cut using a cryostat. Standard immunohistochemistry procedures were employed utilizing the following antibodies and reagents: Rabbit anti-ASIC3 (Alomone) 1:500, Biotinylated goat anti-rabbit IgG (Invitrogen) 1:250, Alexa 568 strepavidin (Invitrogen) 1:500. Sections were viewed with Olympus BX-51 microscope. For each FB-labeled neuron, ASIC3 expression was examined and quantified as the percent of total FB-labeled neurons. Soma size of FB-labeled neurons was measured using Image J software.

Statistical analysis

Repeated measures ANOVA followed by Tukey’s post-hoc test for behavior experiments, student’s t-test for DRG cell counting, and Kolmogorov-Smirnov test for soma size distribution were used. The level of significance was set at p<0.05.

Results

Figure 1 A) Mechanical sensitivity of the paw. In ASIC3+/+ mice, bilateral secondary mechanical hyperalgesia (paw) occurs 24h after carrageenan injection. However, it does not develop in ASIC3-/- mice. Asterisks indicate a significant difference when compared to ASIC3+/+. B) Mechanical sensitivity of the knee joint. Primary mechanical hyperalgesia (knee) develops similarly in both ASIC3+/+ and ASIC3-/- mice after joint inflammation.

Discussion

Joint pain is uniquely different from cutaneous pain and characterized as diffuse, longer lasting and more unpleasant. It is often accompanied by referred pain and secondary hyperalgesia that are important components of arthritic pain. Based on our results of pain behavior experiments, secondary mechanical hyperalgesia developed after carrageenan-induced arthritis depends on activation of ASIC3.

There is an ASIC3 upregulation in knee joint afferents by carrageenan-induced arthritis. Although there are a few reports of ASIC3 upregulation by inflammation in rat DRG, this is, to our knowledge, the first report about ASIC3 upregulation specifically in knee joint afferents. This finding would help to elucidate the mechanism underlying the development of secondary hyperalgesia in arthritis. It is likely to require not only the presence of ASIC3 but also the ASIC3 upregulation by joint inflammation.

It is unknown why ASIC3 upregulation observed in knee joint afferents had nothing to do with primary hyperalgesia. Based on our results of soma size distribution of ASIC3-IR neurons, ASIC3 is present in a limited population of nociceptors and probably in some non-nociceptors. Because ASIC3 unlikely sensitizes nociceptors that contributes to primary hyperalgesia, non-nociceptors expressing ASIC3 might send signals to dorsal horn neurons through ASIC3 activation that contributes to central sensitization and thus secondary hyperalgesia.

In conclusion, ASIC3 is critical for the development of secondary hyperalgesia that is an important component of arthritic pain. Further, ASIC3 upregulation in knee joint afferents is presumably one of the underlying mechanisms.