Shinoda M, Ozaki N, Sugiura Y. Involvement of ATP and its receptors on nociception in rat model of masseter muscle pain. Pain 2008;134:148–157.

A number of papers have described that the purinergic receptors in the trigeminal ganglion neurons are involved in persistent pain in the orofacial region. P2X receptors (P2XR) are especially thought to be 1 of the important receptors for deep pain such as that associated with temporomandibular or masseter muscle inflammation.

In this paper, the authors studied the underlying mechanisms of P2X involvement in masseter muscle pain. The authors determined the role of P2X(3)R on pressure pain and mechanical hyperalgesia in a newly developed rat model of masseter muscle pain. The pain in the masseter muscle was assessed by the pressure pain threshold (PPT), which was defined as the amount of pressure required to induce head flinching. In naive animals, systemic treatment with morphine was associated with increase of PPTs. Changes in PPTs were examined after administration of P2XR agonists or antagonists into the masseter muscle. The masseter muscle injection of alpha, beta-meATP with PPADS (P2X(1,3,2,5,1/5,2/3)R-specific antagonist). Excessive contraction of the masseter muscle produced by electrical stimulation produced a significant reduction in PPTs, indicating the induction of mechanical hyperalgesia of the muscle. Moreover, administration of PPADS to the muscle produced a complete reversal of reduced PPT. Immunohistochemically, the number of trigeminal ganglion P2X3R-positive neurons innervating the masseter muscles increased in the excessively contracted condition.

The authors suggested that P2X3R plays an important role in pressure pain and mechanical hyperalgesia in masseter muscle caused by excessive muscular contraction.

Nunéz S, Lee JS, Zhang Y, Bai G, Ro JY. Role of peripheral mu-opioid receptors in inflammatory orofacial muscle pain. Neuroscience 2007;146(3):1346–1354.

It is known that the mu opioid receptor (MOR) is involved in the modulation of spinal and central nervous system (CNS) neuronal excitability. Recently, peripheral MORs have been suggested to be target structures to reduce the hyperexcitability of the peripheral nociceptors induced by nerve injury or inflammation.

The authors studied whether inflammation in the masseter muscle alters MOR mRNA and protein expressions in trigeminal ganglia (TG) and assessed the contribution of peripheral MORs under acute and inflammatory muscle pain conditions. mRNA and protein levels for MOR were quantified by RT-PCR and Western blot, respectively, from the TG of naïve rats, and compared with those from rats treated with complete Freund's adjuvant (CFA) in the masseter. TG was found to express mRNA and protein for MOR, and CFA significantly upregulated both MOR mRNA and protein by 3 days following the inflammation. The MOR protein upregulation persisted to day 7 and returned to the baseline level by day 14. They then investigated whether peripheral application of a MOR agonist, D-Ala2, N-Me-Phe4, Gly5-ol-enkephalin acetate salt (DAMGO), attenuates masseter nociception induced by masseter infusion of hypertonic saline (HS) in lightly anesthetized rats. DAMGO (1, 5, 10 μg) or vehicle was administered directly into the masseter 5 to 10 minutes prior to the HS infusion. The DAMGO effects were assessed on mean peak counts (MPC) and overall magnitude was calculated by the area under the curve (AUC) of the HS-evoked behavioral responses. Under this condition, only the highest dose of DAMGO (10 μg) significantly reduced MPC, which was prevented when H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP), a selective MOR antagonist, was coadministered. DAMGO pretreatment in the contralateral masseter did not attenuate MPC. The same doses of DAMGO administered into CFA-inflamed rats, however, produced a greater attenuation of both MPC and AUC of HS-evoked nocifensive responses.

These findings have demonstrated that activation of peripheral MOR provides greater antinociception in inflamed muscle and that the enhanced MOR effect can be partly explained by significant upregulation of MOR expression in TG.

Lennerz JK, Rühle V, Ceppa EP, Neuhuber WL, Bunnett NW, Grady EF, Messlinger K. Calcitonin receptor-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and calcitonin gene-related peptide (CGRP) immunoreactivity in the rat trigeminovascular system: Differences between peripheral and central CGRP receptor distribution. J Comp Neurol 2008;507:1277–1299.

A number of studies have tried to clarify the underlying mechanisms of migraine headache by using a variety of animal models. Although calcitonin gene-related peptide (CGRP) is thought to be a key mediator in primary headaches including migraine, the neuronal mechanisms of migraine headache are not well known. In the present paper, the authors tried to clarify the target structures for peripheral and central CGRP by using animal models of meningeal nociception. To study the distribution of CGRP receptors in the rat trigeminovascular system, the authors used antibodies recognizing 2 components of the CGRP receptor, the calcitonin receptor-like receptor (CLR) and the receptor activity-modifying protein 1 (RAMP1). In the cranial dura mater, CLR and RAMP1 immunoreactivity (-ir) was found within arterial blood vessels, mononuclear cells, and Schwann cells, but not sensory axons. In the trigeminal ganglion, besides Schwann and satellite cells, CLR- and RAMP1-ir was found in subpopulations of CGRP-ir neurons where colocalization of CGRP- and RAMP1-ir was very rare (approximately 0.6%). CLR- and RAMP1-ir was localized to "glomerular structures," partly colocalized with CGRP-ir. However, CLR- and RAMP1-ir was lacking in central glia and neuronal cell bodies.

Based on the present results, the authors concluded that CGRP receptors were associated with structural targets of known CGRP effects (vasodilation, mast cell degranulation) and targets of unknown function (Schwann cells). In the spinal trigeminal nucleus, CGRP receptors were probably located on neuronal processes, including primary afferent endings, suggesting involvement in presynaptic regulation of nociceptive transmission. Thus, in the trigeminovascular system, CGRP receptor localization suggests multiple targets for CGRP in the pathogenesis of primary headaches.

D'Arco M, Giniatullin R, Simonetti M, Fabbro A, Nair A, Nistri A, Fabbretti E. Neutralization of nerve growth factor induces plasticity of ATP-sensitive P2X3 receptors of nociceptive trigeminal ganglion neurons. J Neurosci 2007;27:8190–8201.

The molecular mechanisms of migraine pain are still unknown, although migraine mediators such as nerve growth factor (NGF) and calcitonin gene-related peptide (CGRP) are believed to play an important role for migraine headache. Although NGF block is proposed as a novel analgesic approach, its consequences on nociceptive purinergic P2X receptors of trigeminal ganglion neurons remain unknown.

The authors investigated whether neutralizing NGF might change the function of P2X3 receptors natively coexpressed with NGF receptors on cultured mouse trigeminal neurons. Treatment with an NGF antibody (24 h) decreased P2X3 recep-

tor-mediated currents and Ca2+ transients, an effect opposite to exogenously applied NGF. Recovery from receptor desensitization was delayed by anti-NGF treatment without changing desensitization onset. NGF neutralization was associated with decreased threonine phosphorylation of P2X3 subunits, presumably accounting for their reduced responses and slower recovery. Anti-NGF treatment could also increase the residual current typical of heteromeric P2X2/3 receptors, consistent with enhanced membrane location of P2X2 subunits. This possibility was confirmed with cross-linking and immunoprecipitation studies. NGF neutralization also led to increased P2X2 splicing variant at mRNA and membrane protein levels. These data suggest that NGF controlled plasticity of P2X3 subunits and their membrane assembly with P2X2 subunits. Despite anti-NGF treatment, CGRP could still enhance P2X3 receptor activity, indicating separate NGF- or CGRP-mediated mechanisms to upregulate P2X3 receptors. In an in vivo model of mouse trigeminal pain, anti-NGF pretreatment suppressed responses evoked by P2X3 receptor activation.

These findings outline the important contribution by NGF signaling to nociception of trigeminal sensory neurons, which could be counteracted by anti-NGF pretreatment.

Chiang CY, Wang J, Xie YF, Zhang S, Hu JW, Dostrovsky JO, Sessle BJ. Astroglial glutamate-glutamine shuttle is involved in central sensitization of nociceptive neurons in rat medullary dorsal horn. J Neurosci 2007;27:9068–9076.

Several studies have described that the astroglial activation in the spinal dorsal horn (DH) is involved in the modulation of DH nociceptive transmission. The neuronal mechanisms of neuron-glial interaction have also been extensively studied and glutamine is thought to be an important molecule involved in neuron-astroglial communication. However, no studies have tested their possible involvement in modulating the activity of functionally identified nociceptive neurons. It is very important to know the underlying mechanisms of neuron-astroglial interaction on the state of central sensitization following peripheral inflammation, since central sensitization is considered a crucial process in allodynia and hyperalgesia.

The authors in the present study demonstrated that the central sensitization induced in functionally identified nociceptive neurons in trigeminal subnucleus caudalis (the medullary dorsal horn) by application of an inflammatory irritant to the rat's tooth pulp can be significantly attenuated by continuous intrathecal superfusion of methionine sulfoximine (MSO; 0.1 mM), an inhibitor of the astroglial enzyme glutamine synthetase that is involved in the astroglial glutamate-glutamine shuttle. Simultaneous superfusion of MSO and glutamine (0.25 mM) restored the irritant-induced central sensitization. In control experiments, superfusion of either MSO or glutamine alone, or vehicle, did not produce any significant changes in baseline neuroceptive neuronal properties.

The present findings suggest that the astroglial glutamate-glutamine shuttle is essential for the initiation of inflammation-induced central sensitization but that inhibition of astroglial function may not affect normal nociceptive processing.