PATWARDHAN AM, DIOGENES A, BERG KA, ET AL. PAR-2 AGONISTS ACTIVATE TRIGEMINAL NOCICEPTORS AND INDUCE FUNCTIONAL COMPETENCE IN THE DELTA OPIOID RECEPTOR. PAIN 2006;125:114–124.

It has been reported that the protease-activated receptor-2 (PAR-2) is involved in peripheral nociception. Injection of PAR-2 agonist into the parotid grant gland or nasal mucosa causes c-fos activation in the trigeminal spinal nucleus. These findings suggest that PAR-2 is involved in trigeminal nociceptive mechanisms. However, the neuronal mechanisms underlying the role of PAR-2 activation in trigeminal nociception and in induction of functional competence in the delta opioid receptor (DOR) are not known.

The authors evaluated whether agonists of PAR-2 activate the capsaicin-sensitive subclass of trigeminal nociceptors in a PLC–PKC-dependent manner and induce functional competence in the DOR. Adult male rat trigeminal ganglion (TG) cultured neurons were treated with either a PAR-2 agonist (SL-NH_2) or an enzyme activator of PAR (trypsin), and the activation of TG nociceptors was assessed using 3 independent methods: neuropeptide release, calcium influx, and whole cell patch-clamp. The specificity of SL-NH_2 and trypsin responses was evaluated using TG cultures transfected with short interfering RNA (siRNA) against PAR-2. The in vivo role of PAR-2 activation was determined by measuring SL-NH_2 and trypsin-evoked nocifensive behavior and increase in blood flow. Trigeminal neurons were treated with SL-NH_2/vehicle and then the DOR agonist to test for DOR inhibition of evoked neuropeptide release and cyclic adenosine monophosphate (cAMP) accumulation. The results showed that SL-NH_2 (100 μmol/L) and trypsin (1 to 600 nmol/L) activate TG nociceptors; activation was partly reversible by the PKC inhibitor bisindolylmaleimide (500 nmol/L) and by ruthenium red (10 μmol/L). In cultures treated with siRNA against PAR-2, both SL-NH_2 and trypsin responses were significantly diminished. Both SL-NH_2 and trypsin evoked nocifensive behavior and increases in blood flow in an orofacial pain model. Application of SL-NH_2 rapidly produced functional competence of DOR for inhibiting nociceptor function.

The present paper has clearly demonstrated that PAR-2 is involved in trigeminal nociceptive mechanisms. In inflamed tissue, endogenous proteases may activate TG nociceptors and generate pain. Moreover, activation of PAR-2 can also induce functional competence in DOR. *(KI)*

PAREEK TK, KELLER J, KESAVAPANY S, ET AL. CYCLIN-DEPENDENT KINASE 5 ACTIVITY REGULATES PAIN SIGNALING. PROC NATL ACAD SCI 2006;103:791–796.

A number of molecules expressed in ganglion neurons have been reported to be involved in the intracellular signaling associated with noxious processing. The intracellular molecules and cellular pathways have been implicated in nociceptive signaling, but the precise molecular mechanisms have not been clearly defined. Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase implicated in the development and disease of the mammalian nervous system. The precise role of this kinase in sensory pathways has not been well characterized. The authors demonstrated the molecular role of Cdk5 in nociception. They identified the expression of Cdk5 and its activator p35 in nociceptive neurons, which is modulated during a peripheral inflammatory response. Increased calpain activity in sensory neurons after inflammation resulted in the cleavage of p35 to p25, which forms a more stable complex with Cdk5 and, consequently, leads to elevation of Cdk5 activity. p35 knockout mice ($p35^{-/-}$), which exhibit significantly decreased Cdk5 activity, showed delayed responses to painful thermal stimulation compared with controls (wild type). In contrast, mice overexpressing p35, which exhibited elevated levels of Cdk5 activity, were more sensitive to painful thermal stimuli than were controls.

The authors described a role for Cdk5/p35 activity in primary afferent nociceptive signaling, suggesting that Cdk5/p35 may be a target for the development of analgesic drugs. *(KI)*

BEREITER DA, CIOFFI JL, BEREITER DF, ZARDENETA G, MILAM SB. LOCAL BLOCKADE OF INTEGRINS IN THE TEMPOROMANDIBULAR JOINT REGION REDUCES FOS-POSITIVE NEURONS IN TRIGEMINAL SUBNUCLEUS CAUDALIS OF FEMALE RATS PRODUCED BY JAW MOVEMENT. PAIN 2006;125:65–73.

A number of papers have documented that trigeminal spinal nucleus (Vc) neurons are involved in temporomandibular joint (TMJ) pain. However, the detailed neuronal mechanisms of TMJ pain are not known. This paper presents new insight into Vc mechanisms of TMJ pain by showing the influence of integrins on trigeminal brainstem neural activity evoked by passive jaw movements (JM).

Limited range of motion and pain during jaw opening are common complaints of patients with TMJ disorders. Under barbiturate anesthesia, JM (0.5 Hz, 30 minutes) were produced in ovariectomized (OvX) female rats given estrogen replacement and in males. Quantification of Fos-like immunoreactivity (Fos-LI) after JM served as an index of evoked neural activity. The TMJ was injected locally with either an active (GRGDS, 300 μmol/L, 25 μL) or an inactive integrin antagonist (SDGRG) prior to JM. The effect of prior inflammation of the TMJ region was assessed in separate groups of rats by injecting bradykinin (10 μmol/L, 25 μL) with or without integrin drugs prior to JM. Active integrin antagonist significantly reduced JM-evoked Fos-LI in superficial laminae at the trigeminal subnucleus caudalis/upper cervical cord (Vc/C2) junction in OvX female rats compared to male rats, independent of bradykinin pretreatment. Fos-LI produced in the dorsal paratrigeminal and trigeminal subnucleus interpolaris/caudalis (Vi/Vc) transition regions was not reduced by active integrin antagonist in males or OvX females. Active integrin antagonist did not affect Fos-LI produced after injection of bradykinin alone into the TMJ.

These results suggest that Arg-Gly-Asp binding integrins contribute to JM-evoked neural activity at the Vc/C2 junction under naive and inflamed conditions in a sex-dependent manner. *(KI)*

NAG S, MOKHA SS. ACTIVATION OF α_2-ADRENOCEPTORS IN THE TRIGEMINAL REGION PRODUCES SEX-SPECIFIC MODULATION OF NOCICEPTION IN THE RAT. NEUROSCIENCE 2006;142:1255–1262.

Many papers have described sex differences in pain in humans. It has been recently reported that the estrogen receptors are dominantly expressed in the trigeminal spinal nucleus in female rats compared with male rats. The estrogen receptor is thought to be involved in sex differences in trigeminal pain. Sex-related differences in the sensitivity to pain and in the response to analgesics have also been reported, including higher perceptual responses to experimentally induced pain and the higher prevalence of many pain syndromes in women compared with men.

The present study tested whether α2-adrenoceptor-mediated antinociceptive effects are reduced by estrogen. Such a reduction could account for sex-related differences in pain perception and modulation. Clonidine, an α_2-adrenoceptor agonist, has been shown to inhibit noxious stimulus-evoked nociceptive behavior as well as the responses of nociceptive neurons in the trigeminal subnucleus caudalis (medullary dorsal horn). Intracisternal microinjection of clonidine (7 μg/5 μL) through an implanted PE-10 cannula dorsal to the trigeminal region in male

rats and in ovariectomized (OVX) and diestrous (DiE) female rats produced a strong antinociceptive effect on *N*-methyl-D-aspartic acid (NMDA)-induced nociceptive scratching behavior and heat-induced face withdrawal nociceptive tests. However, it failed to produce any inhibition in the estradiol-treated ovariectomized (OVX+E) group regardless of the dose of estradiol (1, 10, or 100 µg/100 µL, sesame oil) or in a proestrous (ProE) group. Further, clonidine produced dose-dependent effects on the NMDA-induced nociceptive behavior in male and OVX groups but not in the OVX+E group. Finally, the effect of clonidine on thermal nociceptive test was reversed by yohimbine, an α_2-adrenoceptor antagonist, in the male and OVX groups.

The authors conclude that activation of α_2-adrenoceptors produces sex-specific, estrogen-dependent modulation of nociception in the trigeminal region of the rat. A decreased α_2-adrenoceptor-mediated inhibition could be 1 of the factors responsible for the higher prevalence of pain syndromes in females. *(KI)*

AMBALAVANAR R, MORITANI M, MOUTANNI A, PANDURANGA G, YALLAMPALLI C, DESSEM D. DEEP TISSUE INFLAMMATION UPREGULATES NEUROPEPTIDES AND EVOKES NOCICEPTIVE BEHAVIORS WHICH ARE MODULATED BY A NEUROPEPTIDE ANTAGONIST. PAIN 2006;120:53–68.

Substance P (SP) and calcitonin gene-related peptide (CGRP) are important neuropeptides involved in inflammation-related muscle pain as well as cutaneous pain. Recently, some neuropeptide antagonists have been used for pain treatment (eg, a CGRP antagonist for migraine treatment). Therefore, it is important to understand the functional role of neuropeptides in nociception and inflammation. To explore this relationship, the authors examined behavioral changes and primary afferent neuronal plasticity in rats following deep tissue inflammation.

One hour following craniofacial muscle inflammation produced by injection of complete Freund's adjuvant (CFA), ipsilateral as well as contralateral head withdrawal thresholds and ipsi- and contralateral hindpaw withdrawal thresholds were lowered and remained reduced for 28 days. Elevated levels of CGRP within the trigeminal ganglion temporally correlated with this mechanical allodynia. Inflammation also induced an increase in the number of CGRP and SP-immunopositive trigeminal ganglion neurons innervating the inflamed muscle but did not evoke a shift in the size distribution of peptidergic muscle afferent neurons. Trigeminal proprioceptive muscle afferent neurons situated within the trigeminal mesencephalic nucleus did not express CGRP or SP prior to or following inflammation. Intravenous administration of a CGRP receptor antagonist (8-37) 2 minutes prior to CFA injection blocked plasma extravasation and abolished both head and hindlimb mechanical allodynia. Local injection of CGRP antagonist directly into the masseter muscle prior to CFA produced similar, but less pronounced, effects.

The present paper has revealed that unilateral craniofacial muscle inflammation produces mechanical allodynia at distant sites and upregulates CGRP and SP in primary afferent neurons innervating deep tissues. These data further implicate CGRP and SP in deep tissue nociceptive mechanisms and suggest that peptide antagonists may have therapeutic potential for musculoskeletal pain. *(KI)*