Chapter 4
Nociceptive Chemical Mediators in Oral Inflammation

Nalini Vadivelu, Anusha Manje Gowda, Stephen Thorp, Alice Kai, Amarender Vadivelu, and Susan Dabu-Bondoc

Introduction

Orofacial pain is commonly due to inflammation. It is extremely important to understand the chemical mediators of oral inflammation and the pathways of orofacial pain for its effective treatment. This chapter focuses on the pain pathways traversed by impulses causing orofacial pain and the many nociceptive chemical mediators that play a role in oral inflammation.

Nociceptors, the receptors for pain, are unmyelinated nerve endings that are located in bone, skin, muscle, and visceral tissues, the activation of which generates a Ca^{2+} current that depolarizes the distal axonal segment and initiates a self-propagating action potential and an inward current of Na^{+}. The nociceptors of sensory afferent fibers are activated following tissue injury by the release of prostaglandins mainly the prostaglandin (PGE), which is synthesized by the enzyme cyclooxygenase-2 from the damaged cells, bradykinin from damaged vessels, and cellular mediators including hydrogen and potassium ions. Orthodromic transmission in sensitized afferents initiates the release of peptides like substance P (sP), calcitonin gene-related peptide (CGRP), and cholecystokinin (CCK) within and around

N. Vadivelu, M.D. (✉) • S. Thorp, M.D. • S. Dabu-Bondoc, M.D.
Department of Anesthesiology, Yale University School of Medicine and Yale-New Haven Hospital, 333 Cedar Street, 208051, New Haven, CT 06520-8051, USA
e-mail: Nalinivg@gmail.com; Susan.Dabu-Bondoc@yale.edu

A.M. Gowda, M.B.B.S.
Bangalore Medical college and research institute, Bangalore 560003, Karnataka, India

A. Kai, B.A.
Neuroplasticity Unit, National Institutes of Health, Bethesda, MD, USA
e-mail: alicemkai@gmail.com

A. Vadivelu, B.D.S., M.D.S.
Annoor Dental College and Hospital, Muvattupuzha, Kerala 686673, India
e-mail: Amarvadivelu@gmail.com

N. Vadivelu et al. (eds.), *Orofacial Pain: A Clinician's Guide*,
DOI 10.1007/978-3-319-01875-1_4,

the site of tissue damage. Substance P further enhances nociceptor excitability through the release of bradykinin, histamine from mast cells, and serotonin (5HT) from platelets. The above factors combine with other mediators such as cytokines and 5HT which results in the inflammatory response and recruits neighboring nociceptors, leading to primary hyperalgesia. Reflex sympathetic efferent responses cause further release of BK and sP which excite nociceptors by the release of noradrenaline, which also results in peripheral vasoconstriction and trophic changes [1].

Pathophysiology of Orofacial Pain

There are three types of primary peripheral afferents: Ab fibers, Ad fibers, and C fibers. Ab fibers are the quickest conducting fibers due to their myelinated quality. Ad fibers are slightly slower, comprising thinner myelinated axons. The slowest conducting fibers are the C fibers consisting of the thinnest and unmyelinated fibers [2].

Trigeminal nerve mostly innervates the orofacial region, and its primary afferent cell bodies are found within the trigeminal ganglion which consists mostly of Ad fibers and C fibers [3]. The Ab fibers of the trigeminal region respond to pressure and light touch, while pain acts as a stimulus to the less conducting Ad fibers and C fibers, which are jointly termed nociceptors, the excitation of which causes considerable release of sP, which activates neurokinin-1 receptors and thereby modulates sensitivity to pain [4]. These nociceptors can be further classified into mechano-nociceptors, thermo-nociceptors, and chemo-nociceptors. Thermo-nociceptors contain vanilloid receptor 1-like receptors that contribute to the pain due to extreme temperatures [5]. Mechano-nociceptors, which are sensitive to mechanical stimuli, are located in the root pulp and are lined with epithelial Na^+ channels that contribute to sharp pain produced by liquid motion in the dentinal tubules [6]. Chemo-nociceptors are sensitive to chemicals.

The modulation of these nociceptors is either mechanical or chemical. Canine studies have shown that the threshold for mechano-nociception can increase sensitization because it may be lowered by periodontal inflammation [7]. It is possible that this is due to the hydrodynamic mechanism (which activates the nociceptors by increasing pressure on the pulp [8]) of inflammation in the noncompliant environment in the dentine-encased pulp. In addition, there is substantial peripheral and central modulation due to the various neuropeptides released following tissue damage, and it is localized within the trigeminal ganglia. Peptides such as sP and calcitonin-related peptide are crucial in sensitizing the nociceptors: this leads to allodynia, the pain to innocuous stimuli, and hyperalgesia, increased sensitivity to painful stimuli [9].

The primary afferent neurons terminate in the trigeminal spinal tract nucleus in the brain stem. The trigeminal spinal tract nucleus is made up of three subnuclei: the subnucleus oralis, subnucleus interpolaris, and subnucleus caudalis. The subnucleus caudalis is structurally comparable to the spinal dorsal horn and hence often called the trigeminal dorsal horn [10]. This subnucleus functions as the main brain stem relay and is a critical central structure for the modulation of nociception.

The subnucleus oralis and subnucleus interpolaris both receive a versatile input from all three types of fibers, particularly from the quickly conducting Ab fibers. The central neurons found within these subnuclei are further subdivided into nociceptive specific neurons. This consists of either Ad fibers and C fibers that respond only to noxious stimuli or all the three fiber types that respond to both noxious and innocuous stimuli [11]. Deep pain is attributed to the convergence of various types of receptors into a central nociceptive neuron. The intricacy of this convergence results in the misreading of the original sensation, leading to allodynia or hyperalgesia [12]. In addition, various other studies have also found that the transition zone between the subnuclei caudalis and the subnuclei interpolaris also contributes to the central processing of deep orofacial nociception [13].

From the brain stem, the orofacial impulses are conducted through the thalamus to the cortex. In the thalamus, mainly the posterior nucleus, ventral posterior nucleus, and intralaminar nucleus, nociceptive specific neurons and wide dynamic range neurons are located. The ventral posterior nucleus is involved in localization of pain to a region, and the intralaminar nucleus is responsible for the affective and motivational dimension to pain and identification of stimuli as pain is caused by the posterior nucleus. In effect, the lateral thalamus projects to the somatosensory cerebral cortex to narrow down and identify the location of the pain, and the medial thalamus projects to neighboring areas such as the hypothalamus and the cingulated gyrus in order to associate the pain with their relevant emotions [14].

Transduction, Conduction, and Transmission of Nociceptors

Transduction

The activity of nociceptors can be classified into that of transduction, conduction, and transmission. Transduction [15] is the response of peripheral nociceptors to noxious impulses caused by traumatic, mechanical, chemical, or thermal stimuli that are converted within the distal nociceptors into a depolarization current mediated by Ca^2. Cellular damage and neurohormonal response to the injury in the skin, fascia, bone, muscle, and ligaments cause the release of intracellular H^+ and K^+ ions, in addition to arachidonic acid (AA) from cell membranes that have been lysed and other noxious mediators. The accumulated AA activates and up regulates the cyclooxygenase-2 enzyme isoform (COX-2), which leads to conversion of AA into biologically active metabolites like prostaglandin E2 (PGE2) and prostaglandin G2 (PGG2), followed by prostaglandin H2 (PGH2). These metabolites and the intracellular H^+ and K^+ ions cause the sensitization of peripheral nociceptors that initiates inflammatory responses leading to pain and an increase in the swelling of the tissue at the site of injury [16].

Other important primary and secondary noxious sensitizers that are released following tissue injury are 5-hydroxytryptamine (5-HT) [17], bradykinin (BK) [18], and histamine [19]. The 5-HT released in response to thermal stimuli activates

peripheral 5-HT2a receptors causing sensitization of primary afferent neurons leading to mechanical allodynia and thermal hyperalgesia [20]. G-protein-coupled receptors [21] B1 and B2, which are located in the primary nociceptors, mediate bradykinin's role in peripheral sensitization. The receptor–G-protein complex, when activated by BK and kallidin, leads to increase of nociceptor excitability by causing inward Na^+ flux and reduced outward K^+ currents. The resulting primary hyperalgesia is due to the increase in nociceptor irritability, increase in vascular permeability, initiation of neurogenic edema, and activation of adjacent nociceptor endings caused by these locally released substances. In addition, bradykinin, 5-HT, and other primary mediators excite orthodromic transmission in sensitized nerve endings and initiate the release of various peptides and neurokinins like CGRP [22], sP [23], and CCK [24] in and around the injury site.

Substance P enhances sensitization of peripheral nociceptors by inducing further release of histamine from mast cells, bradykinin, and 5-HT through a feedback loop mechanism. Calcitonin gene-related protein, a 37 amino acid peptide, is present in the central and peripheral terminals of greater than 35 % of Ad fibers and 50 % of C fibers [25]. Similar to sP, CGRP [26] produced in the cell bodies of primary nociceptors found in the dorsal root ganglion initiates mechanical and thermal hyperalgesia. CGRP released at peripheral endings has several effects including the inhibition of its peripheral metabolic breakdown, which prolongs the effect of sP [27] and histamine-induced vasodilation and inflammatory extravasation.

Acute tissue injury leads to an increase in the production and release of proinflammatory cytokines including IL-1b and IL-6, which play a critical role in intensifying the edema and irritation associated with pain caused by inflammation [28]. Inflammatory mediators and these proinflammatory cytokines activate transducer molecules including the transient receptor potential (TRP) ion channels [21]. Up to eight types of TRP ion channels have been discovered, and the response of each varies depending on mediators activated by the thermal, chemical, or traumatic stimuli within the surrounding microenvironment. The 4-unit TRP-VI/capsaicin ion channel receptor consists of a central ion channel that allows inward flow of Na^+ and Ca^{2+} after being activated by H^+ ions, heat, and presence of capsaicin [29]. This inward ion flux of Ca^+ activates the generator potential [19], which causes summation and depolarization of distal axonal component. The resulting action potential is conducted centrally to the dorsal horn axon terminals.

Conduction

Conduction is the propagation of action potentials through myelinated and unmyelinated nerve fibers, from peripheral nociceptive endings. Nociceptive and nonnoxious nerve fibers can be classified by the extent of myelination, diameter, and conduction velocity. Ab fibers are the nonnoxious special sensory fibers of greatest diameter that are found in somatic structures like skin and joints. The nociceptive

fibers are of two varieties—the Ad fibers and C fibers, which are distributed in skin and other tissues. The Ad fibers propagate the "first pain," which is defined as a sharp, localized stinging sensation of a duration up to 1 s. This "first pain" serves to warn the person of potential injury and causes withdrawal response. The C fibers, also known as high-threshold polymodal nociceptive fibers, are activated by mechanical, thermal, and chemical stimuli and are responsible for the perception of "second pain" that has a prolonged latency lasting from seconds to minutes [30]. It is described as a non-localized stabbing, burning sensation that becomes progressively more comfortable. Ion channels that are located in nociceptive axons and their terminal endings seem to have particular roles in conduction of noxious impulses. Axonal Na^+ ion channels are classified as either sensitive or resistant (TTX-r) to biotoxin tetrodotoxin of the puffer fish. Axonal conduction in nociceptive fibers results in secretion of excitatory amino acids (EAAs) and peptide neurotransmitters from presynaptic terminals in the dorsal horn. The release of EAAs in these nerve terminals is brought about by neuronal-type (N-type) calcium channels, which are voltage-gated channels composed of four subunits that open following depolarization allowing the rapid influx of Ca^{2+} ions. These channels can be blocked by conotoxins including ziconotide.

Transmission

Transmission is defined as the transfer of noxious impulses from primary nociceptors to cells located in the dorsal horn of spinal cord. Ad and C fibers are axons of the unipolar neurons that have nociceptive endings which enter the dorsal horn and branch within Lissauer's tract and ultimately synapse with second-order cells that are situated mostly in Rexed's laminae II known as substantia gelatinosa and V known as nucleus proprius. There are two kinds of second-order dorsal horn neurons, consisting of the nociceptive specific (NS) neurons and WDR. Nociceptive specific neurons found in lamina I are stimulated only by noxious impulses from C fibers, while WDR that are mostly found in lamina I are responsive to both noxious and innocuous stimuli. There is a wide range of responses that are dependent on frequency stimulation: low-frequency stimulation of C fibers leads to sensory transmission not related to pain, while high-frequency stimulation of WDR neurons results in progressive increases in discharge and transmission of painful impulses [31].

The rapid transmission through synapses and accelerated neuronal depolarization is due to excitatory amino acids like glutamate (Glu) and aspartate. Excitatory amino acids stimulate ionotropic amino-3-hydroxy-5-methyl-4-propionic acid (AMPA) and kainite (Kar) receptors, which modulate the influx of K^+ and Na^+ ions along with intraneural voltage potential. These receptors are rather impervious to other cations like Ca^{2+}. Each AMPA receptor contains the central cation channel surrounded by four subunits that have intrinsic binding sites for glutamate. The interaction of agonists with two or more binding sites on the receptor results

in activation and opening of the channel, permitting Na^+ influx into the cell [32], leading to rapid transmission of noxious impulses to supraspinal sites of perception. Kainate receptors also regulate the influx of Na^+ and K^+ ions and are responsible for postsynaptic excitation. However, KAR receptors also seem to be involved in transmission of synaptic signals that follows brief noxious excitation. In addition, KAR receptors may increase the efficacy of synaptic transmission by multiplying the likelihood of discharge from second-order neurons in conditions of ongoing excitation.

Under conditions of high-frequency noxious stimulation, AMPA and KAR receptors activate the priming of *N*-methyl-D-aspartic acid (NMDA) receptors that is voltage mediated [33, 34]. The NMDA receptor is a ligand-specific ion channel that is voltage gated. It consists of four subunits—two NR2 units with sites for glutamate binding on its extracellular portions and two NRI units with binding sites for glycine and an allosteric site that is reactive to zinc ions. The receptor regulates the influx of Na^+ and Ca^{2+} ions and the outflow of K^+ ions through its intrinsic ion channel. Each subunit has a considerable cytoplasmic part that can be altered by protein kinases and an outer allosteric component that is modifiable by zinc ions. Activation of these receptors requires an AMPA-induced membrane depolarization with a positive change in intracellular voltage in addition to the binding of aspartate or glutamate to the receptor. AMPA receptors that are activated result in excitatory postsynaptic potentials (EPSPs) that span for several hundred milliseconds [35] and accumulate to produce a depolarization that removes a Mg^{2+} "plug" that inhibits the NMDA ion channel, permitting Ca^{2+} ion influx. The accumulation of Ca^{2+} ions leads to series of neurochemical and neurophysiological events that influence processing of acute pain. In a process known as "windup" that specially involves excitation of dorsal horn neurons independent of transcription, second-order spinal neurons become highly sensitized and fire rapidly without the need for further activation.

Studies by Woolf have found that the activation of NMDA receptors, the process of "windup," and central sensitization play a critical role in clinical hyperalgesia and can be incited by trauma, nerve injury, and inflammation. The central sensitization is found in supraspinal regions of the CAN that include the amygdala, anterior cingulated gyrus, and rostroventral medulla [36]. The influx of Ca^{2+} ions also activates inducible enzymes that include COX-2 and nitric oxide synthase (NOS). Peptides including sP and CGRP result in the delayed and prolonged depolarization of second-order dorsal horn neurons. When sP binds to metabotropic neurokinin 1 (NK-1), NMDARs are activated: this seems essential for the establishment of long-term potentiation (LTP) [37]. Upon stimulation of NK-1, phosphokinase A (PKA) and cyclic adenosine monophosphate (cAMP) are synthesized which mediate various changes in the cell such as the slow priming of NMDA receptors, genome activation, and second-messenger cascades. The increase in intracellular and extracellular PGE and NO and the synthesis of acute-phase proteins lead to transcription-dependent central sensitization and are associated with responses that facilitate changes in neural plasticity.

Conclusion

To prevent neural plasticity and long-term orofacial pain it is therefore important to control inflammation and resultant morbidity at the earliest. The effects of neurotransmitters can be blocked at specific levels in the pain pathways of the head, neck, and face region that are involved in the development of orofacial pain by medical and interventional pain management. Controlling inflammation and blocking of the pain pathways can result in decrease of peripheral and central sensitization and LTP of pain.

Acknowledgement The authors wish to thank Nirmal Kumar, Amarender Vadivelu, Gopal Kodumudi, and Vijay Kodumudi for their help in the preparation of the manuscript.

Conflict of Interest None declared.

References

1. Vadivelu N, Whitney CJ, Sinatra RS. Pain pathways and acute pain processing. New York: Cambridge Univ. Press; 2009.
2. Hunt CC. Relation of function to diameter in afferent fibers of muscle nerves. J Gen Physiol. 1954;38(1):117–31.
3. Dubner R, Sessle BJ, Store AT. The neural basis of oral and facial function. New York: Plenum; 1978.
4. Jessel TM. Substance P, in nociceptive sensory neurons. Ciba Found Symp. 1982;91:225–48. Review.
5. Ichikawa H, Sugimoto T. Vanilloid receptor 1-like receptor-immunoreactive primary sensory neurons in the rat trigeminal nervous system. Neuroscience. 2000;101(3):719–25.
6. Ichikawa H, Fukuda T, Terayama R, Yamaai T, Kuboki T, Sugimoto T. Immunohistochemical localization of gamma and beta subunits of epithelial Na+ channel in the rat molar tooth pulp. Brain Res. 2005;1065(1–2):138–41. Epub 2005 Nov 17.
7. Matsumoto H. Effects of pulpal inflammation on the activities of periodontal mechanoreceptive afferent fibers. Kokubyo Gakkai Zasshi. 2010;77(2):115–20.
8. Mathews B, Sessle BJ. Peripheral mechanisms of orofacial pain. In: Sessle BK, Lavigne GL, Lund JP, et al., editors. Orofacial pain. 2nd ed. Chicago: Quintessence; 2008. p. 27–43.
9. Meyer RA, Ringkamp M, Campbell JN, et al. Peripheral mechanisms of cutaneous nociception. In: McMahon SB, Koltzenburg M, editors. Wall and Melzacks textbook of pain. 5th ed. Amsterdam: Elsevier; 2006. p. 3–34.
10. Gobel S, Bennett GJ, Allen B, Humphrey E, Seltzer Z, Abdelmoumene M, Hayashi H, Hoffert MJ. Synaptic connectivity of substantia gelatinosa neurons with reference to potential termination site of descending axons. In: Sjolund B, Bjorkland A, editors. Brain stem control of spinal mechanisms. NewYork: Elsevier/North Holland; 1982.
11. Tenebaum HC, Mock D, Gordon AS, Goldberg MB, Grossi ML, Locker D, Davis KD. Sensory and affective components of orofacial pain: is it all in your brain? Crit Rev Oral Biol Med. 2001;12(6):455–68. Review.
12. Ness TJ, Gebhart GF. Visceral pain: a review of experimental studies. Pain. 1990;41(2):167–234. Review; PMID: 2195438.

13. Shimizu K, Guo W, Wang H, Zou S, LaGraize SC, Iwata K, Wei F, Dubner R, Ren K. Differential involvement of trigeminal transition zone and laminated subnucleus caudalis in orofacial deep and cutaneous hyperalgesia: the effects of interleukin-10 and glial inhibitors. Mol Pain. 2009;5:75. PubMed PMID: 20025765; PubMed Central PMCID: PMC2806354.
14. Willis WD. Nociceptive functions of thalamic neurons. In: Sterlade M, Jones EG, McCormick DA, editors. Thalamus. Oxford: Elsevier Science; 1997.
15. Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis. 2001;8(1):1–10.
16. Ito S, Okuda-Ashitaka E, Minami T. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res. 2001; 41(4):299–332.
17. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5584):1871–5.
18. Millan MJ. Serotonin and pain: evidence that activation of 5-HT1A receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. Pain. 1994;58(1):45–61.
19. Wang H, Kohno T, Amaya F, Brenner GJ, Ito N, Allchorne A, et al. Bradykinin produces pain hypersensitivity by potentiating spinal cord glutamatergic synaptic transmission. J Neurosci. 2005;25(35):7986–92.
20. Sasaki M, Obata H, Kawahara K, Saito S, Goto F. Peripheral 5-HT2A receptor antagonism attenuates primary thermal hyperalgesia and secondary mechanical allodynia after thermal injury in rats. Pain. 2006;122(1–2):130–6.
21. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. New York, NY: Science; 2000. p. 1765–9.
22. Bennett AD, Chastain KM, Hulsebosch CE. Alleviation of mechanical and thermal allodynia by CGRP(8-37) in a rodent model of chronic central pain. Pain. 2000;86(1–2):163–75.
23. Liu H, Mantyh PW, Basbaum AI. NMDA-receptor regulation of substance P release from primary afferent nociceptors. Nature. 1997;386(6626):721–4.
24. Noble F, Derrien M, Roques BP. Modulation of opioid antinociception by CCK at the supraspinal level: evidence of regulatory mechanisms between CCK and enkephalin systems in the control of pain. Br J Pharmacol. 1993;109(4):1064–70.
25. McCarthy PW, Lawson SN. Cell type and conduction velocity of rat primary sensory neurons with calcitonin gene-related peptide-like immunoreactivity. Neuroscience. 1990;34(3):623–32.
26. Evans BN, Rosenblatt MI, Mnayer LO, Oliver KR, Dickerson IM. CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors. J Biol Chem. 2000;275(40):31438–43.
27. Tzabazis AZ, Pirc G, Votta-Velis E, Wilson SP, Laurito CE, Yeomans DC. Antihyperalgesic effect of a recombinant herpes virus encoding antisense for calcitonin gene-related peptide. Anesthesiology. 2007;106(6):1196–203.
28. Bessler H, Shavit Y, Mayburd E, Smirnov G, Beilin B. Postoperative pain, morphine consumption, and genetic polymorphism of Il-1beta and Il-1 receptor antagonist. Neurosci Lett. 2006;404(1–2):154–8.
29. Winter J, Bevan S, Campbell EA. Capsaicin and pain mechanisms. Br J Anaesth. 1995;75(2): 157–68.
30. Djouhri L, Koutsikou S, Fang X, McMullan S, Lawson SN. Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors. J Neurosci. 2006;26(4):1281–92.
31. Hogan QH, Abram SE. Neural blockade for diagnosis and prognosis: a review. Anesthesiology. 1997;86(1):216–41.
32. Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. Annu Rev Neurosci. 2002;25:103–26.
33. Woolf CJ. An overview of the mechanisms of hyperalgesia. Pulm Pharmacol. 1995;8(4–5): 161–7.

34. Mannion RJ, Woolf CJ. Pain mechanisms and management: a central perspective. Clin J Pain. 2000;16(3 Suppl):S144–56.
35. Silvilotti LG, Thompson SW, Woolf CJ. Rate of rise of the cumulative depolarization evoked by repetitive stimulation of small-caliber afferents is a predictor of action potential windup in rat spinal neurons in vitro. J Neurophysiol. 1993;69(5):1621–31.
36. Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci. 2002;25(6):319–25.
37. Ikeda H, Heinke B, Ruscheweyh R, Sandkuhler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science. 2003;299(5610):1237–40.