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Peripheral Sympathetic Component of the Temporomandibular Joint Inflammatory Pain in Rats

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Abstract: **The aim of this study was to further validate our carrageenan-induced temporomandibular joint (TMJ) inflammatory hyperalgesia model in rats by showing that administration of indomethacin before the initiation of inflammation would diminish the TMJ hyperalgesia. Using this model, we investigated whether norepinephrine and local β-adrenoceptors contribute to the development of inflammatory TMJ hyperalgesia. Carrageenan-induced TMJ hyperalgesia was assessed by measuring the behavioral nociceptive responses, such as rubbing the orofacial region and flinching the head, induced by the injection of a low dose of 5-hydroxytryptamine into the TMJ sensitized 1 h before by a TMJ injection of carrageenan. Blockade of prostaglandin synthesis by indomethacin prior to initiation of inflammation by carrageenan significantly attenuated the TMJ hyperalgesia. The guanethidine depletion of norepinephrine or the blockade of β_2 but not the blockade of the β_1-adrenoceptor by the selective adrenoceptor antagonists ICI 118.55 and atenolol, respectively, significantly reduced carrageenan-induced TMJ hyperalgesia. In the present study, we further validated our carrageenan-induced TMJ hyperalgesia model to study the mechanisms involved in inflammatory TMJ hyperalgesia and to test the analgesic effect of different types of peripheral analgesics. We also demonstrated that norepinephrine released at the site of injury contributes to the development of the inflammatory TMJ hyperalgesia by the activation of β_2-adrenoceptors.**

Perspective: ***The findings that local sympathomimetic amines contribute to the inflammatory TMJ hyperalgesia by activating β_2-adrenoceptors may be relevant to clinical TMJ inflammatory pain states less sensitive to nonsteroidal anti-inflammatory drugs.***

Key words: *TMJ, carrageenan, chemical hyperalgesia, sympathomimetic amines, β-adrenoceptors.*

Temporomandibular joint disorders, especially those associated with acute trauma, internal derangement, or arthritis are commonly associated with acute or chronic inflammation.[1,43] They represent a group of chronic painful conditions involving the muscles of mastication and the temporomandibular joint (TMJ) with a prevalence in the general population up to 12%.[9,22,49]

Inflammatory TMJ conditions can result in TMJ hyperalgesia produced by peripheral sensitization of TMJ nociceptors[1,29,35,36,38] and by central sensitization of the nociceptive neurons of the trigeminal brainstem sensory nuclear complex.[21,30,41] Peripheral sensitization as well as central sensitization are characterized by an increase in the neuronal membrane excitability by inflammatory mediators released at the site of injury[1,29,43] and by neuropeptide and excitatory amino acid released at the tri-

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geminal brainstem sensory nuclear complex,[3,4,7,52] respectively. Some of the inflammatory mediators released at the site of injury including PGE_2 are present at high levels in the synovial fluid of patients with temporomandibular disorders.[29] During hyperalgesic states, nociceptive threshold is lowered and a nonnoxious stimulus such as jaw movement can induce pain[1] as well as noxious stimulus can induce increased pain.[18] Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to manage inflammatory pain.[19,32,44] The analgesic action of these drugs results from the blockade of prostaglandins synthesis, thus preventing the peripheral sensitization of nociceptors.[23,24] Considering that many patients are intolerant to prolonged treatment with NSAIDs and that not all patients with TMJ inflammatory pain respond to its effects,[44] a better understanding of the neurochemicals and receptors involved in these conditions is necessary.

It is well known that inflammatory pain has a sympathetic component[31,33] that may predominate in pain less sensitive to NSAIDs and that TMJ receives a rich sympathetic innervation.[28,50,51] However, whether sympathomimetic amines play a role in the development of TMJ hyperalgesia remains to be investigated. Our recently described model of carrageenan-induced hyperalgesia in the rat TMJ[36] may contribute to that. In this model a low dose of 5-hydroxytryptamine (5-HT) that induces minimal nociceptive behavior is injected into the TMJ sensitized 1 h before by a TMJ injection of carrageenan. The chemical stimulation induced by the TMJ injection of 5-HT is used to detect carrageenan-induced hyperalgesia in the TMJ region. To distinguish this type of inflammatory hyperalgesia from that detected by mechanical stimulation, the experimental model was termed carrageenan-induced chemical hyperalgesia in the rat TMJ. The aim of this study was to further validate this model by showing that administration of indomethacin before the initiation of inflammation would diminish the TMJ hyperalgesia. Using this model, we investigated whether norepinephrine contributes to the development of inflammatory TMJ hyperalgesia by activating articular β-adrenoceptors.

Materials and Methods

Animals

A total of 154 male Wistar rats (200-300 g) was used in this study. The animals were housed in plastic cages with soft bedding (5 per cage) on a 12:12 light cycle (lights on at 6:00 AM) with food and water available ad libitum. They were maintained in a temperature-controlled room (±23°C) and handled for at least 1 week prior to the experiments. Experimental protocols were approved by the Committee on Animal Research of the University of Campinas and conformed to IASP guidelines for the study of the pain in animals.[53]

General Procedures

Each animal was placed in a test chamber (30 × 30 × 30 cm mirrored-wood chamber with a glass at the front side) for a 15-min habituation period. Each animal was used in only one experiment and was sacrificed at the end of the experiment. Testing sessions took place during the light phase (between 9:00 AM and 5:00 PM) in a quiet room maintained at 23°C.[39]

TMJ Injection

Animals were briefly anesthetized by inhalation of halothane and the posteroinferior border of the zygomatic arch was palpated. The needle was inserted immediately inferior to this point and was advanced in an anterior direction until reaching the posterolateral aspect of the condyle. TMJ injections were performed via a 30-gauge needle introduced to the left TMJ at the moment of the injection. A cannula consisting of a polyethylene tube was connected to the needle and also to a 50 μL syringe (Hamilton, Reno, NV). Volume per injection was 15 μL. Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber.

After the conclusion of each experiment, animals were anesthetized with an intraperitoneal injection of a mixture of urethane (1 g/kg) and α-chloralose (50 mg/kg). The Evans blue dye (0.1%, 5 mg/kg) was then administered systemically to visualize the inflammation-induced plasma extravasation of Evans blue dye bound to plasma protein upon postmortem examination of the injected TMJs.[25] The correct site of injection was indicated by the observation that the plasma extravasation induced by the TMJ injections was restricted to the TMJ region.

Measurement of Behavioral Nociceptive Responses

Two TMJ injections were given at 1-h intervals and each rat was returned to the test chamber following the last TMJ injection for an observation period of 30 min. Rats immediately recovered from the anesthesia after each TMJ injection. The recording time was divided into 10 blocks of 3 min and a pain score was determined for each block by measuring the number of seconds that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore- and hindpaw and/or flinching the head in an intermittent and reflexive way characterized by high-frequency shakes of the head as previously described.[12,36,40] Rats did not have access to food or water during the test.

Carrageenan-Induced Chemical Hyperalgesia in the TMJ

Carrageenan-induced TMJ hyperalgesia was assessed by measuring these behavioral nociceptive responses induced by application of a low dose of 5-HT (75 μg) into the TMJ challenged 1 h prior by carrageenan injection (100 μg).[36]