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Physiology & Behavior 90 (2007) 782–789

PHYSIOLOGY & BEHAVIOR

Capsaicin sensitive neurons role in the inflamed TMJ acute nociceptive response of female and male rats

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Received 25 August 2006; received in revised form 14 December 2006; accepted 10 January 2007

Abstract

Computerized meal pattern analysis, and more specifically meal duration, has recently been used as a non-invasive biological marker of nociception in the temporomandibular joint (TMJ). Cells responsible for the nociceptive response in the inflamed TMJ may include capsaicin (CAP) sensitive neurons. To test the role of CAP sensitive neurons in acute nociceptive responses first, male and female rats were treated neonatally with vehicle or CAP, an agent known to destroy a majority of C fibers. Second, after 56 days the rats were divided into four groups: neonatal vehicle-injected and treated with and without complete Freund's adjuvant (CFA). Treatment groups included neonatal non-CAP vehicle treated and TMJ not-injected (CON); vehicle treated and TMJ CFA injected (CFA); CAP-treated and not-injected (CAP); and CAP-treated and CFA injected (CAP+CFA). Meal patterns were analyzed for two days after injection. CFA-injection in non-CAP-treated rats lengthened meal duration on the first and second day after treatment in the males, but only on the first day in the females. CAP treatment in male and female rats prevented significant lengthening of meal duration induced by CFA. CAP treatment attenuated the CFA-induced increase in calcitonin gene-related peptide expression in the trigeminal ganglia similarly in males and females. The data suggests CAP-sensitive neurons are responsible, in part, for transmission of acute nociceptive responses associated with CFA administration and suggest gender can affect nociception in the inflamed TMJ region.

Keywords: Gender; Orofacial; Sensory neuron; Trigeminal ganglion; Interleukin-1β; Pain

1. Introduction

Temporomandibular joint disorders (TMD) are often characterized by pain and dysfunction of the temporomandibular joint (TMJ) and surrounding muscles. Physical conditions causing TMD can include muscle pain, disc displacements and joint conditions that include arthralgia and osteoarthritis. TMD associated with acute trauma, internal derangement, or osteoarthritis often includes an inflammatory component [1,2]. Males and females may respond to this inflammation differently because TMJ pain in humans is reported more often in reproductive age females than males [3–5] suggesting that hormones modulate disease mechanism and/or pain experienced by TMD patients.

Inflammation in the TMJ region entails the production and release of inflammatory substances, including interleukin-1β (IL-1β), tachykinins such as substance P, and inflammatory-related neuropeptides, such as calcitonin gene-related peptide (CGRP) [6–12]. Inflammation/nociception causes an increase in trigeminal ganglia CGRP and substance P [12–14]. Increased trigeminal ganglia CGRP levels have been linked to craniofacial nociception and can be used as a marker for craniofacial nociception. Pain typically accompanies severe inflammation [15–17] and patients with longstanding TMD are frequently in severe and chronic pain [18].

When injected into the TMJ, complete Freund's adjuvant (CFA) causes synovitis characterized by proliferation of synovial

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0031-9384/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2007.01.002

cells, lymphocytic infiltration of the articular and periarticular tissues, destruction of bone and articular cartilage by pannus formation, and periosteal bone formation [19]. After phagocytosis of immune complexes by polymorphonuclear leukocytes and synovial macrophages, inflammatory mediators are released into the extracellular fluid. These include lysosomal enzymes, oxygen free radicals, oxidative products of arachidonic acid, and matrix metalloproteinases that degrade the cartilage matrix [20,21]. These mediators in turn act on synovial macrophages and fibroblast-like cells to release additional proinflammatory cytokines such as IL-1β, other arachidonic acid metabolites, and oxygen free radicals that can contribute to TMJ area swelling [21,22].

To date, most TMJ nociceptive animal models have induced TMJ inflammation/nociception following the injection of a variety of agents that have included substance P, interleukin-1α, collagen, mustard oil, carrageenan, and CFA [10,14,17,23–30]. Behavioral measurement of mechanical allodynia using Semmes–Weinstein monofilaments [31] or vocalization following pressure on the TMJ [32] have been used to record nociception, but the animal must be manipulated during the measurement and these techniques measure surface and not deep tissue nociception. Recently, our laboratory has demonstrated that meal patterns, and more specifically a lengthening of meal duration, can be used as a non-invasive biological marker of CFA-induced TMJ nociception (surface and deep) in male and female rats [7–9]. The premise of our animal model is that the CFA-induced TMJ nociception affects the rat, such that, when a hungry animal initiates a meal the animal eats slowly due to the TMJ nociception associated with the movement of the mandible during the chewing process. This is exactly what was observed following bilateral TMJ CFA injections, the injected male and female rats had longer meal durations. Further rationale for suggesting meal pattern analysis as a nociceptive marker stems from impaired eating in TMD patients [33] and a clinical study of juvenile rheumatoid arthritic children [34]. The latter study looked at chewing performance as an objective measure of masticatory function and showed that the juvenile rheumatoid arthritic children with TMD symptoms changed their chewing habits to presumably "guard" against pain [35].

In support of our model we showed that a variety of drugs, such as, ibuprofen or cyclooxgenase-II inhibitors normalize meal duration in both male and female rats with TMJ inflammation [7,8]. However, ibuprofen and cyclooxgenase-II inhibitors modulate the immune response as well as having analgesic properties. Thus several questions arose. Could the normalization of meal duration following administration of these drugs in rats with TMJ inflammation be attributed to either 1) decreasing TMJ swelling and thus reducing the physical impediment of TMJ mobility and/or 2) a reduction in non-nociceptive (e.g., mechanoreceptors) neuronal signals due to swelling and/or 3) decreased nociception.

The TMJ is innervated with nerve endings including unmyelinated C and thinly myelinated A-δ axons [24,32,36–38]. Activation of C and A-δ nerve endings has been associated with nociceptive responses [39]. Capsaicin (CAP) given to neonatal rats or mice destroys >80% unmyelinated C-fibers and up to 40% of A-δ fibers and thus would remove many neurons thought responsible for TMJ inflammation-induced nociception [40–43], while leaving other sensory neurons intact that could transmit non-nociceptive signals [44,45]. If our hypothesis is correct that meal duration is a measure of TMJ nociception, then removal of these nociceptive neurons with CAP treatment should be manifested by a reduction in nociceptive signaling and normal meal duration following injection of CFA into the TMJ. If on the other hand, meal duration is still lengthened following CAP and CFA treatment it would suggest that factors associated with TMJ swelling and not nociception lengthens meal duration. This was tested in the present study and experiments were completed in male and female rats, because of reports in the literature that indicate reproductive age females experience more TMD than males [3–5] and thus, females may respond to TMJ inflammation differently than males.

2. Materials and methods

This study was approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee. Female Sprague Dawley rats were bred in the Baylor College of Dentistry's Animal Resource Unit. When their pups were two days of age, the pups were injected subcutaneously with 50 mg/kg of 95% CAP in a 25 μl solution of 20% v/v of Tween 80, 10% v/v of ethanol and 80% v/v of 0.9% saline, whereas non-CAP control animals were injected with only the vehicle [46]. Prior to injection the pups were anesthetized with isoflurane gas (5%) and respiration was monitored after CAP injection. If necessary the pups were ventilated with a straw for <60 s. Care was taken so none of the solution leaked from the injection site. The pups were handled with gloves and rubbed in their bedding prior to giving them back to their Dam. The injections were repeated at 10 days of age. When the animals were weaned at approximately 26 days of age they were placed in individual boxes with soft bedding and maintained on a 12:12 h light/dark schedule with lights out at 2000 h. When the female rats weighed 260 g they received daily vaginal smears to determine their estrous cycle stage. The rats were placed in individual sound-attenuated food intake monitoring chambers linked to a computer. The animals were given several days to adjust to their new environment, and then handled daily to minimize stress. The estrous stage was recorded, and food intakes and body weights were recorded daily.

The sound attenuated chambers are equipped with photobeam computer-activated pellet feeders (Med Assoc. Inc., East Fairfield, VT). The rats were given 45 mg rodent chow pellets (Product No. FO 165, Bioserv, Frenchtown, NJ). When a rat removes a pellet from the trough of the feeder, a photobeam placed at the bottom of the trough is no longer blocked, signaling the computer to drop another pellet, record the date and time, and keep a running tally of the total daily food consumption. The computer record of pellets dropped over time established meal patterns, i.e. meal frequency, inter-meal interval, meal size, meal duration and total food intake [9]. These fore-mentioned parameters were then computer analyzed for differences between groups. Note: when representing meal duration in minutes, meal duration differs between male and female rats, because of gender differences [9,30]. Thus, to compare the genders the meal duration data was represented as a percentage of change from their respective non-CFA injected control group [9].