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Measuring persistent temporomandibular joint nociception in rats and two mice strains

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ABSTRACT

Temporomandibular joint (TMJ) pain has been reported to last for prolonged periods in humans. In rodents a variety of methods have been used to measure TMJ nociception, but for most of these methods the period of measurement has been minutes to a couple of hours. In addition, most measurement protocols required restraint or training of the animal. Previous studies from our laboratory demonstrated that feeding behavior, particularly meal duration, was an indicator of TMJ nociception in unrestrained and untrained male and female Sprague–Dawley rats for up to two days. In this study, we first found that injection of complete Freund's adjuvant (CFA) into the TMJ of rats significantly lengthened meal duration for 19 days and also decreased meal frequency for 42 days. Interestingly, the meal duration varied significantly from day to day within the 19 day period. TMJ interleukin-1β (IL-1β) and calcitonin gene-related peptide (CGRP) were significantly elevated in the TMJ tissues of CFA-injected animals and the level of these markers was attenuated as the meal duration decreased with time. Control animals injected with saline into the TMJ or CFA into the knee did not show a significant lengthening in meal duration but did show a decrease in meal frequency. In a second study, DBA/1LacJ mice given TMJ CFA injections showed a significantly lengthened meal duration on four of the seven days measured using end-of-the meal definition of 5 or 10 min. No other meal pattern changed significantly. Two days post-CFA injection, the DBA/1LacJ mice showed significantly elevated interleukin-6 (IL-6), but not elevated IL-1β. Seven days post-injection, both IL-6 and IL-1β were significantly elevated. No change in CGRP was detected. In this study C57Bl/6 mice also received TMJ CFA injections, but they did not show a lengthening in any meal pattern or significant increases in IL-1β, IL-6 or CGRP. Our data show, for the first time, that meal duration can be used to measure CFA-induced nociception in the TMJ over the course of several weeks in unrestrained rats and for up to seven days in the DBA/1LacJ mouse strain. In addition, C57Bl/6 mice are resistant to CFA-induced TMJ nociception at the same dose used in the DBA/1LacJ mice.

1. Introduction

Temporomandibular joint disorders (TMD) are often characterized by pain and dysfunction of the temporomandibular joint (TMJ) and surrounding muscles. A portion of the individuals with TMJ pain show an accompanying inflammatory component of the joint. For example, patients with cartilage degradation are often observed to have some level of synovitis [1].

In male and female rats, meal duration was a measure of nociception resulting from injection of complete Freund's adjuvant (CFA) into the TMJ but our previous studies were only for 2 days [2–6]. Injection of CFA into the TMJ significantly lengthened meal duration in rats for two days, while the same amount of CFA in the knee did not lengthen meal duration [5]. Food intake and meal frequency were reduced in animals after injecting CFA into the TMJ but these meal patterns were also reduced after injecting the knee suggesting these meal patterns were not specific for orofacial pain [5]. Another meal pattern, meal size, remained unchanged by administration of CFA. For these reasons, meal duration was a focus of study in our lab. When the rats received prior treatment with ibuprofen meal duration was normal after CFA injection in both male and female rats [6]. Interestingly IL-1β remained significantly elevated in the TMJ of the ibuprofen treated animals injected with CFA [6], suggesting that some inflammation from the CFA injection remained, but because the ibuprofen attenuated the nociception meal duration normalized as the nociception subsided. In another study, cyclooxygenase-II (COX-2) inhibitors normalized meal duration in rats after CFA injection [2]. In this study the COX-2 inhibitor also attenuated the inflammation, i.e., TMJ tissue IL-1β normalized [2]. In still another study, rats were given capsaicin or vehicle at 2 and 10 days of age; capsaicin permanently destroyed afferent nociceptive fibers in these animals [7]. When these male rats reached adulthood saline or CFA

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was injected into the TMJ and their meal duration was measured. Capsaicin treatment alone had no effect on meal duration, because saline injected, non-capsaicin treated rats had the same meal duration as saline injected, capsaicin treated rats. Non-capsaicin treated rats injected with CFA had longer meal durations than rats that were pre-treated with capsaicin, which demonstrated that meal duration after CFA injection was normalized due to a capsaicin-induced loss of afferent nociception neuronal fibers [7]. The lack of change in meal duration in these capsaicin treated male rats occurred despite CFA inducing greater TMJ swelling, which demonstrated that the physical and mechanical changes in the inflamed TMJ synovial joint did not affect meal duration measurements. Another rationale for suggesting that meal duration is a measure of nociception stems from the finding that eating is impaired in patients with TMD [8] and from a clinical study of juvenile rheumatoid arthritic children [9] that examined chewing performance as an objective measure of masticatory function. It showed that the juvenile rheumatoid arthritic children with TMD symptoms changed their chewing habits presumably to "guard" against pain. Most recently, meal duration in the rat was shown to be increased over the course of a week following pulp exposures demonstrating meal duration can also be used as a measure of tooth nociception [10].

Using meal duration to measure TMJ nociception in rats offers several advantages over other methods used today: 1) the animal does not have to be trained prior to testing as compared to a previous thermal sensitivity test [11,12]; 2) the animal is no longer handled once the TMJ inflammation is induced, thus reducing further compounding stresses that might arise in bite force and Von Frey filament tests [13,14]; 3) no artificial test-induced competing behaviors are generated [11,12]; 4) testing continues 24 h a day, thereby eliminating any artifacts of testing in the light phase, as has occurred in many previous studies, when the rodent normally sleeps [13–15]; and 5) testing can continue in the undisturbed animal for days in contrast to a test for scratching behavior [15]. All of these factors make the TMJ meal duration measurement a powerful tool for studying the mechanisms of TMD nociception.

As mentioned above, CFA-induced changes in TMJ nociception, as measured by meal duration, lasted only a few days. For the first time we investigated the effect for over three weeks. Also, we measured inflammatory mediators interleukin 1β (IL-1β) and interleukin-6 (IL-6) and calcitonin gene-related peptide (CGRP) in the TMJ and CGRP in the trigeminal ganglia (TG) to determine if there was an association between expression of one of these molecules and nociception.

The second study was based on the fact that mice have been used to study arthritis and inflammatory nociception and that differences in species have been shown to impact the incidence and severity of these measurements. The mouse strain DBA/1 has the major histocompatibility complex H-2q resulting in the animal being highly sensitive to heterologous and homologous collagen-induced arthritis [16,17]. In addition to collagen-induced arthritis, CFA can induce an arthritic response in certain mouse strains [18]. Because the DBA/1 strain has H-2q injection of the adjuvant CFA into the TMJ was expected to elicit a robust nociceptive response. In contrast, the C57Bl/6 mouse strain does not carry this allele and may be more resistant to artificially induced TMJ nociception. C57Bl/6 mice are important because many knockout mice have this background and future studies using feeding behavior to test for a gene's role in nociception would use a knockout having a C57Bl/6 background. In our studies, we wanted to determine if the DBA/1 mice were more sensitive to TMJ CFA-induced nociception than the C57Bl/6 strain.

The first goal of the following experiments was to show for the first time that a meal pattern can measure a persistent increase in TMJ nociception in rats and a second goal was to show that a meal pattern is a measure of nociception in the TMJ of mice, particularly DBA/1 mice. A third primary goal was to determine the protein concentration of inflammatory molecules in the joint during which the nociceptive measurements were being completed.

2. Materials and methods

These studies were approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee in accordance of the guidelines of the USDA and National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). In the first study, male Sprague–Dawley rats (250 g) were purchased from Harlan Industries (Houston, TX) and in the second study, six-week-old male C57Bl/6 mice (22 g) and DBA/1LacJ mice (18 g) were purchased from Jackson Laboratory (Bar Harbor, ME). The rodents were housed individually in a constant-temperature room at 23 °C with standard food pellets (Harlan Industries) and water available *ad libitum* for at least three days. All the animals were maintained on a 12:12 h light/dark cycle with the lights on at 06:00. The animals were transferred to the feeding modules and left undisturbed for five days before experimentation. Their body weights were recorded daily.

2.1. Meal pattern analysis

The computer record of pellets dropped over time establishes the meal patterns (meal duration, meal frequency, food intake and meal size). Meal patterns were characterized using data acquired from 32 feeding modules that were situated within sound-attenuated chambers equipped with photobeam computer-activated pellet feeders (Med Assoc. Inc., East Fairfield, VT). The rats were given 45 mg rodent chow pellets, and the mice were fed 20 mg rodent chow pellets (Bioserv, Frenchtown, NJ). When the animal removed a pellet from the feeder trough, a photobeam placed at the bottom of the trough would no longer be 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 record of pellets dropped over time was computer-analyzed with a proprietary computer program to establish the meal patterns [19]. In the meal pattern calculations for the rat, the end-of-the-meal was defined as when no pellets were removed from the feeder for 10 min [20]. In the mice the end-of-the-meal has not been defined and thus, three end-of-the-meal definitions were used in our meal pattern calculations: 5, 10 or 15 min. The minimum meal size needed to be at least three pellets.

2.2. CFA injection

At 08:00 the Sprague–Dawley rats or DBA/1LacJ or C57Bl/6 mice were anesthetized with isoflurane (5% flow), and given bilaterally either saline or CFA injections into the TMJ or knee. The injections were made using a 29-gauge, one-half-inch needle (Becton Dickinson, Franklin Lakes, NJ). The TMJ injections were completed by guiding the needle, angled at 30–40° to the sagittal plane, under the zygomatic arch, and inserting it 2–3 mm, followed by injection into the peri-articular space of the TMJ within 5 s. For the intracapsular injection of the knee, the needle was inserted anteriorly.

The rats received 250 μg of CFA (Chondrex, Redmond, WA) or 0.9% saline in a 50 μl volume into the TMJ or the knee. Previously we observed [21,22] that a similar injection caused a persistent TMJ inflammation for up to 6 weeks; in that study meal duration was not measured.

The mice of each strain were injected bilaterally into the TMJ with 30 μg of CFA or 0.9% saline in a 6 μl volume. An additional group of DBA/1LacJ mice also received a bilateral CFA injection (30 μg/6 μl) into the knee. The dosage of CFA injected into the mice was based on a dose–response experiment indicating that this dose produced a significant decrease in food intake compared to the controls one day after injection (Table 1). A saline knee injection was not performed since previous studies showed that saline knee injections did not affect meal patterns [5].