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Capsaicin avoidance as a measure of chemical hyperalgesia in orofacial nerve injury models

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Abstract

Many patients suffer from trigeminal neuralgia and other types of orofacial pain that are poorly-treated, necessitating preclinical animal models for development of mechanisms-based therapies. The present study assessed capsaicin avoidance and other nocifensive behavioral responses in three models of orofacial nerve injury in rats: chronic constriction injury (CCI) of the mental nerves, partial tight ligation of mental nerves, and CCI of lingual nerves. We additionally investigated if nerve injury resulted in enhanced capsaicin-evoked activation of neurons in trigeminal caudalis (Vc) or nucleus of the solitary tract (NTS) based on expression of Fos-like immunoreactivity (FLI). Mental nerve CCI resulted in an enhancement of capsaicin avoidance in a two-bottle preference paradigm, while neither mental nerve injury produced thermal hyperalgesia or mechanical allodynia. CCI of lingual nerves did not affect capsaicin avoidance. Counts of FLI in Vc were significantly higher in the lingual sham and mental nerve CCI groups compared to mental shams; FLI counts in NTS did not differ among groups. Mental nerve CCI may have induced central sensitization of chemical nociception since increased capsaicin avoidance was accompanied by greater activation of Vc neurons in response to oral capsaicin.

INTRODUCTION

Orofacial pain of differing etiologies affects millions of patients worldwide [18, 23] and is challenging to treat, partly due to a lack of understanding of the underlying mechanisms generating the pain [2]. Animal models of neuropathic pain following peripheral nerve injury result in behavioral signs of thermal hyperalgesia and mechanical allodynia in the affected limbs [e.g., 3, 15, 19]. Most animal models of trigeminal nerve injury involve constriction, ischemic injury or transection of the infraorbital or inferior alveolar nerves [7, 11–13, 24] or the mental nerve [9, 10, 16–18, 23]. The aim of the present study was to investigate and compare manifestations of thermally-, mechanically- and chemically-evoked pain using two injury models of the mental nerves, as well as a lingual nerve injury, in rats. Lingual and mental nerve innervation of the tongue and lower lip are critical for sensing the chemesthetic properties of food and liquids ingested into the oral cavity. We also tested if altered sensation was correlated with central sensitization of neurons in trigeminal

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subnucleus caudalis (Vc) and nucleus of the solitary tract (NTS) as assessed by Fos immunohistochemistry.

METHODS

Surgery

Experiments were conducted under a protocol that was approved by the UC Davis Institutional Animal Care and Use Committee. A total of 58 adult male Sprague-Dawley rats was used. Prior to surgery, all animals were tested in each of the behavioral paradigms (see below). Animals were divided into 5 experimental groups: (1) bilateral chronic constriction injury (CCI) of the lingual nerves, (2) bilateral CCI of the mental nerves, (3) partial tight ligation (Selzer model) of the mental nerves bilaterally, (4) sham exposure of the lingual nerves bilaterally and (5) sham exposure of the mental nerves bilaterally.

For all surgical procedures, rats were anesthetized with sodium pentobarbital (60 mg/kg ip). For lingual nerve CCI, the lingual nerves on each side were isolated central to their separation from the chorda tympani (CT) as described previously [5]. Two 5-0 chromic gut sutures (Ethicon, Somerville, NJ) were threaded underneath each lingual nerve and tied to produce a loose ligation. In sham-operated control rats the lingual nerve was exposed bilaterally in the same manner, but not ligated. For mental nerve CCI and partial tight ligation (Selzer), each mental nerve was surgically isolated near its exit from the mental foramen in the lower mandible. The nerves were either ligated loosely with chromic gut suture as above, or a needle with 7-0 silk surgical suture was passed through the nerve to tightly ligate approx. 40–50% of the nerve. The mental nerves were exposed in the same manner but not ligated in sham-operated control animals. The rationale for bilateral ligation of the lingual and mental nerves was that any spreading hyperalgesia resulting from unilateral nerve damage would likely be expressed bilaterally. In addition, the capsaicin drinking test involves bilateral stimulation of peri- and intraoral tissue by the ingested fluid, making it impossible to evaluate lateralized sensitivity differences if a unilateral nerve injury were produced.

Behavioral testing

Capsaicin 2-bottle drinking paradigm—Prior to surgery, and at 1 and 6 months post-surgery, rats were tested as previously described [22]. Briefly, rats were water-restricted for 22 hr and then transferred to a test box with two bottles, one with capsaicin and the other vehicle. The sipper tube of each bottle, and the floor of the metal box, were connected to a lickometer (DM-8; Columbus Instruments, Columbus OH) to simultaneously monitor licking from both bottles. Fluid consumption from each bottle, and numbers of licks, were recorded during a 2-hr access period. We recorded licks as an adjunct measure for comparison with volume consumption. We used an ascending series of capsaicin concentrations ranging from 0.1 ppm (0.33 μ M) to 10 ppm (33 μ M), with matching vehicle (0.005–0.5% ethanol in distilled water) in the other bottle. Each concentration of capsaicin was tested over 2 consecutive days, with the position of capsaicin- and vehicle-containing bottles switched each day to avoid positional preference. A 2-day period of unrestricted access to water intervened before testing the next capsaicin concentration. Capsaicin consumption (% of total volume consumed from both bottles) and lick counts for each group (except mental Selzer and sham groups) at each capsaicin concentration and time point were subjected to analysis of variance (ANOVA) with post-hoc Least-Significant Difference (LSD) tests.

Thermally- and mechanically-evoked head withdrawal—We used a modification of the method reported by Imamura et al. [12] to measure head withdrawal latency to chin

heating. Animals rested in a plastic restrainer fitted at the front end with a cardboard covering over the face to occlude vision with an opening to expose the chin and lower snout (Fig. 1). The rat's chin rested comfortably on a glass surface heated to 30°C $\pm$ 0.5°C. A radiant heat source (Plantar test 390; IITC, Woodland Hills CA) was positioned beneath the glass to direct a beam of light to the chin. The latency for withdrawal of the head was measured. Using the same restrainer, an electronic von Frey filament (IITC) was pressed against the rat's lower lip and the force at the moment of head withdrawal was measured. The incidence of attempts to bite the von Frey filament was also recorded. Between-group differences in thermal withdrawal latency, mechanical withdrawal threshold, and number of bites, was assessed by analysis of variance (ANOVA) with post-hoc Least Significant Difference (LSD) tests.

c-fos immunohistochemistry—After behavioral testing was completed, rats were anesthetized with sodium pentobarbital (60 mg/kg ip). The mouth was gently held open and 0.1 ml of capsaicin (100 ppm; 330 μ M) was placed on the anterior tongue bilaterally. This concentration was chosen because it, but not lower (10 ppm) concentrations, excited Vc neurons when applied to the tongue of barbiturate-anesthetized rats [6]. Underlying gingiva was covered with Parafilm® to avoid stimulation of tissue other than the tongue. Two hr after capsaicin application, the animal was perfused transcardially with 250 ml of phosphate-buffered saline (PBS) followed immediately by 500 ml of 4% paraformaldehyde. The brainstem was removed, postfixed, and transferred to 30% sucrose for cryoprotection. Brainstems were cut in 50 μ m frozen sections and immunohistochemically processed as described previously [4]. Cell nuclei displaying black Fos-like immunoreactivity (FLI) were counted bilaterally at 150 μ m intervals in the dorsomedial aspect of Vc, as well as in the NTS at levels caudal and rostral to the area postrema. The investigator counting FLI was blinded as to the experimental treatment. Counts of FLI in 5 representative sections with Vc and NTS were made in each animal. Selected sections were imaged with a digital camera (MP-5, Q-Imaging, Technical Instruments, San Francisco CA) using Scion Image QCapture-Pro 6.0 software (Q-Imaging). Counts of FLI in Vc and NTS were compared between treatment groups using ANOVA followed by post hoc LSD tests. $P < 0.05$ was considered to be significant.

RESULTS

Mental nerve injury

In general, there was a linear concentration-dependent increase in capsaicin avoidance (Fig. 2). One month after mental nerve CCI or partial tight ligation, there were no significant between-group differences, and no significant pre- vs. post surgery within-group differences (Fig. 2A). Six months post-surgery, there was a significant group effect with CCI exhibiting significantly greater capsaicin avoidance compared to pre-surgery (Fig. 2B, ● vs. ■). Total capsaicin consumption by the CCI group was significantly less 6 months post-surgery compared to pre-surgery consumption ($p < 0.01$). The proportion of licks of the capsaicin-containing bottles was also significantly less in the mental CCI group 6 (Fig. 2D; ●) but not 1 month post-surgery (Fig. 2C; ●), consistent with the consumption data.

Thermally- and mechanically-evoked head withdrawals, and incidence of bites, did not significantly change following mental nerve CCI or partial ligation, indicating an absence of thermal hyperalgesia or mechanical allodynia.

Lingual nerve CCI

At 1 and 6 mo post-surgery, there was a significant difference between the lingual CCI and sham-operated groups with total capsaicin consumption (Fig. 3A) and licks (Fig. 3C)

significantly less in the sham group. The enhanced capsaicin avoidance (i.e., chemical hyperalgesia) observed in the sham but not lingual CCI group might be explained by reduced afferent chemesthetic transmission in the injured lingual nerves that counterbalanced the sensitizing effect of surgery.

Both the lingual CCI and sham-operated groups exhibited significant decreases in thermal withdrawal latency and mechanical withdrawal threshold, and significant increases in biting, 3, 6 and 9 days post-surgery ($p < 0.05$, repeated-measures ANOVA with post-hoc LSD test) complete (thermal) or partial (mechanical) recovery at 180 days post-surgery. There was no difference between sham and lingual CCI groups for any of these measures. This indicates that the surgical procedure was sufficient to induce significant heat hyperalgesia and mechanical allodynia regardless of whether the lingual nerves were ligated or not. The data also suggest that lingual nerve surgery was more invasive than mental nerve surgery, since the mental sham-operated group did not exhibit thermal hyperalgesia, mechanical allodynia or increased biting.

Fos immunohistochemistry

Capsaicin produced FLI in the dorsomedial aspect of Vc as previously reported [4]. Examples from a mental nerve CCI and mental sham-operated rat are shown in Fig. 4A (left and right photomicrographs, respectively). Fig. 4B plots mean counts of FLI in dorsomedial Vc for each group. Notably, the mental nerve CCI group exhibited significantly higher FLI counts in Vc compared to Selzer or sham-operated groups (which were not different from each other). The lingual sham-operated group exhibited a mean FLI count in Vc that was not significantly different from that of the mental CCI group, but was significantly greater compared to lingual CCI and lingual sham groups (Fig. 4B). FLI counts in Vc were comparable in lingual CCI, mental nerve Selzer and mental sham groups. There were no significant differences among any of the treatment groups for counts of FLI in NTS (data not shown).

DISCUSSION

Mental nerve ligations

CCI of the mental nerves resulted in enhanced capsaicin avoidance at 6 but not 1-month post-surgery (Fig. 2), with good concordance between assessments of volume consumption and lick counts. Neither CCI nor partial tight ligation of the mental nerves resulted in thermal hyperalgesia or mechanical allodynia. These findings contrast with models of neuropathic pain involving CCI [3], partial tight ligation of the sciatic nerve [21] or tight ligation of lumbar spinal nerves [15], which usually result in mechanical allodynia and less commonly thermal hyperalgesia. The present absence of thermal hyperalgesia and mechanical allodynia is also at odds with reports that CCI of the rat's infraorbital nerve resulted in thermal hyperalgesia [12] and mechanical hypersensitivity [11], and that partial ischemic injury to the infraorbital nerve resulted in mechanical allodynia with a weaker and transient heat hyperalgesia [7], as well as reports of mechanical allodynia following bilateral mental nerve CCI [9, 10, 23] or transection of the inferior alveolar nerve [13] or inferior alveolar and mental nerves [17] that resulted in increased expression of TRPV1 and ATF3 [16]. We presently do not have a good explanation for the lack of allodynia following mental nerve CCI, and only suggest that chemesthetic sensory input generating chemofensive behavior [8] may be most sensitive to this nerve injury. A reduction in capsaicin avoidance was observed 6 months post-CCI indicating a delayed onset [20] for the development of chemical sensitization. Partial mental nerve ligation did not presently result in hyperalgesia or allodynia. While this is at odds with effects of partial sciatic nerve

ligation [21], we are not aware of similar studies of partial tight ligation of the mental nerves which was presently insufficient to produce measurable behavioral effects.

CCI, but not partial tight ligation of the mental nerves produced a chemical hyperalgesia as evidenced by significantly increased avoidance of capsaicin in the two-bottle paradigm. Presumably, CCI was more effective than partial tight nerve ligation in sensitizing the perioral skin to capsaicin. As the mental nerve innervates the lower lip and chin, increased avoidance of capsaicin may reflect an enhanced sensitivity to capsaicin when it contacts the lips during sipping, or possibly a spreading hyperalgesia into the oral cavity. Mental nerve CCI also resulted in a significant increase in FLI compared to sham controls in the dorsomedial aspect of Vc (Fig. 4) which receives chemonociceptive input from the tongue and perioral skin [4, 6]. This suggests that the constrictive nerve injury may have resulted in central sensitization of capsaicin-responsive Vc neurons to account for the increased avoidance of oral capsaicin.

We presently did not assess spontaneous pain, although there were no overt signs such as reduced feeding. It was previously reported that 4 wk after bilateral CCI of the mental nerves (using 2, 7-0 silk sutures), rats exhibited increased grooming behavior directed toward the mandibular area which was suggested to reflect spontaneous pain or dysesthesia [9, 10]. Unilateral CCI of the infraorbital nerve in rats also resulted in increased grooming behavior directed to the affected side as well as hyperresponsiveness to mechanical stimuli, suggestive of spontaneous pain or dysesthesia [24].

Lingual nerve CCI

Unilateral crush of the lingual nerve which travels with the CT in the tongue reduced thermal and mechanical head withdrawal thresholds in rats [14]. Our present study is the first, to our knowledge, to assess effects of lingual nerve damage central to its bifurcation from the CT. We wished to avoid damage to the CT, since gustatory nerve damage was proposed to disinhibit trigeminal nociceptive pathways, thereby leading to increased oral pain [1]. We observed that “sham” surgical exposure of the lingual nerves alone resulted in a significant and persistent enhancement of capsaicin avoidance (Fig. 3) as well as reduced thermal head withdrawal latency and mechanical withdrawal threshold. This suggests that the surgical trauma alone was sufficient to trigger thermal and chemical hyperalgesia and mechanical allodynia. The sensitization may be at least partly central, since the lingual sham group exhibited FLI in the dorsomedial aspect of Vc comparable to that of the mental nerve CCI group, and significantly greater compared to the lingual CCI or mental nerve sham-operated groups (Fig. 4B). The low level of FLI in the lingual nerve CCI group was consistent with a lack of enhancement of capsaicin avoidance in this group (Fig. 3). We speculate that constriction of the lingual nerves resulted in partial denervation to reduce the central transmission of chemesthetic information from the oral cavity. This effect may have negated the sensitization due to surgical trauma, resulting in no net change in capsaicin sensitivity. The nerve injury may have also affected descending modulatory control of trigeminal chemesthetic transmission, a possibility not addressed in the present study.

Acknowledgments

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Highlights

- A 2-bottle preference paradigm assessed capsaicin avoidance following nerve injury
- Constriction but not partial ligation of mental nerves enhanced capsaicin avoidance
- Mental nerve constriction increased Fos expression in trigeminal subnucleus caudalis

Fig. 1.

Behavioral assessment of thermal hyperalgesia and mechanical allodynia. Rats rested comfortably in a restrainer with a covering over the head and face that exposed the chin. The covering was sufficiently loose to allow limited upward and lateral head movements. The restrainer was angled so that the chin rested on a warmed glass surface and could be stimulated with radiant heat to elicit head withdrawals. This also allowed mechanical von Frey stimulation to record head withdrawal thresholds.

Fig. 2.

Effect of mental nerve injury on capsaicin avoidance. A: capsaicin consumption per group plotted vs. concentration 1 mo post-surgery. There were no significant between-group differences (ANOVA, $F_{2,417}=0.5$, $p>0.05$), and no significant pre- vs. post surgery within-group differences. B: As in A, 6 mo post-surgery. There was a significant group effect ($F_{3,697}=4.18$, $p<0.05$) with capsaicin consumption by the CCI significantly less compared to pre-surgery (*, $p<0.05$, LSD). C, D: % licks from the capsaicin-treated bottle vs. capsaicin concentration at 1 and 6 months post-surgery, respectively. The CCI group licked significantly less capsaicin (*, $p<0.05$) at 6 months compared to pre-surgery (Selzer and sham groups omitted).

Fig. 3. Effects of lingual nerve injury and sham surgery on capsaicin avoidance. A: graph as in Fig. 2A, 1 month post-surgery. There was a significant group effect ($F_{1,268}=15.3$, $p<0.001$). Total consumption for the sham (*; $p<0.001$) but not lingual CCI group, was significantly less vs. pre-surgery D: 6 month post-surgery results were similar, with a significant group effect ($F_{1,158}=8.1$, $p=0.005$). Total consumption by the sham ($p<0.001$) but not CCI group was significantly different vs. pre-surgery

FIG. 4.

Capsaicin-evoked FLI in Vc. A: photomicrographs of representative sections through Vc showing greater FLI in an animal with bilateral mental nerve CCI (left) compared to sham-operated control (right). B: graph plots mean counts of FLI/section for each treatment group. Error bars: SEM. There was an overall significant effect (ANOVA; $F_{4,48} = 5.4$, $p < 0.05$) with the sham lingual group significantly greater compared to lingual (*; $p < 0.04$, LSD) and mental CCI group significantly greater compared to mental Selzer or Sham mental groups (#; $p = 0.01$ and 0.007 , respectively). Counts of FLI in NTS did not differ significantly across groups (data not shown).