Effects of Pre-Emptive Morphine, Ibuprofen or Local Anesthetic on Fos Expression in the Spinal Trigeminal Nucleus Following Tooth Pulp Exposure in the Rat

Maria T. Locher-Claus, DDS, MS, Ty E. Erickson, DDS, M.S., Alan S. Law, DDS, PhD, William T. Johnson, DDS, MS, and G.F. Gebhart, PhD

Abstract

In the present study, we used Fos expression as an index of nociceptive input to the spinal trigeminal nucleus after exposure of the coronal pulp tissue of maxillary right first molars and examined the effects of pretreatment with an opioid, a nonsteroidal anti-inflammatory drug or a local anesthetic before pulp exposure. Exposure of the tooth pulp produced a significant increase in Fos-like immunoreactivity in the superficial laminae of subnucleus caudalis; pretreatment with a control infiltration injection of saline directly above the maxillary molar 30 min before pulp exposure had no effect on Fos expression. Pretreatment with morphine 30 min before pulp exposure dose-dependently (2.5, 5, and 10 mg/kg subcutaneously) reduced Fos expression in subnucleus caudalis whereas pretreatment with ibuprofen (10–100 mg/kg subcutaneously) did not significantly affect Fos expression. Local anesthetic pretreatment was effective in reducing Fos expression only for the long acting bupivacaine; lidocaine without and with epinephrine (1:100,000) failed to significantly affect Fos expression. These results suggest that pre-emptive opioid treatment can decrease postoperative central nervous system changes associated with tooth pulp injury, and therefore, may decrease postoperative pain. Given the effects of local anesthetic on Fos expression, a combination of long acting local anesthetic with pre-emptive opioid would likely be most efficacious in decreasing postoperative dental pain.

Key Words

c-Fox, morphine, ibuprofen, local anesthetics, trigeminal nucleus caudalis

Address requests for reprints to Dr. Gebhart, Ph.D., Department of Pharmacology, Bowen Science Building, Carver College of Medicine, The University of Iowa, Iowa City, IA 52242; E-mail address: gf-gebhart@uiowa.edu.

Although significant advances have been made in prevention of dental caries and resultant pulpal tissue inflammation, there remains a significant percentage of the population that suffers from pulpal pathosis and pain. Several studies have shown that postoperative pain after root canal therapy is common (1–3). The presence of preoperative pain is a risk factor for the occurrence of postendodontic pain; patients with moderate to severe pain report greater pain for 3 days after endodontic cleaning and surgery (4) and 55% of patients with asymptomatic pulpitis complain of pain 24 h after the local anesthetic has worn off (5). Accordingly, strategies to reduce postoperative pain remain a priority in dentistry.

Tissue injury typically results in local inflammation and altered sensations to noxious and sometimes non-noxious stimuli. The altered sensations represent hyperalgesia and arise from changes in the excitability of nociceptors (e.g. receptors for tissue-damaging, noxious stimuli) and in the second order neurons in the central nervous system upon which nociceptors terminate. These processes are termed nociceptor sensitization and central sensitization, respectively. The principal objective in management of postinjury pain is prevention or reduction of central sensitization, a mechanism by which peripheral input from nociceptors and non-nociceptors can be amplified and include altered sensations in adjacent, uninjured tissue.

The purpose of this study was to examine potential mechanisms by which peripheral and/or central sensitization could be reduced, thus reducing postprocedure pain and pain medications. To this end, we examined pharmacologic modulation of peripheral input into the brainstem trigeminal nucleus using the expression of Fos as a marker of neuronal activation. Fos is a nuclear phosphoprotein encoded by the immediate early gene c-fos and has been used widely as a marker for central neuronal activity after noxious peripheral stimulation (6–9). The expression of Fos in the spinal cord and brain after peripheral noxious stimulation matches known anatomic pathways that convey nociceptive information, and thus has been used to map pathways of neuronal activation after noxious peripheral stimulation. Because the trigeminal nerve is primarily responsible for detection, transmission, and encoding of painful stimuli in the orofacial reigon (10), and because nociceptors in tooth pulp are principally associated with A delta and C fibers, we examined Fos expression in the spinal trigeminal nucleus complex (11, 12).

The main pharmacologic strategies for management of dental pain are local anesthetics (typically given before a procedure) and nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids given after procedures. Opioids are the most efficacious analgesic drugs available, but are used sparingly in dentistry despite evidence that opioids given in advance of a procedure (i.e. pre-emptively) effectively reduce postprocedure pain and central sensitization (13, 14). NSAIDs are widely used in dentistry, principally for postprocedure control of pain and/or inflammation. Ibuprofen, a popular and effective NSAID, has been evaluated in numerous clinical trials. For example, Cooper et al. (15) reported that 400 mg ibuprofen was consistently more effective in the reduction of postoperative dental pain when compared to 650 mg of aspirin, 600 mg acetaminophen, or both aspirin and acetaminophen combined with 60 mg codeine. Generally, however, other clinical studies testing pre-emptive effects of NSAIDS have not revealed significant differences between preoperative and postoperative dosing (16–18). Local anesthetics are an intrinsic part of clinical practice in dentistry; over 300 million car-

tridges are administered by dentists in the United States every year (19). Although not widely investigated, Gordon et al. (20) reported that preoperative local anesthetic and postprocedure bupivacaine resulted in reduced need for postoperative pain medications, suggesting that postoperative pain can be reduced with local anesthetic, thus reducing central sensitization.

In the present report, we examined Fos expression in the rat spinal trigeminal nucleus complex after tooth pulp exposure as an index of central neuronal excitation, and evaluated the efficacy of pre-emptive local anesthetic, NSAID, and opioid treatments.

Materials and Methods

Rats (150–250 g; Harlan Sprague-Dawley, Indianapolis, IN) were housed two per cage. They were allowed to acclimate for 1 wk before use, and were fed a standard diet of Teklad Rat Chow and water ad libitum and kept on a 12 h light-dark cycle (lights on 6 A.M.). The experimental protocols were approved by the Animal Care Use Review Committee at The University of Iowa.

Rats were deeply anesthetized with halothane (via a nose cone) and placed in either an oral restraining rig for visualization and access to the teeth or held by an assistant. The tongue was gently retracted by an assistant, and with the aid of a dissecting microscope, the coronal pulp of one maxillary molar was exposed (as evidenced by hemorrhage) using a high-speed dental hand piece and air water coolant.

Treatment Groups

Rats (four per group) received either morphine (2.5, 5, or 10 mg/kg in sterile, preservative-free saline) or the active S-enantiomer of ibuprofen (25, 50, or 100 mg/kg) subcutaneously, local anesthetic (0.3 ml, 2% lidocaine plain, 2% lidocaine with 1:100,000 epinephrine, or 0.5% bupivacaine with 1:200,000 epinephrine) in the vestibule, or vehicle (saline for morphine, methanol for S-ibuprofen and saline, pH 4.6 for local anesthetics) 30 min before pulp exposure. As a control for treatment with the active isomer of ibuprofen, one group of four rats was treated subcutaneously with 100 mg/kg of the inactive R-enantiomer of ibuprofen. Three groups of rats ($n = 12$ total, distributed across treatment protocols) were anesthetized as described above but received no pretreatment or pulp exposure (i.e. sham procedure groups). Two additional rats were anesthetized as described above and received a needle stick in the vestibule (without injecting anything), but no pulp exposure, as an additional control. There were no significant differences in Fos expression in the brainstem between sham and control procedures in the absence of pulp exposure. To establish the selectivity of action of morphine at the mu opioid receptor, one group of four rats was pretreated with naltrexone (2.5 mg/kg subcutaneously; Lyphomed, Melrose Park, IL) 5 min before morphine (10 mg/kg) administration.

Two hours after tooth preparation (or sham/control procedures), rats were deeply anesthetized with pentobarbital (100 mg/kg, ip 2.5% sodium thiopental) and transcardially perfused with 100 ml saline followed by 400 ml 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.3). The brainstem containing the trigeminal nucleus (an approximate 15 mm long piece of tissue) was removed, postfixed in 4% paraformaldehyde for 2 h at 4°C. After the tissue was equilibrated in 30% sucrose for 24 h, cryostat sections (40 μm) were cut serially between subnuclei oralis and caudalis (from ~ -11 to -15 mm relative to Bregma; 21); every 6th section was reserved and stored in 0.1M PB. Free-floating sections were rinsed in 0.1M PB and preincubated for 30 min in 3% normal goat serum (NGS) in phosphate-buffered saline (PBS) at pH 7.4. To block endogenous peroxidase activity, the sections were placed in 5% H_2O_2 for 10 min before preincubation (to quench peroxidase activity). Sections were incubated for 24 h at 4°C with a primary antibody for Fos (1:1,000) in 1% NGS (with 0.75% Triton X-100 in PBS). After two 10 min washes in PB, sections were washed with 3% NGS for 30 min, incubated for 1 h at room temperature with the secondary antibody (biotinylated goat anti-rabbit-IgG, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and diluted in 1% NGS with 0.75% Triton X-100. After two 10 min rinses with PBS and a 30 min rinse with 3% NGS, sections were incubated with ABC solution (peroxidase-conjugated egg-white avidin, Jackson ImmunoResearch Laboratories). Reaction products were visualized with diaminobenzidine DAB (0.05%)-H_2O_2 (0.01%) in 0.1M PB for 5 to 10 min. Sections were then rinsed with PB and mounted, dehydrated, and cover-slipped. Some sections adjacent to those reserved for evaluation of Fos expression were reacted without the primary antibody, or with preimmune serum, as negative controls.

Tissue sections were then evaluated by an examiner blinded as to treatment for the distribution of Fos-li cells in the spinal trigeminal nucleus. An imaging program was used to display light microscopic histological sections and only cells with a characteristic darkly stained nucleus were considered Fos positive. The total number of cells in the left and right subnuclei oralis, interpolaris, and caudalis, reported as the mean $\pm$ SEM, was determined for each rat. Differences between experimental groups were tested using an ANOVA followed by pairwise comparisons when appropriate using either Student's *t* or Scheffe tests; statistical significance was set at $p < 0.05$.

Results

When examined 2 h after tooth pulp exposure, there was a statistically significant increase in Fos expression in the trigeminal nucleus. This increase was restricted to the side of the brainstem ipsilateral to the pulp-exposed tooth and most prominent in the subnucleus caudalis. Fos-like immunoreactive (li) neurons were distinguished by their darkly stained nuclei and unlabeled nucleoli through light microscopy at magnifications of 4×, 10×, and 20×. Neurons expressing Fos-li in the trigeminal nucleus in naïve rats or those following a sham procedure were infrequent, as in a study by Byers et al. (22). Figure 1 shows photomicrographs of Fos expression in the brainstem in the absence of (Fig. 1A, C) and 2 h after tooth pulp exposure (Fig. 1B, D).

After tooth pulp exposure the mean numbers of Fos-li nuclei in the ipsilateral trigeminal nucleus were: subnucleus oralis, 4.3 $\pm$ 0.9; sub-

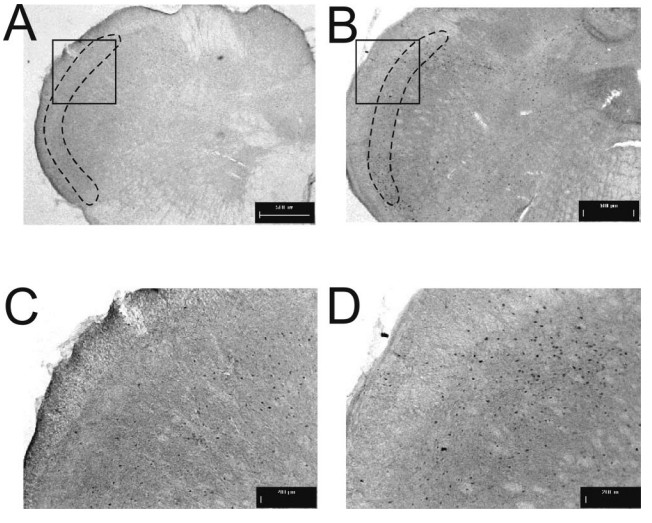

Figure 1. Fos-li in laminae I and II of subnucleus caudalis. Photomicrographs showing that sham (A, C; anesthesia, but no pulp exposure) treatment produces very few Fos-li expressing cells whereas pulp exposure (B, D) produces a significant number of Fos-li expressing cells in subnucleus caudalis.