NOS Inhibitor Antagonism of PGE$_2$-Induced Mechanical Sensitization of Cutaneous C-Fiber Nociceptors in the Rat

XIAOJIE CHEN AND JON D. LEVINE

National Institutes of Health Pain Center and Departments of Anatomy, Medicine, and Oral and Maxillofacial Surgery, Division of Neuroscience, University of California, San Francisco, California 94143-0440

Chen, Xiaojie and Jon D. Levine. NOS inhibitor antagonism of PGE$_2$-induced mechanical sensitization of cutaneous C-fiber nociceptors in the rat. J. Neurophysiol. 81: 963–966, 1999. Prostaglandins, metabolites of arachidonic acid, released during tissue injury and inflammation sensitize primary afferent nociceptors. While it has been suggested that this effect on nociceptors is mediated mainly via the cAMP second messenger system, recent evidence suggests that nitric oxide (NO) is also involved in peripheral pain mechanisms. To test the hypothesis that NO contributes to the sensitization of nociceptors to mechanical stimuli induced by hyperalgesic prostaglandins, we compared von Frey hair mechanical threshold as well as the response evoked by 10-s sustained threshold mechanical stimulation before and after injection of prostaglandin E$_2$ (PGE$_2$) alone, and NOS inhibitor N$_G$-methyl-L-arginine (L-NMA) or its inactive stereoisomer N$_G$-methyl-d-arginine (d-NMA) plus PGE$_2$, adjacent to the receptive field of C-fiber nociceptors. The reduction of mechanical threshold and increase in number of action potentials to sustained mechanical stimulation induced by intradermal application of PGE$_2$ was blocked by L-NMA, but not d-NMA. It is suggested that NO contributes to nociceptor sensitization induced by hyperalgesic prostaglandins.

INTRODUCTION

Tissue injury and inflammation result in the release of prostaglandins and other inflammatory mediators, some of which cause pain and hyperalgesia. The peripheral administration of prostaglandin E$_2$ (PGE$_2$), the most thoroughly studied hyperalgesic inflammatory mediator, produces mechanical hyperalgesia in animals (Ferreira 1981; Ferreira et al. 1978; Taiwo and Levine 1988) and humans (Moncada et al. 1978), and sensitizes primary afferent nociceptors to mechanical stimuli (Ahlgren et al. 1997; Martin et al. 1987; Schaible and Schmidt 1988).

Nitric oxide (NO), a second messenger produced from L-arginine by the calcium-calmodulin-requiring enzyme NO synthase (NOS), contributes to a variety of biological functions (Brenman and Bredt 1997; Moncada and Higgs 1993). Increasing evidence suggests that NO is involved in both central (Machelska et al. 1997; Moore et al. 1991; Salter et al. 1996) and peripheral (Carrado et al. 1992; Haley et al. 1992; Holthusen and Arndt 1994, 1995; Ialenti et al. 1992; Kingdgen-Milles and Arndt 1996; Larson and Kitto 1995; Lawand et al. 1997; Thomas et al. 1996) pain mechanisms.

Although the central effect of NO is to enhance nociception, the peripheral effect is still controversial. Whereas an antinociceptive effect of NO in the periphery was reported (Duarte et al. 1992; Kamei et al. 1994; Kawabata et al. 1992; Lorenzetti and Ferreira 1996), other studies have demonstrated that local application of NO solutions produces pain (Holthusen and Arndt 1994, 1995). Moreover, although behavioral studies have shown that NOS inhibitors block hyperalgesia (Aley et al. 1998; Lawand et al. 1997), there is thus far no evidence showing the role of NO in nociceptor sensitization by inflammatory mediators. In this study, we used a NOS inhibitor N$_G$-methyl-L-arginine (L-NMA) and its inactive stereoisomer N$_G$-methyl-d-arginine (d-NMA) to explore the role of NO in mechanical sensitization of cutaneous C-fiber nociceptors by PGE$_2$, a direct-acting hyperalgesic inflammatory mediator (England et al. 1996; Gold et al. 1996).

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley rats (280–380 g) from Bantin and Kingman (Fremont, CA) were anesthetized with pentobarbital sodium (initially 50 mg/kg ip, with additional doses given throughout the experiment to maintain areflexia) and their trachea were cannulated. Anesthetized rats were positioned supine, with the left hindlimb secured at the ankle and the skin on the medial aspect of the thigh incised and retracted to form a pool which was filled with warm mineral oil. The saphenous nerve was then exposed and dissected free for electrophysiological studies. At the end of experiments, rats were killed with an overdose of pentobarbital. Animal care and use conformed to NIH guidelines for care and use of laboratory animals.

Electrophysiological recording

Extracellular recordings were made from C-fiber afferents in the fascicle of the saphenous nerve in vivo. This nerve was dissected free from accompanying blood vessels at two sites 20–32 mm apart. At the distal site, bipolar silver-wire stimulating electrodes were placed under the nerve to allow electrical stimulation of the nerve, to determine conduction velocity. At the proximal site, a portion of the nerve was desheathed and fine filaments were dissected from the nerve with sharpened jeweler’s forceps, and placed on a silver-wire recording electrode. The conduction velocity of a fiber was determined by dividing the distance between the stimulating and recording electrodes by the latency from the stimulus to the first action potential. The nerve was crushed proximal to the recording site to block the conduction of action potentials to the spinal cord, which would otherwise evoke hindlimb reflexes. Fascicles were teased from the nerve, with the use of a voltage amplitude (window) discriminator, until a single C-fiber was activated at a given receptive field or when a single unit could be easily distinguished by the height of its action potential. There were between one and three C-fibers per fascicle. Fibers with conduction velocities <2 m/s were classified as C-fibers and used in experiments.
The action potential corresponding to the C-fiber whose receptive field was identified was determined by the latency delay technique, in which a mechanically induced orthodromic spike produced a delay in the electrically induced orthodromic spike (Handwerker et al. 1991; Iggo 1958).

Mechanical stimuli

The mechanically sensitive receptive field of each C-fiber was located and mapped using a 75 g von Frey hair (VFH). This intensity of VFH was previously demonstrated to be able to activate more than 95% of all mechanically sensitive C-fibers (Ahlgren et al. 1992). The location on the C-fiber’s receptive field that was most sensitive to mechanical stimulation was marked by a felt tip pen and was the target for all further mechanical stimulation. Mechanical threshold was determined by the use of stimuli of ascending and descending intensity with calibrated VFH (Ainsworth; London, UK) and defined as the lowest force that elicited two or more spikes within 1 s, in ≥6 of 10 trials. Sustained threshold stimulation was performed using a calibrated VFH. The VFH was placed by hand on the receptive field for 10 s. The C-fiber activity was recorded on video tape (Vetter; Rebersburg, PA). Triplicate trials of sustained stimulation were given at 1 min intervals. Acute and sustained (10 s) threshold intensity stimulation was performed before and 3 and 5 min after the intradermal injection of agents.

Drug injection

Injection of saline, PGE₂ (100 ng), and L-NMA (1 µg) plus PGE₂ (100 ng) or D-NMA (1 µg) plus PGE₂ (100 ng) was performed 1 mm away from the center of the fiber’s mechanical receptive field. All injections were in a volume of 2.5 µl. PGE₂ and L-NMA or D-NMA were separated in the injection syringe by a tiny air bubble to prevent mixing before injection, and injected in the order of L-NMA or D-NMA then PGE₂. PGE₂ (Sigma; St. Louis, MO) was dissolved in ethanol and diluted to a final concentration with saline; and the final ethanol concentration was <1%. L-NMA and D-NMA (both Sigma; St. Louis, MO) were dissolved in saline to a concentration of 1%, as their stock solutions.

Analysis of data

Data are expressed as means ± SE. Statistical analyses were done using analysis of variance (ANOVA), t-test, χ² test and Wilcoxon Signed Rank test, as appropriate. Differences were considered significant at the P < 0.05 level.

R E S U L T S

Effect of NOS inhibitor on PGE₂-induced reduction of mechanical threshold

Before injection of saline, the range and average of the baseline mechanical threshold was 0.22–12.6 g and 1.93 ± 0.53 g (n = 38), respectively; before injection of PGE₂ the range and average of the baseline mechanical threshold of saline and PGE₂ treated group was 0.22–12.6 g and 1.89 ± 0.33 g (n = 38), respectively. The range and average of the baseline mechanical threshold of L-NMA group was 1.0–4.75 g and 2.02 ± 0.46 g (n = 8). The range and average of the baseline mechanical threshold of D-NMA group was 1.66–4.75 and 2.82 ± 0.60 g (n = 8). There were no statistically significant differences between baseline mechanical thresholds for these groups of C-fibers (all P > 0.05). Intradermal injection of PGE₂ (100 ng) alone decreased the mechanical threshold in 18 of 38 (47.4%) C-fibers whereas intradermal injection of saline decreased the mechanical threshold in only 5 of 38 (13.2%) C-fibers (P < 0.005, Fig. 1A). Following intradermal injection of L-NMA (1 µg) plus PGE₂ (100 ng), the mechanical threshold decreased in only 1 of 8 (12.5%) C-fibers and was unchanged in 7 of 8 (87.5%) C-fibers (P > 0.05, Fig. 1A). However, following application of D-NMA (1 µg) plus PGE₂ (100 ng), the mechanical threshold decreased in 4 of 8 (50%) C-fibers and was unchanged in 4 of 8 (50%) C-fibers (P < 0.05, Fig. 1A).

Effect of NOS inhibitor on PGE₂-induced change in response to sustained threshold mechanical stimulation

The average number of action potentials elicited by sustained threshold mechanical stimulation before saline and PGE₂ was 17.0 ± 3.5 and 15.1 ± 3.6 (both n = 15, imp/10 s), respectively. After intradermal injection of PGE₂ (100 ng), the number of action potentials was increased in 12 of 15 (80.0%) C-fibers whereas the number of action potentials was increased in only 5 of 15 (33.3%) C-fibers after saline. The increase in
number of action potentials after PGE$_2$ was statistically significant ($P < 0.005$, Fig. 1B). Before intradermal injection of L-NMA (1 µg) plus PGE$_2$ (100 ng) the average number of action potentials was 18.4 ± 5.4 (imp/10 s, $n = 8$) and it increased in only 2 of 8 (25%) C-fibers. However, before injection of d-NMA (1 µg) plus PGE$_2$ (100 ng) the average number of action potentials was 14.5 ± 2.1 (imp/10 s, $n = 8$) and it increased in 5 of 8 (62.5%) C-fibers. The increase in number of action potentials produced by PGE$_2$ in the presence of d-NMA but not L-NMA was statistically significant ($P < 0.05$ and $P > 0.05$, respectively; Fig. 1B).

**Discussion**

Accumulating evidence suggests that peripheral administration of PGE$_2$ sensitizes nociceptors (Ahlgren et al. 1997; Handwerker 1975; Kumazawa et al. 1993; Martin et al. 1987; Mizumura et al. 1993; Schaible and Schmidt 1988) by direct action on the nociceptor (Englund et al. 1996; Gold et al. 1996), and that this effect is mediated by the cAMP second messenger system (Feferre and Nakamura 1979; Taiwo and Levine 1991; Taiwo et al. 1989). However, recent studies have shown that intracutaneous application of another signaling molecule, NO, also evokes pain in humans (Holthuisen and Arndt 1994, 1995). In behavioral studies local administration of NOS inhibitors blocked hyperosmolar solution-induced (Kindgen-Milles and Arndt 1996), bradykinin-induced pain (Carrado et al. 1992; Kindgen-Milles and Arndt 1996), formalin-induced mechanical hyperalgesia and acetic acid-induced abdominal writhing (Moore et al. 1993), neuropathy-induced thermal hyperalgesia (Thomas et al. 1996), PGE$_2$-induced mechanical hyperalgesia (Aley et al. 1998), and carrageenan-induced thermal hyperalgesia (Lawand et al. 1997). Our results demonstrate that the NOS inhibitor, L-NMA, blocks PGE$_2$-induced reduction of C-fiber mechanical threshold and increase in number of action potentials evoked by sustained threshold stimulation. This result is consistent with the recent finding that NOS inhibitors block the allodynia induced by intrathecal administration of PGE$_2$ (Minami et al. 1995), which suggests that the hyperalgesia induced by PGE$_2$ acting at both central and peripheral terminals of nociceptors is mediated by NO.

Some studies have shown a peripheral antinociceptive effect of NO (Duarte et al. 1992; Kamei et al. 1994; Kawabata et al. 1992; Lorenzetti and Ferreira 1996), which may be the result of high doses of l-arginine (10 µg and 2 mg, respectively) because in the study that evaluated low and high doses, injection of the high dose (≥10 µg l-arginine) attenuated nociception whereas injection of the low dose (0.1–1 µg) enhanced nociception (Kawabata et al. 1992). In addition, administration of a NOS inhibitor is fundamentally different from administration of a NO precursor; for example, l-arginine may be metabolized to kyotorphin, an antinociceptive endogenous neuropeptide (Ueda et al. 1987).

Although NO often produces several of its effects by activation of guanylyl cyclase (Brenman and Bredt 1997; Moncada and Higgs 1993), we recently have shown that the contribution of NO to PGE$_2$-induced hyperalgesia is not mediated by a guanylyl cyclase-dependent modulation of the cAMP second messenger system (Aley et al. 1998). Because PGE$_2$ is a direct-acting hyperalgesic agent and NO is present in small-diameter dorsal root ganglion neurons (Qian et al. 1996; Zhang et al. 1993) we suggest that the NOS mediating PGE$_2$-induced sensitization of C-fibers is in the peripheral terminal of these neurons. Nevertheless, we cannot exclude other potential sources of NO. For example, prostaglandin-induced aqueous flare (Hiraki et al. 1996) and hyperosmolar solution-and bradykinin-induced pain (Kindgen-Milles and Arndt 1996) may be mediated by NO released from vascular endothelium.

In conclusion, the data for the present study supports the suggestion that PGE$_2$-induced decrease in mechanical threshold and increase in response to sustained threshold mechanical stimulation in C-fiber nociceptors is dependent on the NO second messenger system.

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Present address and address for reprint requests: J. D. Levine, NIH Pain Center, Box 0440, C-555, University of California, San Francisco, CA 94143-0440.

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