NMDA Receptor Mechanisms Contribute to Neuroplasticity Induced in Caudalis Nociceptive Neurons by Tooth Pulp Stimulation

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Chiang, Chen Yu, Soo Joung Park, Chun L. Kwan, James W. Hu, and Barry J. Sessle. NMDA receptor mechanisms contribute to neuroplasticity induced in caudalis nociceptive neurons by tooth pulp stimulation. J. Neurophysiol. 80: 2621–2631, 1998. We recently demonstrated that application of mustard oil (MO), a small-fiber excitant and inflammatory irritant, to the rat maxillary molar tooth pulp induces significant and prolonged increases in jaw muscle electromyographic (EMG) activity that are suggestive of central sensitization. Because small-fiber afferents, including pulp afferents, access nociceptive neurons in trigeminal (V) subnucleus caudalis, this study examined whether pulpal application of MO induces neuroplastic changes in caudalis nociceptive neurons (wide dynamic range and nociceptive specific) and whether central N-methyl-D-aspartate (NMDA) receptor mechanisms are involved in these MO-induced neuroplastic changes. After pretreatment with vehicle (saline, 10 μl i.t.) to the surface of the medulla, the pulpal application of MO to the maxillary molar tooth pulp produced a significant increase in neuronal spontaneous activity, a significant expansion of the pinch and/or tactile mechanoreceptive field (RF), a significant decrease in mechanical threshold, and significant increases in neuronal responses to graded pinch stimuli. Compared with vehicle-treated rats, pretreatment with the NMDA receptor antagonist MK-801 (10 μg/10 μl i.t.) followed by MO application to the pulp in another group of rats significantly reduced or abolished these MO-induced neuroplastic changes in nociceptive neurons. In another group of rats pretreated with saline (intrathecally), mineral oil application to the pulp did not show any significant changes in spontaneous activity or RF properties over the 40-min observation period. The pulpal application of MO in other rats (pretreated with saline, intrathecally) did not produce any significant neuroplastic changes in caudalis low-threshold mechanoreceptive neurons. These results indicate that the MO-induced activation of molar pulp afferents can produce profound NMDA receptor-related neuroplastic changes in caudalis nociceptive neurons. Such neuroplastic changes may contribute to the hyperalgesia and spread of pain that can be associated with pulpal inflammation.

INTRODUCTION

Toothache is one of the most common pain conditions, but the pathophysiological mechanisms underlying the pain and the pulpal inflammation that are usually associated with toothache are still unclear. Very little information is available of the central mechanisms associated with pulpal inflammation because most of the studies investigating these mechanisms focused on peripheral processes of acute dental pain and used a variety of pulp stimuli, including mechanical, thermal, electrical, and chemical (Byers 1984, 1992; Närhi et al. 1992; Pasley et al. 1992; Sessle 1987). The cutaneous application of mustard oil (MO), a small-fiber excitant and inflammatory irritant, was found to induce local inflammation and pain and hence is now widely used to study central as well as peripheral nociceptive mechanisms related to cutaneous, deep, and visceral pain (Al-Chaar et al. 1996; Handwerker et al. 1991; Koltzenburg et al. 1994; Woolf 1992; Woolf and Wall 1986; Yu et al. 1993). We recently demonstrated that application of MO to the rat maxillary molar tooth pulp induces pulpal inflammation and significant and prolonged increases in electromyographic (EMG) activity of jaw muscles; this latter effect appears to be due to activation of molar pulp afferents because MO application to the molar tooth in which the pulp was extirpated does not induce such EMG changes (Hu et al. 1997; Sunakawa et al. 1993). Because trigeminal (V) subnucleus caudalis receives substantial tooth pulp afferent inputs and is considered to be an important brain stem relay of orofacial nociceptive information (see Dubner and Bennett 1983; Gobel et al. 1981; Hannam and Sessle 1994; Hu et al. 1981; Sessle 1987, 1996; Takeamura et al. 1991; Yokota 1985), an aim of this study was to test whether pulpal application of MO induces neuroplastic changes in nociceptive neurons in caudalis. Also, because the N-methyl-D-aspartate (NMDA) receptor ion-channel blocker MK-801 can effectively block MO-induced increases in EMG activity of jaw muscles (Yu et al. 1996) and was implicated in neuroplastic changes that can be induced by peripheral injury or inflammation in central nociceptive pathways and that are thought to reflect a “central sensitization” (Coderre et al. 1997; Dubner 1991; Dubner and Basbaum 1994; Willis 1993; Woolf 1992), an additional aim was to test whether central NMDA receptor mechanisms are involved in any MO-induced neuroplastic changes. The data were partly published in abstract form (Chiang et al. 1997b; Kwan et al. 1997).

METHODS

Animals preparation and recording

The study was carried out on 35 Sprague-Dawley male rats (250–410 g). The methods used for animal preparation, anesthesia, and neuronal recording and classification were similar to those detailed previously (Chiang et al. 1994, 1997b; Hu 1990), and so only a brief description of these methods follows. The animals were anesthetized by a mixture of chloralose (50 mg/kg ip) and urethane (1 g/kg ip). A tracheal cannula was inserted, and the left external jugular vein was cannulated. The coronal pulp of the right maxillary first molar was exposed through an occlusal cavity with a dental high-speed drill and a small-diameter (0.5 mm) bur, and
the cavity was temporarily filled with a saline-soaked cotton pellet. Then the animal was placed in a stereotaxic apparatus, the caudal medulla was surgically exposed, and the dura overlying subnucleus caudalis was removed. After a supplemental dose of urethane (200–300 mg/kg iv), the animal was immobilized with gallamine triethiodide (initial dose 35 mg/kg, followed by 14 mg/h iv) and artificially ventilated throughout the experimental session. Heart rate, percentage expired CO2, and rectal temperature were continuously monitored and maintained at physiological levels of 333–430/min, 3.5–5%, and 37–37.5°C, respectively.

Single neuronal activity was recorded extracellularly by a tungsten microelectrode from histologically confirmed sites in laminae III–VI of caudalis. The recording sites were 1.2–1.6 mm posterior to the obex, 1.2–1.4 mm from the midline, and down to a depth of 1.3 mm below the medullary surface. A wide range of graded mechanosensory stimuli (brush, pressure, and pinch) and noxious radiant heat (51–53°C) was used to classify units according to previously outlined criteria (Chiang et al. 1994; Hu 1990) into wide dynamic range (WDR) or nociceptive-specific (NS) neurons, or low-threshold mechanoreceptive (LTM) neurons or primary afferents. Nociceptive as well as LTM neurons were studied.

**Mechanical and electrical stimulation**

For each neuron, the mechanoreceptive field (RF) size and location were carefully delineated with a brush (for tactile RF) and/or a blunt forceps (for pinch RF), and the extent of the cutaneous RF was then outlined on life-size drawings of the rat’s head. The areas of the different RF components (touch and pinch) were measured by a computer-aided device (SigmaScan, Jandel, CA) (Chiang et al. 1997b; Yu et al. 1993). Single-pulse electrical stimuli were applied to the center (or most mechanically sensitive part) of the RF to activate A-fiber afferent inputs (0.2 ms, <1 mA) and C-fiber afferent inputs (2 ms, <5 mA). Spontaneous activity was also initially recorded for 2 min. The mechanical threshold of the unit was determined with the use of a set of von Frey nylon monofilaments (0.1–92 g) applied to the center of the neuronal RF. The threshold was defined as the monofilament with the lowest value that elicited one or two spikes per trial in at least five of six trials. After drug administration, the threshold was retested at different time points, beginning with a filament one value below that of the previous threshold. If a marked drop in threshold occurred, the new value was determined by the use of a descending and then an ascending series of monofilaments. For LTM neurons, the responses to low-intensity stimuli (each for 2 s at 2-s intervals; 6 trials) were determined. The pinch stimulus was quantitatively delivered by means of a modified forceps with an attached strain gauge that monitored force levels up to 600 g/mm². The responses to graded pinch stimuli (50, 100, and 200 g in ascending order, each for 3 s at 13-s intervals) applied to the RF were assessed in NS neurons. A less intense and graded series of mechanical stimuli (20, 40, and 80 g) was used for WDR neurons. These various determinations for each of these three classes of neurons were made before and after MO application, at the time intervals specified subsequently.

**Chemical application**

To establish an acute chemical irritant-induced pulpal inflammation, MO (allyl isothiocyanate, 95%; Aldrich Chemical, CA) was applied locally to the exposed tooth pulp (see RESULTS). A similar application of commercially available mineral oil served as control. To test for possible NMDA receptor mechanisms, a freshly prepared 0.1% solution of (+)-MK-801 (Research Biochemicals, Natick, MA; 10 μg/10 μl), a noncompetitive blocker of the NMDA receptor, was applied as a pretreatment to the medullary surface close to the recording electrode (intrathecally). As a control for MK-801, its vehicle (saline, 10 μl) was similarly applied intrathecally.

**Experimental paradigm**

Only one neuron was studied in each experiment. During the neuron recording, each animal received pretreatment with saline or MK-801 (intrathecal) followed by the pulpal application of MO or mineral oil. This study involved four groups of animals: two groups received saline and MO (one for testing of nociceptive neurons and the other group for testing of LTM neurons); another group received MK-801 and MO, and another group received saline and mineral oil (both these groups were used for testing of nociceptive neurons only). A similar experimental paradigm was applied for all four groups; after a nociceptive or LTM neuron was identified by mechanical, thermal, and electrical stimuli, the neuronal spontaneous activity and RF properties including RF size, mechanical threshold, and responses to pinch stimuli were determined and served as baseline values. A bolus (10 μl) of either saline or MK-801 was applied with a Hamilton syringe to the medullary surface overlying subnucleus caudalis ipsilateral to the exposed maxillary molar pulp. The dose of MK-801 (10 μg/10 μl i.t.) was in accordance with that shown to be effective for antinociception in a recent study (Coderre and Van Empel 1994). Five minutes later, the determination of spontaneous activity and RF properties was repeated. Then the saline-soaked cotton pellet in the molar was carefully replaced by a segment of endodontic paper point (1 mm) soaked with either MO (95%; 0.2 μl) or mineral oil (0.2 μl), and the occlusal cavity was quickly sealed by application of CAVIT (ESPE, D-8031, Germany). Thereafter, the spontaneous activity and RF properties were determined at 5-min intervals for the first 10 min and at 10-min intervals for the subsequent 40-min observation period after MO or mineral oil application.

**Histological and statistical analyses**

Recording sites were marked by electrolytic lesions (anodal current 8 μA, 10 s) at the termination of each experiment and verified with conventional histological procedures. Differences in drug effects among groups were treated statistically by a two-way analysis of variance (ANOVA) with posthoc multiple comparisons (Tukey’s test) or ANOVA on ranks or Mann-Whitney rank sum test. Differences between baseline (predrug) values and values at different postdrug time points in each group were treated by a repeated measures ANOVA. Values were presented as means ± SE, and P < 0.05 was considered to indicate statistical significance.

**RESULTS**

A total of 35 caudalis nociceptive (18 NS, 12 WDR) and nonnociceptive (5 LTM) neurons was studied. All nociceptive and nonnociceptive neurons were found histologically to be located in the deep laminae of the rostral subnucleus caudalis (akin to laminae V/VI and III/IV of spinal dorsal horn). Because of the technical difficulties of maintaining a stable single unit recording for the >2 h required for full characterization of the neuron and the drug effects, only two-thirds of the neurons were studied with the full range of tests (see METHODS).

**Pulpal application of MO induces neuroplastic changes in caudalis nociceptive neurons**

Pulpal application of MO induced profound changes in spontaneous activity and RF properties in nearly all (90%) of the NS and WDR neurons tested in the saline and MO group.
TRIGEMINAL NEUROPLASTICITY AND NMDA RECEPTOR MECHANISMS

FIG. 1. Example of a caudalis nociceptive-specific (NS) neuron showing changes in spontaneous activity and mechanoreceptive field (RF) properties after mustard oil (MO) application to the right maxillary molar pulp. A: neuronal responses to mechanical and thermal stimuli applied to the cutaneous RF. Top trace: marker of brush (Br), pressure (Pr), pinch (Pi) and radiant heat (RH). Middle trace: neuronal responses in control conditions (i.e., Pre-MO, before MO application). Bottom trace: neuronal responses to graded mechanical stimuli (50, 100, and 200 g). Each stimulus lasts for 3 s, and the y-axis scale (omitted) is the same for middle and bottom traces in A and B. Binwidth is 1 s. Note that after MO application this NS neuron became responsive to light brushing and radiant heating of the cutaneous RF and strongly responsive to graded pinch stimuli. C: profound decrease in mechanical threshold at 20 min and a partial recovery at 40 min after MO application; 0-min value represents the baseline value before MO application; arrow represents the time when MO was applied to the molar pulp. D: MO-induced a brief burst of discharges followed by higher firing rate than the baseline level (0 min). E: MO-induced an expansion of cutaneous pinch RF as well as a temporary appearance of tactile RF. Values at 0 min represent the RF size before MO application. Insets: histologically retrieved recording site and RF sizes before (0 min) and 10 min after MO application (i.e., solid area represents pinch RF and shaded area represents a novel tactile RF that also responded to pinch stimulus after MO application).

(n = 14) but did not produce any significant neuroplastic changes in LTM neurons (n = 5). The neuroplastic changes of the nociceptive neurons included increases in spontaneous firing rate, RF size, and responses to pinch stimuli and a decrease in mechanical threshold. An example is shown in Fig. 1, and details of these changes are outlined subsequently.

INCREASE IN SPONTANEOUS ACTIVITY. The baseline level of spontaneous activity was low in most of the caudalis nociceptive neurons studied, 0–4 spikes/2 min in six of eight NS neurons and in three of six WDR neurons; the remaining five neurons had relatively higher spontaneous activity (8–48 spikes/2 min). In 43% of the nociceptive neurons tested, pulpal application of MO produced immediately a brief burst (latency 15–40 s; duration 3–5 min) of discharges followed by a long-lasting (20–40 min) firing (6–8 spikes/2 min) that was higher than baseline level. In 34% of the neurons, MO induced no immediate burst of firing and only a mild (2–6 spikes/2 min) long-lasting firing; in the remaining two neurons it produced no changes in background activity. In those neurons (6 NS, 3 WDR) with a low baseline spontaneous activity, the mean spontaneous firing rate increased significantly (P < 0.05) at 5 min after MO application, and then over the next 35 min it gradually declined close to, but still significantly higher than, baseline level (P < 0.05; Fig. 2 and Table 1).

INCREASE IN TACTILE AND PINCH RF SIZE. The NS neurons normally were mechanically responsive only to pinch stimulation of the RF, consistent with earlier findings (Chiang et al. 1994; Hu 1990). After pulpal application of MO, however, a novel area responsive to both tactile and pinch stimuli appeared within or adjoining the previous pinch RF in six of eight NS neurons tested; this tactile RF reached its peak size at 10 min (P < 0.05) and disappeared by 20–40 min (Fig. 3). In three of four WDR neurons tested, the mean tactile RF size also increased (from 0.08 ± 0.03 cm² to 0.28 ± 0.19 cm²) at 5 min after MO application, and the time course of this change was similar to that of the novel tactile area of NS neurons. Another tested WDR neuron showed no marked changes in its tactile RF.

Pulpal application of MO produced a significant and long-lasting increase in pinch RF size in 92% of NS (n = 8) and all WDR (n = 4) neurons tested. As shown in Fig. 4 and Table 1, the pinch RF size increased significantly throughout
FIG. 2. Time course of MO-induced changes in mean spontaneous activity of caudalis nociceptive neurons in 3 groups of rats. The 1st group (●; n = 9) received saline (10 µl i.t.) pretreatment followed by pulpal application of MO (SAL and MO group). The 2nd group (■; n = 9) received MK-801 (10 µg/10 µl i.t.) pretreatment followed by the same MO application (MK-801 and MO group). The 3rd group (□; n = 6) received saline (10 µl i.t.) pretreatment followed by pulpal application of mineral oil (SAL and MIN group). Arrows represent saline or drug injection time. Note that differences between baseline (predrug) values and values at different postdrug time points in each group are treated by a repeated measures analysis of variance (ANOVA, *P < 0.05). Significant differences in time course values between these 3 groups are treated by 2-way ANOVA with multiple comparisons and indicated in Table 1. This legend and all symbols apply also to Figs. 3–6.

DECREASE IN MECHANICAL THRESHOLD. After pulpal application of MO, the mean mechanical threshold of the NS neurons tested (n = 7) significantly decreased at 20 and 40 min (P < 0.05; see Fig. 5 and Table 1). Three of four WDR neurons also showed a marked decrease in their mechanical threshold; the fourth WDR neuron, however, showed a marked increase in its mechanical threshold (despite an expansion of both its tactile and pinch RFs).

INCREASE IN RESPONSES TO PINCH STIMULUS. Before MO application, a group of five NS neurons responded to graded pinch stimuli of 50, 100, and 200 g. These responses significantly increased after the MO application (Fig. 6 and Table 1).

| TABLE 1. Changes in spontaneous activity and RF properties of caudalis nociceptive neurons produced by pulpal application of MO (or mineral oil) associated with pretreatment by MK-801 (or saline) intrathecally |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment                                      | Spontaneous Activity, spikes/2 min | Tactile RF, cm² | Pinch RF, cm²   | Mechanical Threshold, g |
| Neuron sample                                  | 6 NS, 3 WDR     | 6 NS            | 8 NS, 4 WDR     | 7 NS, 3 WDR     | 5 NS            |
| Baseline value                                 | 1.8 ± 0.9       | 0.0             | 1.0 ± 0.4       | 15.3 ± 6.5      | 35.2 ± 15.6     |
| Peak value after saline/MO                    | 21.3 ± 13.2*    | 0.3 ± 0.1*      | 2.0 ± 0.3*      | 4.1 ± 1.9*      | 114.4 ± 23.5*   |
| Value 40 min after saline/MO                  | 6.2 ± 3.6*      | 0.05 ± 0.04     | 1.7 ± 0.6*      | 8.6 ± 5.1*      | 72.0 ± 25.9     |
| MK-801 and MO                                  | 5 NS, 4 WDR     | 5 NS            | 5 NS, 4 WDR     | 6 NS, 4 WDR     | 5 NS            |
| Neuron sample                                  | 2.4 ± 1.5       | 0.0             | 1.3 ± 0.3       | 8.4 ± 2.4       | 113.4 ± 62.7    |
| Baseline value                                 | 0.2 ± 0.1       | 0.0             | 0.8 ± 0.2*      | 9.9 ± 2.7       | 38.4 ± 10.6*    |
| Peak value after MK-801/MO                    | 1.1 ± 0.6       | 0.0             | 1.0 ± 0.2       | 8.7 ± 2.4       | 41.8 ± 12.2*    |
| Value 40 min after MK-801/MO                  | 3 NS, 3 WDR     | 4 NS            | 3 NS, 4 WDR     | 4 NS, 4 WDR     | 4 NS            |
| Neuron sample                                  | 2.2 ± 1.6       | 0.0             | 1.2 ± 0.3       | 9.1 ± 6.5       | 139.3 ± 81.3    |
| Baseline value                                 | 2.5 ± 1.9       | 0.0             | 1.1 ± 0.4       | 9.1 ± 6.5       | 153.8 ± 78.1    |
| Peak value after saline/mineral oil            | 1.7 ± 1.0       | 0.0             | 1.1 ± 0.4       | 9.1 ± 6.5       | 146.0 ± 77.4    |
| Value 40 min after saline/mineral oil         | 3 NS, 3 WDR     | 4 NS            | 3 NS, 4 WDR     | 4 NS, 4 WDR     | 4 NS            |
| Two-way ANOVA                                  | F(2, 147) = 6.9 | F(2, 91) = 11.1 | F(2, 175) = 4.8 | T = 115.5       | H = 18.1 |
|                                              | P < 0.001       | P < 0.001       | P < 0.01        | P < 0.05        | P < 0.001 |

All values are shown as means ± SE. RF, mechanoreceptive field; MO, mustard oil; NS, nociceptive-specific neuron; WDR, wide dynamic range neuron; T, sum of ranks used in Mann-Whitney test; H, variability among average ranks used in ANOVA on ranks. *P < 0.05 for comparison between control value and peak value or value at 40 min after MO in each group [repeated measures analysis of variance, ANOVA]; two-way ANOVA is used for comparison of values among different groups except values of threshold and pinch responses, which are treated with Mann-Whitney rank sum test and ANOVA on ranks, respectively.
FIG. 3. Time course of temporary appearance of tactile RF of caudalis NS neurons (n = 6) induced by MO application. Note that after MO application a novel tactile RF started to appear at 5 min; its size peaked at 10 min and subsided at ~20–40 min. Because none of the NS neurons in the MK-801 and MO and SAL and MIN groups showed a novel tactile RF, only data of the MK-801 and MO group in association with those of the SAL and MO group are displayed. Significant differences among these groups are shown in Table 1.

1) All 4 WDR neurons tested with 20-, 40-, and 80-g pinch stimuli showed graded increases in responses before MO application, but after MO application they paradoxically revealed a decrease in their responses, to levels of 44.3, 30.1, and 54.5% of control at 20 min; there was only a partial recovery back to control levels at 40 min after the application of MO.

**MK-801 (intrathecal) antagonizes MO-induced neuroplastic changes in nociceptive neurons**

**MK-801 abolishes MO-induced changes in nociceptive neuronal spontaneous activity.** In all nine nociceptive (5 NS, 4 WDR) neurons tested in animals pretreated with MK-801 (intrathecal), MO failed to induce any changes in spontaneous activity throughout the 40-min observation period (Fig. 2). A two-way ANOVA analysis with multiple comparisons showed significant differences between the MK-801 and MO group and the saline and MO group (P < 0.001, 2-way ANOVA; Table 1). Indeed the baseline level of spontaneous activity (2.4 ± 1.5 spikes/2 min) was depressed, although insignificantly, by MK-801 pretreatment itself for a few minutes (0.8 ± 0.5 spikes/2 min, see Fig. 2).

FIG. 4. Time course of MO-induced changes in mean pinch RF size of caudalis nociceptive neurons. Note that the pinch RF sizes of the SAL and MO group (n = 12) increased throughout 40-min observation period after MO application, whereas those of the other 2 groups (n = 7–9) remained unchanged. Significant differences among these 3 groups are shown in Table 1. In addition, in the time course of the MK-801 and MO group, the values at 0-, 5-, and 10-min time points are significantly lower than baseline value, which indicates MK-801 (10 μg i.t.) pretreatment itself depresses the pinch RF size (see Discussion for details).
FIG. 5. Time course of MO-induced changes in mean mechanical threshold of caudalis NS neurons. Note that mean mechanical thresholds significantly decreased throughout the 40-min period in the SAL and MO group (n = 7), whereas those in the other two groups remained unchanged (n = 4–6). Significant differences among these 3 groups are shown in Table 1.

FIG. 6. Time course of MO-induced changes in mean responses to graded pinch stimuli of caudalis NS neurons. Note that the mean neuronal responses (●) to graded pinch stimuli (200 g, top trace; 100 g, bottom trace) significantly increased at 20 min after MO application in the SAL and MO group; those in SAL and MIN group (○) remained unchanged. In contrast, mean neuronal responses to graded pinch stimuli significantly decreased throughout 40 min after MK-801 (10 μg i.t.) pretreatment followed by MO application (■). Significant differences along these 3 groups are shown in Table 1.
There was no consistent pattern in the mechanically evoked response of the four WDR neurons tested with MK-801 and MO. The mechanical threshold was markedly increased in two neurons, decreased in one neuron, and not changed in one neuron; responses to graded pinch were markedly decreased in three neurons but much increased in one neuron. Neuronal threshold data were pooled for WDR and NS neurons and treated statistically as shown in Table 1; data of WDR and NS neuronal responses to pinch stimuli were not pooled because different forces of pinch stimuli were used for WDR neurons (20–80 g) and NS neurons (50–200 g).

**Pulpal application of mineral oil does not induce any significant neuroplastic changes in caudalis nociceptive neurons**

In the group of rats pretreated with intrathecal saline followed by pulp application of mineral oil, all eight (4 NS and 4 WDR) neurons tested showed no significant changes in nociceptive neuronal spontaneous activity, RF size, mechanical threshold, and responses to graded pinch stimuli (see Figs. 2–6). A two-way ANOVA analysis revealed significant differences between this group and the saline and MO group but not between this group and the MK-801 and MO group (Table 1).

**Pulpal application of MO does not induce any significant neuroplastic changes in caudalis nonnociceptive (LTM) neurons**

In the group of rats pretreated with intrathecal saline followed by the pulp application of MO, all five LTM neurons studied showed no significant changes in neuronal spontaneous activity, tactile RF size, mechanical threshold, and responses to von Frey monofilament stimuli. Their baseline values were 0.4 ± 0.24 spikes/2 min, 5.29 ± 1.18 mm², 2.42 ± 0.93 g, and 12.4 ± 2.79 spikes/6 trials, respectively; their peak values, occurring at different time points within the 40-min observation period after MO application, were 1.0 ± 0.45 spikes/2 min, 5.37 ± 0.86 mm², 1.32 ± 0.45 g, and 31.6 ± 9.32 spikes/6 trials, respectively (P = 0.07–0.85).

**Discussion**

Central sensitization is thought to be reflected in neuroplastic changes that can be triggered by nociceptive afferent inputs and that manifest in spinal dorsal horn nociceptive neurons as a prolonged reduction in mechanical and thermal threshold, an expansion of the RF, and an increase in the responsiveness to RF stimuli (Coderre et al. 1997; Dubner 1991; Dubner and Basbaum 1994; Willis 1993; Woolf 1992). The application to cutaneous, visceral, and deep tissues of MO, a small-fiber excitant and inflammatory irritant, can produce an acute inflammatory pain in association with these central neuroplastic changes (Al-Chaer et al. 1996; Cevero and Laird 1996; Handwerker et al. 1991; Woolf 1992; Woolf and King 1990; Yu et al. 1993). Consistent with these previous findings, this study demonstrated that pulp application of MO can produce neuroplastic changes in nociceptive, but not nonnociceptive, neurons in subnucleus caudalis, the V brain stem analog of the spinal dorsal horn (Dubner and Bennett 1983; Gobel et al. 1981; Hannam and Sessle 1994; Hu et al. 1981; Sessle 1987, 1996; Takemura et al. 1991; Yokota 1985). Pulpal application of MO was also shown (Hu et al. 1994; Sunakawa et al. 1993) to evoke an increase in jaw muscle EMG activity with a time course comparable with the caudalis neuroplastic changes revealed in this study. This study furthermore indicated that NMDA receptor mechanisms are involved in this central sensitization in caudalis induced by pulp application of MO because MK-801, which is a potent noncompetitive NMDA antagonist that binds to the phencyclidine site in the NMDA receptor ion-channel (Coderre et al. 1997; Kolhekar et al. 1994; Wong et al. 1986), could antagonize the MO-induced caudalis neuroplastic changes.

Numerous anatomic and electrophysiological studies documented that the tooth pulp is predominantly innervated by both Aβ- and C-fibers (Bishop 1981; Mengel et al. 1993; Nähi et al. 1992; for review, see Byers 1984; Sessle 1987). In the case of the innervation of the molar tooth pulp in the rat, a recent quantitative electromicroscopic study revealed that the ratio of myelinated/unmyelinated fibers is ~1:3 (Naftel et al. 1994). The pulp application of MO induces a neurogenic inflammation in the rat molar tooth pulp and also has been shown to activate pulp small-fiber afferents but not sympathetic efferents (Di Carlo et al. 1992; Komrowski et al. 1996; Nähi et al. 1997). In addition, MO applied to the molar pulp can reflexly increase jaw muscle activity, as noted previously.

Application of MO to skin or deep tissues such as the temporomandibular joint has also been shown to induce pain and other nociceptive behaviors and to excite selective C-fiber afferents in humans and laboratory animals for a duration of ~4 min (Handwerker et al. 1991; Hartwig et al. 1996; Woolf and Wall 1986). A similar short time course of evoked activity is also a feature of caudalis nociceptive neurons and jaw muscle EMG activity in the rat when MO is applied to the skin or deep craniofacial tissues (Chiang et al. 1997b; Hu et al. 1994; Yu et al. 1993). In contrast, we observed in this study that, after an initial short burst of activity, a significantly long-lasting (40 min) and moderate increase in spontaneous firing of many caudalis nociceptive (both NS and WDR) neurons occurred after MO application to the pulp. A recent study also demonstrated that, after MO application to the colon, the increased spontaneous firing of vescerosensitive neurons in the thalamus may last for 25 min (Al-Chaer et al. 1996). These findings suggest that pulp and visceral afferent inputs may be especially potent in producing a central hyperexcitability state. Because the majority of pulp and visceral sensory afferents are composed of Aβ- and in particular C-fibers, the potent central effects of pulp afferents in this study may not be unexpected. In addition, the pulp application of MO produced an immediate, transient burst of activity in only 43% of nociceptive neurons studied, although it did increase the RF size for >40 min in >90% of the neurons. This may indicate that different mechanisms underlie the increase in spontaneous activity and the expansion of the RF, although these two phenomena may often appear together in central sensitization.

Mechanical hypersensitivity (alldynia) may occur after some peripheral tissue injuries such that low-threshold pri-
mary afferent activation elicits pain. Its pathophysiological mechanisms were recently studied by several groups (for review see Cervero and Laird 1996; Perl 1993; Willis 1993; Woolf 1992). Consistent with the findings of Woolf et al. (1994) in the spinal cord, our findings indicate that a novel cutaneous tactile RF, within or adjoining the pinch RF, can be evoked in NS neurons of the rat caudalis by MO application to the pulp; this particular RF change may be associated with a prolonged and dramatic reduction in the mechanical threshold to levels found in WDR or LTM neurons. Although peripheral mechanoceptor sensitization may also occur (Handwerker and Reeh 1992; LaMotte 1992; Levine and Taiwo 1994; Meyer et al. 1994; Schmidt et al. 1994), the “‘allodynia-like’” caudalis neuroplastic changes induced by MO application to the pulp are most unlikely to be solely the consequence of a change in peripheral mechanonociceptor threshold because the MO was applied well away from the cutaneous RF site of the tested neurons; moreover, most of these neurons also had no pulpal input as evidenced by the lack of an evoked response to MO (present data) or to electrical stimulation (unpublished data). These allodynia-like changes cannot be explained either by “‘sprouted connections’” or by a “‘phenotype switch’” in the central terminals of Aβ-fibers because these anatomic changes typically take several days to appear (Neumann et al. 1996; Woolf et al. 1992). In normal conditions, the size of the firing zone of the RF of a neuron in the spinal dorsal horn can be augmented by a recruitment of the subliminal zone through depolarization of the neuron (Brown et al. 1987; Woolf and King 1990). Thus the responsiveness of a neuron, both spatially and in terms of the pattern and magnitude of the evoked discharge, has the potential to change. Such a postsynaptic mechanism could conceivably explain our findings, on the assumption that, in the MO-induced central sensitization state, NS neurons could be activated by “‘weak synaptic’” Aβ-fiber inputs, which are ineffective in normal conditions. Such a possibility is indeed supported by our earlier findings suggesting extensive afferent convergence in caudalis nociceptive neurons that may be unmasked or disinhibited by peripheral injury or MO (Hu and Sessle 1989; Hu et al. 1992; Sessle et al. 1986), and by recent findings in humans that MO induces a burning pain signaled by C-fibers but can produce conditions that may result in a brush-evoked burning pain transmitted by Aβ-fibers (Koltzenburg et al. 1994). In addition to postsynaptic mechanisms, presynaptic mechanisms should also be considered because it was reported that, after a brief period of noxious stimulation, Aβ-afferent fibers may activate C-fiber nociceptive primary afferents by means of Aβ-evoked primary afferent depolarization (Cervero and Laird 1996). Analogous putative processes involving presynaptic and postsynaptic mechanisms could also explain the other neuroplastic changes (e.g., RF expansion and enhanced neuronal excitability) that we documented in both WDR and NS caudalis neurons.

Our findings demonstrated that, in the central sensitization state induced by pulpal application of MO, increases in spontaneous activity and RF size occurred in all the NS and most of WDR neurons tested. However, some WDR neurons were exceptional in that either their mechanical threshold increased or their responsiveness to mechanical stimuli decreased; such changes would suggest not an increase but a decrease in neuronal sensitivity. It was documented that the membrane depolarization of spinal dorsal horn neurons is much increased by cutaneous application of MO, and eventually spike inactivation may occur (Woolf and King 1990). Such effects may conceivably be associated with subtle changes in differential neuronal sensitivity to different types of afferent inputs that might account for these properties of some caudalis WDR neurons that we observed after MO application to the pulp, but further investigation (e.g., with intracellular recordings) would be needed to clarify this.

Through the administration of specific antagonists, NMDA receptor involvement in central sensitization was well documented in recent studies (Coderre et al. 1997; Kolhekar et al. 1994; Ren et al. 1992; Urban et al. 1994; Woolf 1992; Yu et al. 1996). MK-801 is a noncompetitive NMDA receptor antagonist that binds to the phencyclidine site in the NMDA receptor ion channel (Coderre et al. 1997; Kolhekar et al. 1994; Wong et al. 1986); it can prevent the establishment of windup and other neuroplastic changes in spinal dorsal horn (Ren et al. 1992; Woolf 1992; Willis 1993). NMDA receptor mechanisms appear to be involved in V nociceptive transmission because NMDA expression was reported in caudalis neurons (Dohrn and Beitz 1994; Petralia et al. 1994; Tallekes-Greene et al. 1992), NMDA-activated channels were documented in caudalis neurons in vitro (Chen and Huang 1992), systemic or local caudalis application of MK-801 can antagonize increased EMG activity in jaw muscles induced by MO applied to the temporomandibular joint region (Hu et al. 1997; Yu et al. 1996), and we also recently found that peripheral NMDA mechanisms may be involved in the enhanced jaw muscle activity (Cairns et al. 1998). The findings of the involvement of central NMDA receptor mechanisms in V nociceptive phenomena are thus consistent with the current findings that the local caudalis application of MK-801 can antagonize the caudalis neuroplastic changes reflecting central sensitization induced by MO application to the tooth pulp. It should be noted that the dose (10 µg equivalent to 29 nmol i.t.) of MK-801 used in this study was one-half the volume of the effective dose for antinociceptive behavioral effects in rats in which a smaller dose (2 µg i.t.) was ineffective (Coderre and Van Empel 1994). It is known that MK-801 acts also as a blocker of nicotinic acetylcholine receptor ion channels (nAChR) (Ramoa et al. 1990). However, its anticholinergic action is unlikely responsible for antagonizing the MO-induced neuroplasticity in this study. First, in vitro studies have shown that, when the membrane potential is held at −50 mV, MK-801 has ∼100 times greater affinity for the NMDA receptor (Kd 22 nM) than for the nAChR (Kd 2.4 µM), and when it is held at 0 mV an affinity factor of 40 still exists (Amador and Dani 1991; Huettner and Bean 1988). Second, the slow onset and long-lasting blocking effect of MK-801 in in vitro studies is consistent with our in vivo observations, whereas its cholinergic blocking effect has a rapid onset and short duration because MK-801 appears to become trapped in the NMDA receptor but not in the nAChR (Amador and Dani 1991; Huettner and Bean 1988). Nonetheless, MK-801 does produce a concentration-dependent relaxation on normal pial arterioles (Huang et al. 1994) that suggests a direct action on the NMDA receptors located on smooth muscle fibers. Because the vasorelaxation effective

clinical relevance are our findings that the neuroplastic inflammation or nerve injury. In: Rep.
neuroplastic changes in the RF and response properties of pulpitis reflecting pulpal inflammation, and by intense pain 259 ± 275.
ceedings of the Eighth World Congress on Pain these findings may be relevant to dental pain conditions such as pain states elsewhere in the body is that pathological pain may be the consequence of peripheral and central sensitization in the somatosensory system (Coderre et al. 1997; Dubner 1991; Dubner and Basbaum 1994; Handwerker and Reeh, 1992; LaMotte 2007; Levine and Taiwo 1994; Meyer et al. 1994; Schmidt et al. 1994; Willis 1993; Woolf 1992). Thus these findings may be relevant to dental pain conditions such as an acute toothache, which is usually characterized by pulpitis reflecting pulp inflammation, and by intense pain and hyperalgesia, often with pain radiation, even referral, to adjacent orofacial tissues (e.g., Grushka and Sessle 1984; Sharav 1974; Sigurdsson and Maixner 1994). These phenomena suggest spatial and temporal summation that reflect, at least in part, a central sensitization phenomenon akin to that we documented in this study with MO/pulp-induced neuroplastic changes in the RF and response properties of V nociceptive neurons in subnucleus caudalis. Of further clinical relevance are our findings that the neuroplastic changes in caudalis can be abolished with the NMDA receptor antagonist MK-801 because it was recently suggested that NMDA receptor antagonists may be useful in preempting pain (Coderre et al. 1997; Dubner and Basbaum 1994; Price et al. 1994).

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REFERENCES


TRIGEMINAL NEUROPLASTICITY AND NMDA RECEPTOR MECHANISMS


