

P2X Receptors in Trigeminal Subnucleus Caudalis Modulate Central Sensitization in Trigeminal Subnucleus Oral

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Hu, Bo, Chen Yu Chiang, James W. Hu, Jonathan O. Dostrovsky, and Barry J. Sessle. P2X receptors in trigeminal subnucleus caudalis modulate central sensitization in trigeminal subnucleus oral. *J Neurophysiol* 88: 1614–1624, 2002; 10.1152/jn.00164.2002. This study investigated the role of trigeminal subnucleus caudalis (Vc) P2X receptors in the mediation of central sensitization induced in nociceptive neurons in subnucleus oral (Vo) by mustard oil (MO) application to the tooth pulp in anesthetized rats. MO application produced a long-lasting central sensitization reflected in neuroplastic changes (i.e., increases in neuronal mechanoreceptive field size and responses to innocuous and noxious mechanical stimuli) in Vo nociceptive neurons. Twenty minutes after MO application, the intrathecal (i.t.) administration to the rostral Vc of the selective P2X₁, P2X₃, and P2X_{2/3} receptor antagonist, 2'-(or 3'-)-O-trinitrophenyl-ATP (TNP-ATP), significantly and reversibly attenuated the MO-induced central sensitization for more than 15 min; saline administration had no effect. Administration to the rostral Vc of the selective P2X₁, P2X₃, and P2X_{2/3} receptor agonist, α,β -methylene ATP (α,β -meATP, i.t.) produced abrupt and significant neuroplastic changes in Vo nociceptive neurons, followed by neuronal desensitization as evidenced by the ineffectiveness of a second i.t. application of α,β -meATP and subsequent MO application to the pulp. Administration to the rostral Vc of the selective P2X₁ receptor agonist β,γ -methylene ATP (β,γ -meATP, i.t.) produced no significant neuroplastic changes per se and did not affect the subsequent MO-induced neuroplastic changes in Vo nociceptive neurons. These results suggest that P2X₃ and possibly also the P2X_{2/3} receptor subtypes in Vc may play a role in the initiation and maintenance of central sensitization in Vo nociceptive neurons induced by MO application to the pulp.

INTRODUCTION

P2X receptors are a family of ligand-gated ion channels responsive to ATP, and seven subtypes that form homomeric or heteromeric channels have been identified (for review, see Brake and Julius 1996; Burnstock 2000, 2001; Ding et al. 2000; Khakh et al. 2001; North and Surprenant 2000; Ralevic and Burnstock 1998; Robertson et al. 2001). All of the P2X receptor subtypes except P2X₇ have been shown immunocytochemically to be located in the dorsal horn of the spinal cord and the dorsal root ganglion/terminals of primary afferents as well as in the V ganglion (Collo et al. 1996; Cook et al. 1997; Llewellyn-Smith and Burnstock 1998; Petruska et al. 2000a,b; Vulchanova et al. 1996–1998). Since trigeminal subnucleus

caudalis (Vc) is considered to be the structural and functional analog of the spinal dorsal horn and indeed has been termed the “medullary dorsal horn” (see Dubner and Bennett 1983; Sessle 2000), it seems reasonable to presume that these P2X receptor subtypes also exist in Vc.

Whereas the role of P2X receptors at peripheral sites has been extensively studied in several models of nociception (Bland-Ward and Humphrey 1997; Bradbury et al. 1998; Dowd et al. 1998; Eriksson et al. 1998; Hamilton et al. 1999; Jahr and Jessell 1983; Sawynok and Reid 1997; Tsuda et al. 2000; Xu and Huang 2002), there has been limited identification of their role at central sites involved in nociceptive transmission (Driessen et al. 1994; Edwards et al. 1992, 1997; Fyffe and Perl 1984; Salter and Henry 1985; Stanfa et al. 2000; Tsuda et al. 1999a,b). For example, intrathecal administration (i.t.) of the selective P2X₁, P2X₃, and P2X_{2/3} receptor agonist α,β -methylene ATP (α,β -meATP), but not the selective P2X₁ receptor agonist β,γ -methylene ATP (β,γ -meATP), has been reported to elicit dose-dependent thermal hyperalgesia in both the rat and mouse, and this effect can be prevented by i.t. pretreatment with the selective P2X₁, P2X₃, and P2X_{2/3} receptor antagonist, 2',3'-O-(2,4,6-trinitrophenyl) ATP (TNP-ATP) (Driessen et al. 1994; Tsuda et al. 1999a). These findings suggest the central involvement of P2X₃ and P2X_{2/3} receptor subtypes in mediating thermal hyperalgesia. No significant changes in behavioral responses to noxious mechanical and thermal stimuli were found in a P2X₃-knockout mouse model in comparison to the wild mouse, indicating that P2X₃ receptors may not be involved in acute spinal nociceptive processing (Cockayne et al. 2000; Souslova et al. 2000). Moreover, Stanfa et al. (2000) have reported that i.t. application of α,β -meATP does not produce any significant facilitation of the C fiber-evoked responses of neurons in deep laminae of the rat spinal dorsal horn. On the other hand, spinal P2X receptors are involved in mediating central hyperalgesia following inflammation (e.g., induced by subcutaneous injection of formalin or carrageenan) in both normal and P2X₃-knockout animals (Cockayne et al. 2000; Stanfa et al. 2000; Tsuda et al. 1999b). These effects may be mediated by presynaptic P2X receptors since P2X receptors localized at central presynaptic terminals can be activated by ATP and this activation evokes glutamate release in co-cultured preparations (Gu and MacDermott 1997;

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MacDermott et al. 1999). So far, the role in nociception of ATP and P2X receptors in the trigeminal (V) brain stem sensory complex has not been investigated.

Our previous studies have shown that application of the small-fiber excitant and inflammatory irritant mustard oil (MO) to the tooth pulp induces a prolonged (>40 min) "central sensitization" reflected in *N*-methyl-D-aspartate (NMDA)-dependent neuroplastic changes [i.e., enhancement of neuronal mechanoreceptive field (RF) and response properties] in nociceptive neurons of Vc, as well as in more rostral nociceptive neurons in subnucleus oralis (Vo) (Chiang et al. 1998; Park et al. 2001). This pulp-induced central sensitization in Vo can be attenuated by microinjection into Vc of the synaptic blocker CoCl₂, suggesting its dependency on the functional integrity of Vc (Chiang et al. 2002). In view of the possibility noted above of a central role of P2X receptors in mediating central neuroplastic nociceptive mechanisms, the aims of this study were to test 1) whether application of a P2X receptor antagonist to Vc can attenuate the MO-induced central sensitization in Vo nociceptive neurons, and 2) whether application of a P2X receptor agonist to Vc can produce central sensitization in Vo and also P2X receptor desensitization that prevents the MO-induced central sensitization in Vo. The data have been briefly presented in abstract form (Chiang et al. 2001; Hu et al. 2000).

METHODS

Animal preparation

The experiments were performed in 39 anesthetized rats. The methods used for animal preparation, stimulation, and neuronal recording and classification were similar to those described previously in detail (Chiang et al. 1998; Park et al. 2001) and so will only be briefly outlined here. Male Sprague-Dawley adult rats (275–400 g) were anesthetized by a single intraperitoneal injection of a mixture of α -chloralose (50 mg/kg) and urethane (1 g/kg). A tracheal cannula was inserted, and the left external jugular vein was cannulated. To expose the pulp of the right maxillary first molar, an occlusal cavity was prepared with a dental drill (Rotex 780) and immediately filled with a small piece of cotton pellet soaked in normal saline. After the rat was placed in a stereotaxic apparatus, the posterior part of the right cerebrum and the caudal medulla were surgically exposed, and the overlying dura and subarachnoid membrane were removed. Just before the recording session, a supplemental dose of urethane (200–300 mg/kg, iv) was administered, and the rat was immobilized with gallamine triethiodide (initial dose, 35 mg/kg; maintenance dose, 14 mg/h; iv) and artificially ventilated throughout the whole experimental procedure. An adequate level of anesthesia was confirmed periodically by the lack of spontaneous movements and responses to pinching the paw when gallamine-induced muscle paralysis was allowed to wear off. Heart rate, percentage of expired CO₂, and rectal temperature were constantly monitored and maintained at physiological levels of 333–430 beats/min, 3.5–5%, and 37–38°C, respectively. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

Recording and stimulation procedure

Single neuronal activity was recorded extracellularly by means of an epoxy resin-coated tungsten microelectrode (FHC). As the microelectrode was advanced with a rostral inclination of 26° through the cortex into the brain stem, natural stimuli were applied to the orofacial tissues to search for brain stem Vo neurons receiving an orofacial sensory input. The brain stem was explored 2.4–3.0 mm lateral to the

midline and between frontal planes P1.1 and P2.6, with reference to the interaural line (Paxinos and Watson 1986). Neuronal activity was amplified, displayed on oscilloscopes, and also led to a window discriminator connected to an A/D converter (CED 1401 plus; Cambridge Electronic Design) and a personal computer. Data were analyzed off-line with Spike 2 software (Cambridge Electronic Design).

A wide range of mechanical (brush, pressure, and pinch), electrical, and noxious thermal (radiant heat, 51–53°C) stimuli was applied to the facial skin or intraoral mucosa to classify each neuron as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), or nociceptive-specific (NS) (Chiang et al. 1998; Hu 1990; Park et al. 2001). Electrical stimuli of constant-current single pulses (0.2 ms and <1 mA for A-fiber inputs; 2 ms and <5 mA for C-fiber inputs) were applied within the delineated RF to determine the existence of A- or C-fiber inputs, and monopolar cathodal single pulses (0.2 ms, <2 mA) were applied to the exposed maxillary molar tooth pulp to determine the existence of a molar input to each neuron. Based upon a conduction path from molar to brain stem of 40- to 50-mm and an allowance of 1 ms for peripheral activation time, central narrowing of the afferents in the trigeminal spinal tract, and synaptic delay, a neuronal discharge that could be consistently evoked at a latency >30 ms was attributed to C-fiber inputs to the neuron (Chiang et al. 1998; Hu 1990; Park et al. 2001; Price et al. 1976). As Price et al. (1976) have argued, it is unlikely that the long latency C-fiber responses represent multi-synaptic inputs or repetitive firing of neurons as a result of A-fiber activation because of the higher threshold for eliciting the late discharges, their long latencies, and the failure to find any evidence of late or repetitive firing in LTM neurons receiving an A-fiber afferent input alone. Spontaneous activity was determined from a 2-min recording period. As mentioned in our previous studies (Chiang et al. 1997, 1998; Park et al. 2001), the cutaneous perioral and facial RF of each neuron was determined through the use of a brush, blunt probe, and a pair of nonserrated forceps. Noxious stimulation was used sparingly to avoid damage to the skin and peripheral sensitization. A burst response consisting of ≥ 2 spikes during each stimulus trial (touch, pressure, or pinch) was accepted as the criterion for the RF boundary of the neuron tested. A deep nociceptive input was considered to occur if the application of a blunt probe to the skin overlying muscle, bone, tendon, or temporomandibular joint (TMJ) evoked a neuronal response at a mechanical threshold above 5 g, but no response could be evoked by the wide range of mechanical or thermal stimuli applied to the skin itself (Chiang et al. 1994; Iggo 1960; Park et al. 2001; Schaible and Schmidt 1983; Yu et al. 1993). To assess an orofacial RF that included an intraoral component, we empirically divided the intraoral, perioral, and facial regions into 20 small areas (see Fig. 1 in Chiang et al. 2002); the size of the orofacial RF was quantified by summing the number of areas included in the RF. Responses to von Frey monofilament applications or graded mechanical pinch or pressure stimuli (5, 10, and 20 g for WDR neurons; 50, 100, and 200 g for NS neurons applied for 3 s at an interval of >30 s) applied to the neuronal orofacial RF with a force-monitoring forceps were determined as previously described (Park et al. 2001). The lower stimulus intensity range for WDR neurons was chosen since these neurons have a lower mechanical activation threshold and intensity for mid-range response than NS neurons (Chiang et al. 1994, 1998; Hu et al. 1981). An intensity eliciting a mid-range suprathreshold response was chosen and applied to the same site within the RF three times at the duration and interval specified above. The responses were quantified as the average number of spikes produced during the 3-s stimulation.

Chemicals

The chemicals used included MO (allyl isothiocyanate, 95%; Aldrich Chemical Co., Milwaukee, WI); TNP-ATP (2 μ g/10 μ l; Sigma, St. Louis, MO), which is a selective antagonist of P2X₁, P2X₃, and P2X_{2/3} receptor subtypes; α , β -meATP (1 μ g/10 μ l; Research Bio-

chemicals International, Natick, MA), which is a selective agonist of P2X₁, P2X₃, and P2X_{2/3} receptor subtypes, β , γ -meATP (1 μ g/10 μ l; Research Biochemicals International), which is a selective agonist of P2X₁ receptor subtypes; and isotonic saline (Driessen et al. 1994; Tsuda et al. 1999a). The P2X agonists and antagonist were freshly dissolved in isotonic saline adjusted at pH 7.4.

Experimental paradigm

In each animal, only one neuron was tested with application to the medullary surface overlying the rostral part of Vc of TNP-ATP, α , β -meATP, β , γ -meATP, or saline. Animals were divided into four groups: two groups for the antagonist experiments and two groups for the agonist experiments. In the antagonist experiments, MO application to the pulp was followed either by saline application to Vc (Sal/Vc group $n = 8$) or by TNP-ATP application to Vc (TNP/Vc group, $n = 8$). In the agonist experiments, two applications over Vc of α , β -meATP (α , β -meATP/Vc group, $n = 8$) or β , γ -meATP (β , γ -meATP/Vc group, $n = 8$) were made prior to MO application to the pulp. For all these experimental groups, a standard assessment of neuronal spontaneous activity, orofacial RF size, and pinch- or pressure-evoked responses was performed periodically throughout the experiment.

The specific experimental paradigm used for the P2X antagonist experiments was as follows: 10 min after a Vo nociceptive neuron was identified, the standard assessment was carried out and the data obtained were used as baseline values. Then the saline-soaked cotton pellet was carefully removed from the molar pulp cavity and replaced with a segment of dental paper point soaked with MO (0.2 μ l). The cavity was promptly sealed with CAVIT (ESPE) to prevent MO leaking out of the tooth and to ensure the chemical's sustained action on pulp afferents. The standard assessment was carried out again 3 min after the MO application, and repeated at 8- to 10-min intervals for 60 min. At 20–22 min after the MO application, a bolus (10 μ l) of TNP-ATP solution or saline was applied to the surface of the ipsilateral (right side) Vc with a Hamilton syringe driven by a manual microinjector (Model 5000; David Kopf) over 45–60 s. For the P2X agonist experiments, the specific experimental paradigm was as follows: a standard assessment was made 10 min after a Vo nociceptive neuron was identified, and the data were used as baseline values. A bolus (10 μ l) of α , β -meATP or β , γ -meATP solution was applied to the ipsilateral Vc, the standard assessment was carried out at 3 and 13 min after the first application, and a second bolus (10 μ l) of α , β -meATP or β , γ -meATP was applied to the ipsilateral Vc. Three minutes later, the standard assessment was carried out again and was followed by the pulp application of MO as described above. Three minutes after the MO application, the standard assessment was repeated at 8- to 10-min intervals for 60 min.

Histological analysis

At the end of each experiment, an electrolytic lesion was made at the Vo recording site by passing anodal current (8 μ A) for 10 s. The animals were intracardially perfused with isotonic saline followed by 10% buffered formalin (Fisher) and postfixed/preserved in the same formalin solution until tissue sectioning. The lesion was verified within 10 days with conventional histological procedures, and the camera lucida image was drawn under a light microscope mounted with a drawing tube (Nikon).

Statistical analysis

Statistical treatments were performed on the normalized data (percentage), except for that related to spontaneous activity. In each experimental group, differences between baseline values and values at different postdrug time points were treated by repeated measures ANOVAs (RM ANOVA) or RM ANOVA on ranks. In the antagonist

and agonist experiments, differences between groups of drug effects were treated a priori by a two-way ANOVA (ANOVA) or ANOVA on ranks. Differences between groups at a given time point were treated by a priori *t*-test or Mann-Whitney test. All values were presented as mean \pm SE, except those of orofacial RF size, which were expressed as median (25th percentile, 75th percentile). The level of significance was set at $P < 0.05$.

RESULTS

General properties of Vo nociceptive neurons

Thirty-two functionally identified nociceptive neurons (28 WDR and 4 NS) responding to ipsilateral orofacial stimulation were recorded in the right Vo and studied in detail. According to the histological reconstruction of the recording sites, about 70% of the neurons were located in the middle portion of Vo, 20% in the rostral portion of Vo, and 10% in the caudal portion of Vo (Fig. 1). Sixteen (50%) of the neurons had spontaneous activity (1 NS neuron, 15 WDR neurons). Twenty-five percent of the neurons had only a skin RF, 19% only an intraoral RF, 6% only a perioral RF, and 6% only a nasal mucosal RF, whereas 38% had both a perioral RF and intraoral RF and 6% had both a skin RF and intraoral RF. Most (72%) of the 28 WDR neurons and 50% of the 4 NS neurons tested received electrically evoked A-fiber inputs from their cutaneous or intraoral mucosal RFs; 59% of these responsive neurons also received C-fiber inputs. On the basis of responses to electrical stimulation of the pulp, 29% of the 28 WDR neurons tested, but none of the 4 NS neurons, received electrically evoked A- or C-fiber molar inputs.

Saline application to Vc does not affect MO-induced changes in Vo nociceptive neurons

A total of eight Vo nociceptive neurons (5 WDR, 3 NS) were tested in the Sal/Vc group. Two had both a perioral and intraoral pinch RF, one had only a perioral pinch RF, three had only a facial pinch RF, and the other two had only an intraoral pinch RF. All five WDR neurons also had a tactile RF on facial, perioral, or intraoral areas. Pulp application of MO

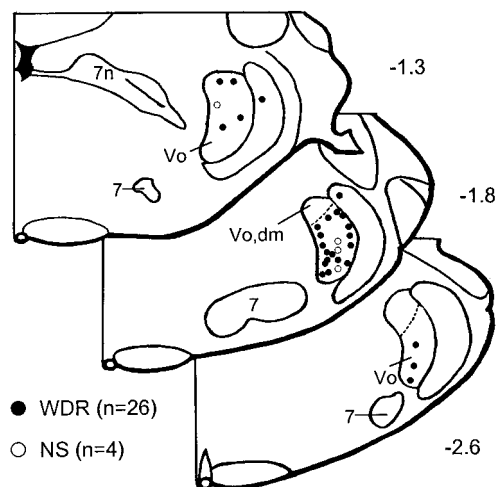


FIG. 1. Coronal sections at 3 different rostrocaudal levels of Vo to show histologically confirmed neuronal recording sites. Numbers on right refer to distance relative to interaural line. Abbreviations: 7, facial nucleus; 7n, facial nerve; Vo dm, dorsomedial portion of Vo.

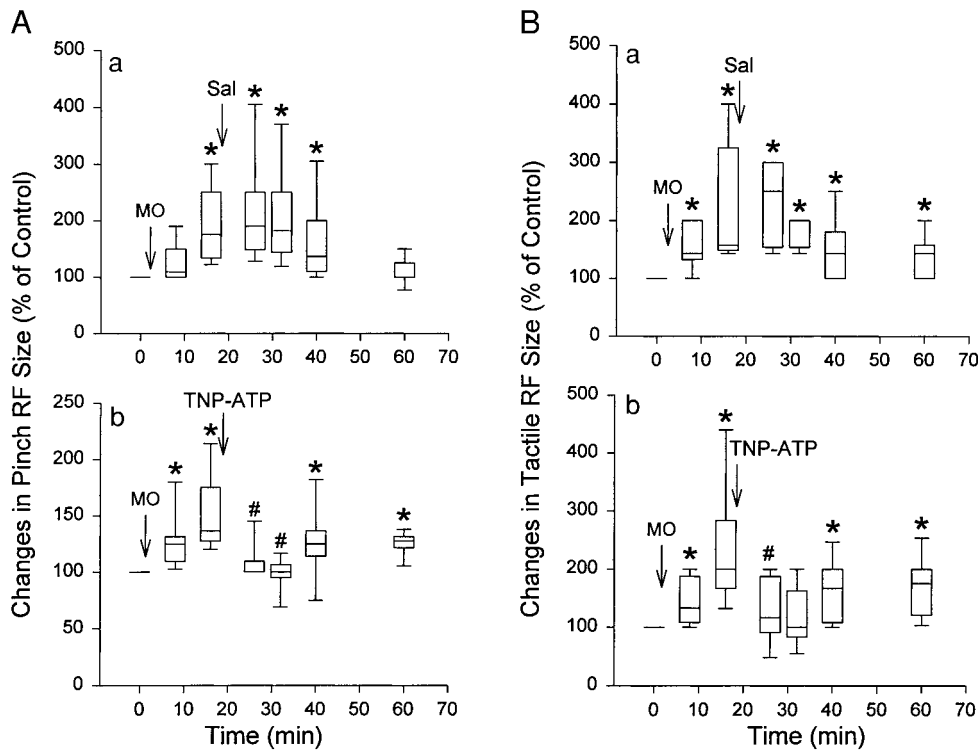


FIG. 2. Time courses of the mustard oil (MO)-induced changes in orofacial mechanoreceptive field (RF) size of subnucleus oralis (Vo) nociceptive neurons and the effects on these changes in the 2'-(or 3')-O-trinitrophenyl-ATP (TNP-ATP)/subnucleus caudalis (Vc) and Sal/Vc groups. **A**: changes in pinch RF size. **B**: changes in tactile RF size. Note significant differences ($*P < 0.05$) between baseline (0 min) values and values at different time points in each group. Differences in pinch RF size (A) at the 24- and 32-min time points between the TNP-ATP/Vc group and the Sal/Vc group were significant ($\#P < 0.05$); similarly, the difference in tactile RF size (B) at the 24-min time point was also significant ($\#P < 0.05$). First arrow represents the time when MO was applied to the pulp. Second arrow represents the time when saline or TNP-ATP was applied to Vc. Median, transverse line within the box; 75th percentile, top of the box; 25th percentile, bottom of the box; 95th percentile, top bar; 5th percentile, bottom bar.

induced neuroplastic changes in all eight nociceptive neurons that were reflected as increases in RF size and pinch- or pressure-evoked response. However, saline application to Vc did not affect the MO-induced neuroplastic changes (Figs. 2 and 3A; Table 1). An example is shown in Fig. 4A.

SPONTANEOUS AND MO-EVOKED ACTIVITY. During baseline recording, three of the five WDR neurons and none of the three NS neurons had spontaneous activity. In two of the WDR neurons, pulp application of MO produced an immediate increase in firing rate. However, no significant difference was found between the mean baseline value and the mean peak value after MO application (see Table 1).

OROFACIAL RF SIZE. Pulp application of MO produced a significant, long-lasting increase in orofacial RF size in all eight nociceptive neurons tested. As shown in Table 1 and Fig. 2Aa, the pinch RF size increased significantly throughout the 40-min period following MO application, with its peak around 26 min (median: 190%, 25th–75th percentile: 149–250%, $P < 0.05$). It was noted that MO application produced a novel intraoral or perioral pinch RF in three WDR neurons that had only an intraoral or a perioral pinch RF prior to the MO application. The orofacial tactile RF size of five WDR neurons also showed a significant increase that peaked at 26 min (median: 250%, 25th–75th percentile:

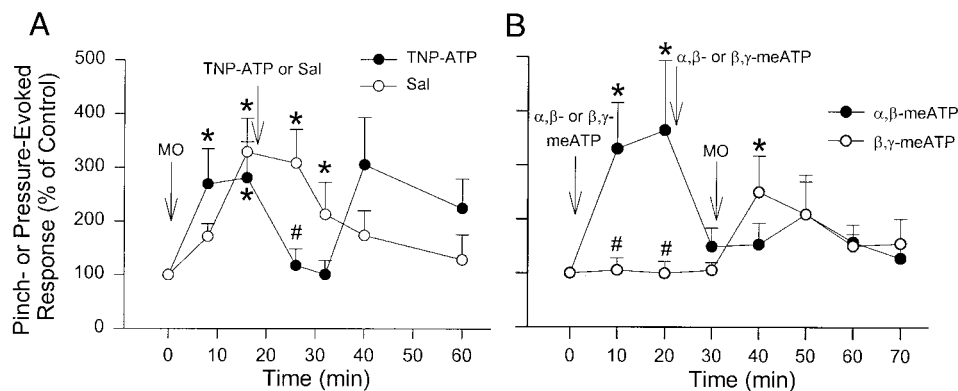


FIG. 3. Time courses of the MO-induced changes in pinch- or pressure-evoked responses of Vo nociceptive neurons in the TNP-ATP/Vc and Sal/Vc groups (A), and the effects of α,β -meATP and β,γ -meATP applications to Vc (B). Note significant differences ($*P < 0.05$) between baseline (0 min) values and values at different time points in each group. **A**: difference in values at the 24-min time point between the TNP-ATP/Vc group and Sal/Vc group was also significant ($\#P < 0.05$). First arrow represents the time when MO was applied to the pulp. Second arrow represents the time when TNP-ATP or saline was applied to Vc. **B**: differences in values at the 10- and 20-min time points between the α,β -meATP/Vc group and β,γ -meATP/Vc group were also significant ($\#P < 0.05$). The 1st 2 arrows represent the 1st and 2nd applications of α,β -meATP or β,γ -meATP to Vc. The third arrow represents the time when MO was applied to the pulp. Note that MO produced a significant increase in pinch-evoked responses in the β,γ -meATP/Vc group, but no effect was seen in the α,β -meATP/Vc group.

TABLE 1. Effects of microinjection of TNP-ATP or saline to Vc on MO-induced neuroplastic changes in spontaneous activity, RF, and response properties of Vo nociceptive neurons in adult rats

Treatment	Spontaneous Activity (Hz)	Tactile RF (number of area)	Pinch RF (number of area)	Pinch or Pressure Responses (spikes/3 s)
TNP-ATP/Vc experiments				
Neuron sample	8 WDR	8 WDR	8 WDR	8 WDR
Baseline value	0.01 \pm 0.01	3.0 (1.0, 3.5)	6.5 (3.5, 10.0)	26.6 \pm 5.8
Value 16 min after MO	1.59 \pm 1.58	5.0 (4.0, 7.5)*	11.5 (4.5, 13.0)*	59.4 \pm 10.3*
Value 6 min after TNP-ATP	0.68 \pm 0.66	2.5 (1.5, 4.5)	8.0 (3.5, 10.5)	24.3 \pm 7.3
Value 20 min after TNP-ATP	0.01 \pm 0.04	3.5 (2.0, 7.5)*	7.5 (3.5, 12.0)*	67.7 \pm 17.0*
Saline/Vc experiments				
Neuron sample	5 WDR, 3 NS	5 WDR	5 WDR, 3 NS	5 WDR, 3 NS
Baseline value	0.09 \pm 0.08	2.0 (1.0, 7.0)	4.0 (1.5, 8.0)	19.1 \pm 7.5
Value 16 min after MO	0.25 \pm 0.24	4.0 (3.8, 10.3)*	6.5 (4.5, 10.5)*	45.4 \pm 9.2*
Value 6 min after Sal	0.15 \pm 0.13	5.0 (3.0, 10.3)*	9.0 (4.5, 11.5)*	55.3 \pm 18.9*
Value 20 min after Sal	0.14 \pm 0.13	5.0 (1.0, 10.3)*	7.0 (3.0, 11.5)*	33.1 \pm 13.2
Two-way ANOVA	$F(1,56) = 1.0$; $P > 0.05$			$F(1,56) = 3.0$; $P < 0.05$
ANOVA on ranks		$H = 26.8$; $P < 0.001$	$H = 39.7$; $P < 0.001$	

All values are shown as mean \pm SE or median (25th percentile, 75th percentile). * $P < 0.05$ for comparison between baseline value and after-treatment values of each indicated time point in each group (repeated measures ANOVA or repeated measures ANOVA on ranks). Two-way ANOVA used for comparison of values between different groups, except values of tactile and pinch RF treated with ANOVA on ranks.

154–300%, $P < 0.05$) after the MO application as shown in Table 1 and Fig. 2Ba. The MO-induced enlargement of the tactile RF lasted ≤ 60 min and longer than that of the pinch RF (Fig. 2Ba).

RESPONSES TO MECHANICAL STIMULI. After MO application, neuronal responses to pinch or pressure stimuli increased in all eight nociceptive neurons tested and peaked at 16 min at a value that was significantly different from baseline ($329 \pm 63\%$, $P < 0.05$; Fig. 3A, Table 1); however, the increased responses lasted < 40 min, much shorter than the duration of the increases in pinch RF size and tactile RF size.

TNP-ATP application to Vc reversibly blocks MO-induced changes in Vo nociceptive neurons

A total of eight WDR neurons was tested in the TNP/Vc group; three had both a perioral and intraoral RF, one had both a facial and intraoral RF, three had only a facial RF, and one neuron had only an intraoral RF. Pulp application of MO induced neuroplastic changes in all eight nociceptive neurons that were reflected as significant increases in RF size and pinch- or pressure-evoked response. Application of TNP-ATP to Vc reversibly blocked the MO-induced neuroplastic changes in the Vo nociceptive neurons. This blockade was reflected in a reduction in neuronal RF size and pinch- or pressure-evoked responses (Table 1; Figs. 2 and 3A). An example is shown in Fig. 4B.

SPONTANEOUS AND MO-EVOKED ACTIVITY. Five of the eight WDR neurons had no baseline spontaneous activity, and the other three had sporadic or periodic bursting activity. The MO application to the pulp elicited immediate responses in three WDR neurons, which had no baseline spontaneous activity, but the mean firing rate was not significantly different from mean baseline at 16 min (Table 1). The increased spontaneous activity induced by MO application was suppressed by TNP-ATP application to Vc in two of the neurons. Overall, there was no significant difference between the mean values at baseline and those at 16 min after MO application or at 6 min after TNP-ATP injection ($P > 0.05$, $n = 8$; Table 1).

OROFACIAL RF SIZE. The neuronal pinch RF and tactile RF size increased from 8 min after the MO application and peaked at 16 min (median: 137%, 25th–75th percentile: 128–175% and median: 200%, 25th–75th percentile: 167–284%, respectively, $P < 0.05$). As shown in Table 1 and Fig. 2, Ab and Bb, the application of TNP-ATP to Vc around 20 min invariably produced a significant blockade of the increased pinch and tactile RF size (median: 100%, 25th–75th percentile: 100–110% and median: 117%, 25th–75th percentile: 92–188%, respectively, $P > 0.05$). The blockade involved only the ex-

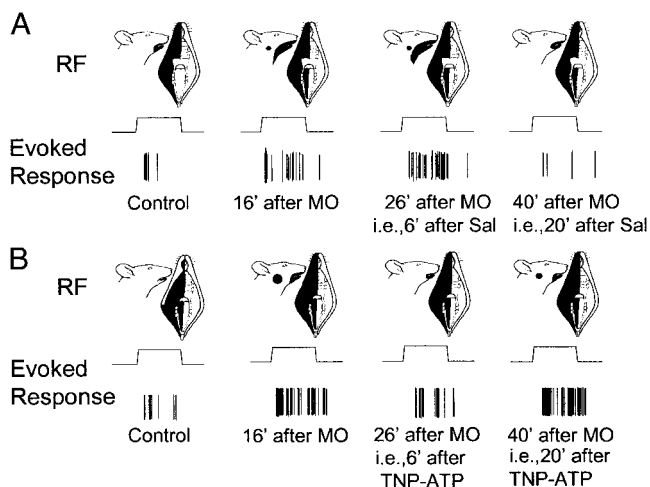


FIG. 4. Examples of changes in orofacial RF and pinch- or pressure-evoked responses of a wide dynamic range (WDR) neuron in each of the Sal/Vc (A) and TNP-ATP/Vc (B) groups. Note that only the pinch RFs are shown on the top panel. Electronic analog signal from the mechanical stimulator is shown on the 2nd line and the neuronal responses on the 3rd line. Note that in both WDR neurons, TNP-ATP applied to Vc reversibly blocked the MO-induced enhanced responses, but saline applied to Vc did not affect these changes, which lasted longer than 40 min.

panded portion of the RF, not the baseline RF, and lasted for 15–20 min. Subsequently, the MO-induced neuroplastic changes recovered around 40 min (median: 125%, 25th–75th percentile: 114–137% and median: 167%, 25th–75th percentile: 109–200%, respectively, $P < 0.05$).

RESPONSES TO MECHANICAL STIMULI. The MO-induced increase of pinch- or pressure-evoked responses was also significantly suppressed after the application of TNP-ATP to Vc. As demonstrated in Table 1 and Fig. 3A, the suppression occurred at 6 min after the TNP-ATP application (i.e., 26 min after MO application, $118 \pm 31\%$, $P > 0.05$), and lasted for 40 min (Fig. 3A, Table 1).

COMPARISONS OF RF SIZE AND RESPONSES BETWEEN THE SAL/Vc AND TNP-ATP/Vc GROUPS. As indicated in Table 1, the differences of pinch RF size, tactile RF size, and responses to pinch or pressure between the Sal/Vc group and TNP-ATP/Vc group were significant ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively). Specifically, as shown in Figs. 2, A and B, and 3A, the differences in pinch RF size, tactile RF size, and pinch- or pressure-evoked responses between the Sal/Vc and TNP-ATP/Vc groups were significant at 26 min after MO application ($P < 0.05$), and the difference in pinch RF between the Sal/Vc and TNP-ATP/Vc groups was also significant at 32 min ($P < 0.05$; e.g., Fig. 2A).

α,β -meATP application to Vc evokes changes in Vo nociceptive neurons

In the α,β -meATP/Vc group, eight WDR neurons were tested. Three had both a perioral and intraoral RF, one had only a facial RF, and the other four neurons had only an intraoral RF. Application of α,β -meATP to Vc induced significant neuroplastic changes in all eight Vo WDR neurons, which were reflected in significant increases of RF size and responses to

pinch or pressure stimulation; no significant increase of spontaneous activity occurred. After the second application of α,β -meATP, however, the RF and responses to pinch or pressure stimulation were significantly reduced, and the spontaneous activity was also markedly suppressed, even though the decrease was not statistically significant. This apparent neuronal desensitization also prevented the MO-induced neuroplastic changes of Vo nociceptive neurons (Figs. 5 and 3B; Table 2). An example is shown in Fig. 6A.

SPONTANEOUS AND MO-EVOKED ACTIVITY. Baseline spontaneous activity occurred in three of the eight WDR neurons. There were no significant changes in mean firing rates following the first or second applications of α,β -meATP or after MO application (Table 2).

OROFACIAL RF SIZE. The first application of α,β -meATP to Vc produced, in all eight WDR neurons tested, a significant increase in size of the facial, perioral, and/or intraoral pinch and tactile RFs that peaked at 20 min after the application (median: 164%, 25th–75th percentile: 150–200% and median: 254%, 25th–75th percentile: 125–350%, respectively, $P < 0.05$). However, pinch and tactile RF size was dramatically reduced 5 min after the second application of α,β -meATP. It reached a value that was not significantly different from baseline (median: 100%, 25th–75th percentile: 100–106% and median: 100%, 25th–75th percentile: 100–150%, respectively, $P > 0.05$) and maintained this value ≤ 40 min after MO application (median: 100%, 25th–75th percentile: 100–106% and median: 150%, 25th–75th percentile: 100–200%, respectively, $P > 0.05$). As in the Sal/Vc group, α,β -meATP application produced a novel perioral pinch RF in two WDR neurons that originally had only an intraoral RF.

RESPONSES TO MECHANICAL STIMULI. After the first application of α,β -meATP to Vc, neuronal responses to pinch or pressure

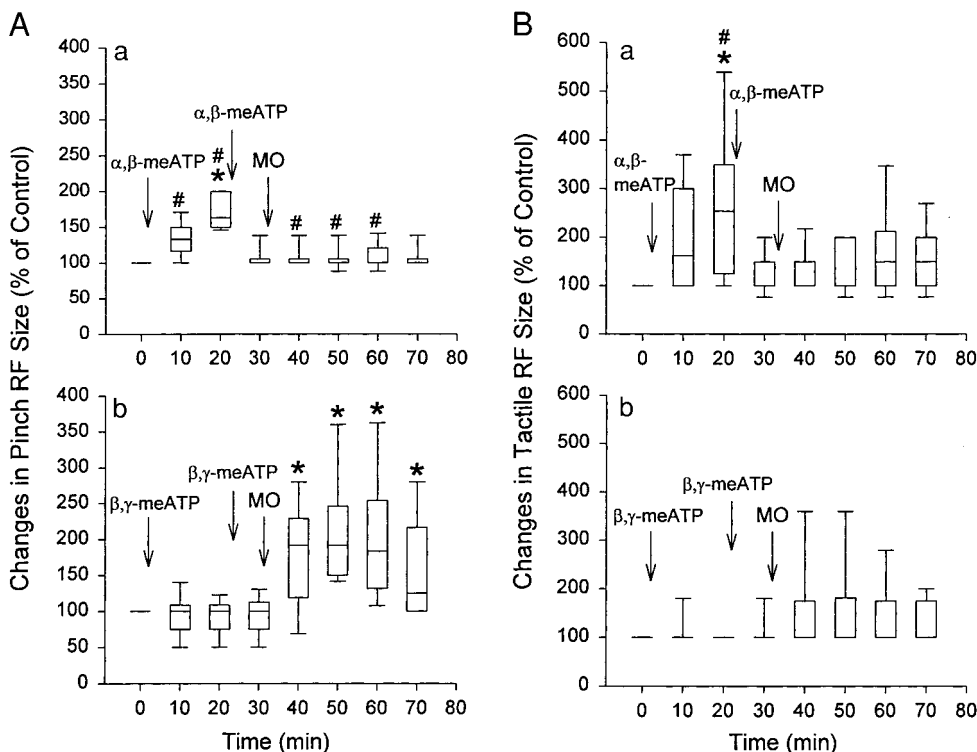


FIG. 5. Time courses of the α,β -meATP (a) or β,γ -meATP (b)-induced changes in orofacial pinch (A) and tactile (B) RF size of Vo nociceptive neurons and the effects of these pretreatments on the subsequent MO-induced changes. Note significant differences ($*P < 0.05$) between baseline (0 min) values and values at different time points in each group. Differences in pinch RF size at the 10-, 20-, 40-, 50-, and 60-min time points between the α,β -meATP/Vc group and the β,γ -meATP/Vc group were significant ($\#P < 0.05$); the difference in tactile RF size at the 20-min time point was also significant ($\#P < 0.05$). The 1st 2 arrows represent the 1st and 2nd applications of α,β -meATP or β,γ -meATP to Vc. The 3rd arrow represents the time when MO was applied to the pulp. Note that MO produced a significant increase in pinch RF size in the β,γ -meATP/Vc group, but no effect was seen in the α,β -meATP/Vc group. Median, transverse line within the box; 75th percentile, top of the box; 25th percentile, bottom of the box; 95th percentile, top bar; 5th percentile, bottom bar.

TABLE 2. Effects of application of α,β -meATP or β,γ -meATP to Vc on Vo nociceptive neuronal spontaneous activity, RF, and response properties and their influence on MO-induced Vo neuroplastic changes in adult rats

Treatment	Spontaneous Activity (Hz)	Tactile RF (number of area)	Pinch RF (number of area)	Pinch or Pressure Responses (spikes/3 s)
α,β -meATP/Vc experiments				
Neuron sample	8 WDR	8 WDR	8 WDR	8 WDR
Baseline value	0.47 \pm 0.47	2.0 (1.0, 2.5)	2.5 (2.0, 5.5)	15.1 \pm 3.4
Value 20 min after 1st α,β -meATP	0.06 \pm 0.06	3.5 (2.5, 6.5)*	4.5 (3.5, 8.5)*	39.0 \pm 7.1*
Value 10 min after 2nd α,β -meATP	0.32 \pm 0.32	2.0 (1.5, 2.0)	3.0 (2.0, 5.5)	21.8 \pm 5.9
Value 20 min after MO	0.56 \pm 0.56	2.0 (2.0, 2.5)	3.0 (2.0, 5.5)	19.3 \pm 5.3
β,γ -meATP/Vc experiments				
Neuron sample	7 WDR, 1 NS	7 WDR	7 WDR, 1 NS	7 WDR, 1 NS
Baseline value	0.02 \pm 0.01	1.0 (1.0, 1.0)	3.5 (1.5, 5.0)	15.2 \pm 3.4
Value 20 min after 1st β,γ -meATP	0.02 \pm 0.01	1.0 (1.0, 1.0)	2.5 (1.0, 6.0)	12.7 \pm 2.3
Value 10 min after 2nd β,γ -meATP	0.02 \pm 0.01	1.0 (1.0, 1.8)	3.0 (1.0, 5.5)	15.9 \pm 3.8
Value 20 min after MO	0.03 \pm 0.02	1.0 (1.0, 3.5)	6.0 (2.5, 9.0)*	29.4 \pm 5.2*
Two-way ANOVA	$F(1,56) = 2.8; P > 0.05$	$H = 20.1; P < 0.01$	$H = 51.1; P < 0.001$	$F(1,56) = 3.8; P < 0.05$
ANOVA on ranks				

All values are shown as mean \pm SE or median (25th percentile, 75th percentile). * $P < 0.05$ for comparison between baseline value and after-treatment values of each indicated time point in each group (repeated measures ANOVA or repeated measures ANOVA on ranks). Two-way ANOVA used for comparison of values between different groups, except values of tactile and pinch RF treated with ANOVA on ranks.

stimuli were increased in all eight WDR neurons tested. As shown in Table 2 and Fig. 3B, responses to pinch or pressure stimuli steadily increased and reached a peak at 20 min that was significantly different from baseline ($365 \pm 129\%$, $P < 0.05$). Ten minutes after the second application of α,β -meATP, however, the responses to pinch or pressure stimuli declined to a value that was not significantly different from baseline ($149 \pm 35\%$, $P > 0.05$) and maintained this value ≤ 40 min after MO application ($128 \pm 28\%$, $P > 0.05$).

β,γ -meATP application to Vc does not evoke changes in Vo nociceptive neurons, nor does it influence MO-induced changes

In the β,γ -meATP/Vc group, seven WDR neurons and one NS neuron were tested. Three had both a perioral and intraoral RF, one had both a facial and intraoral RF, one had only a facial RF, one only a perioral RF, and the other two neurons had only a nasal mucosa RF. Neither the first nor the second application of β,γ -meATP to Vc induced any clear neuroplastic changes in any of the neurons. Moreover, the subsequent application of MO to the pulp caused neuroplastic changes in all eight Vo neurons, showing no clear effect of pretreatment of the two previous applications of β,γ -meATP to Vc (Figs. 5 and 3B; Table 2). An example is shown in Fig. 6B.

SPONTANEOUS ACTIVITY. Baseline spontaneous activity occurred in three of the eight nociceptive neurons. There were no significant changes in mean firing rate following the first or second applications of β,γ -meATP or following MO application (Table 2).

OROFACIAL RF SIZE. As shown in Table 2 and Fig. 5, *Ab* and *Bb*, the first application of β,γ -meATP to Vc produced no significant increases in pinch RF size of all eight nociceptive neurons or in tactile RF size of seven WDR neurons (median: 100%, 25th–75th percentile: 75–109% and median: 100%, 25th–75th percentile: 100–100%, respectively, $P > 0.05$). Similarly, the pinch and tactile RF size underwent no significant change after the second application of β,γ -meATP (median: 100%, 25th–75th percentile: 75–113% and median: 100%, 25th–75th percentile: 100–100%, respectively, $P > 0.05$). Ten minutes after the MO application to the pulp, however, the pinch RF size showed a significant increase (median: 192%, 25th–75th percentile: 119–229%, $P < 0.05$), while the tactile RF size remained unchanged (median: 100%, 25th–75th percentile: 100–175%, $P > 0.05$).

RESPONSES TO MECHANICAL STIMULI. After the first application of β,γ -meATP to Vc, neuronal responses to pinch or pressure

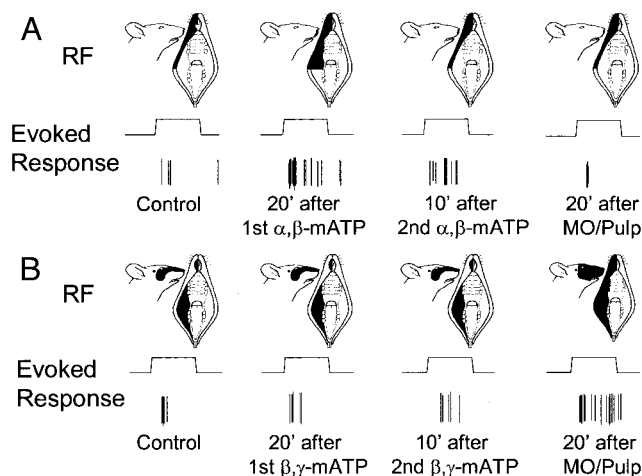


FIG. 6. Examples of changes in orofacial RF and pinch- or pressure-evoked responses of a WDR neuron in each of the α,β -meATP/Vc (A) and β,γ -meATP/Vc (B) groups. Note that only the pinch RFs are shown on the top panel. Electronic analog signal from the mechanical stimulator is shown in the 2nd line and the neuronal responses on the 3rd line. Note that marked changes occurred in the Vo nociceptive neuron after the 1st application of α,β -meATP to Vc, but did not occur after the 2nd application of α,β -meATP to Vc, and the subsequent MO application to the pulp also did not induce changes. In contrast to the effect of α,β -meATP, the 1st and 2nd applications of β,γ -meATP to Vc did not induce any changes, whereas the MO application to the pulp induced significant changes.

stimuli showed no significant changes in the eight nociceptive neurons ($100 \pm 22\%$, $P > 0.05$), as shown in Table 2 and Fig. 3B. Similarly, the second application of β, γ -meATP caused no significant enhancement of the pinch- or pressure-evoked responses ($106 \pm 15\%$, $P > 0.05$). However, the subsequent MO application to the pulp produced a significant increase of the pinch- or pressure-evoked responses ($251 \pm 67\%$, $P < 0.05$).

COMPARISONS OF PINCH RF SIZE, TACTILE RF SIZE AND PINCH OR PRESSURE RESPONSES BETWEEN GROUPS. Overall, as indicated in Table 2, the differences in values of pinch RF size, tactile RF size, and pinch- or pressure-evoked responses between the α, β -meATP/Vc and β, γ -meATP/Vc groups were significant ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively). Specifically, the difference in pinch RF size and pinch- or pressure-evoked responses between the two groups at 10 and 20 min after the first chemical application was significant ($P < 0.05$, Figs. 5A and 3B), and the difference in tactile RF size was significant at 20 min after the first chemical application (Fig. 5B, $P < 0.05$). After MO application to the pulp, the difference in pinch RF size between the two groups was significant at 10, 20, and 30 min ($P < 0.05$, Fig. 5A), and the difference in pinch- or pressure-evoked responses was significant at 10 min after MO application ($P < 0.05$, Fig. 3B). However, no significant difference in tactile RF size was found between the two groups after MO application ($P > 0.05$, Fig. 5B).

DISCUSSION

Our observations confirmed earlier findings that nociceptive neurons occur in Vo (Azerad et al. 1982; Dallel et al. 1998; Greenwood and Sessle 1976; Hu et al. 1992; Raboisson et al. 1995; Woda et al. 2001) and that application of the inflammatory irritant and small-fiber excitant MO to the tooth pulp can induce central sensitization in Vo reflected in enhancement of RF and responses in these neurons (Park et al. 2001). Furthermore, we have provided the first documentation of P2X receptor mechanisms influencing nociceptive transmission in the trigeminal system. Moreover, we have demonstrated that the selective P2X₁, P2X₃, and P2X_{2/3} receptor antagonist TNP-ATP applied to Vc significantly attenuates the MO-induced central sensitization in Vo nociceptive neurons, and furthermore, that application of the selective P2X₁, P2X₃, and P2X_{2/3} receptor agonist α, β -meATP to Vc produces hyperexcitability in Vo nociceptive neurons with features similar to those of the MO-induced central sensitization. The ascending modulatory effects that Vc exerts on Vo and the neural circuitry involved have been extensively discussed in previous reports (Chiang et al. 2002; Greenwood and Sessle 1976; Park et al. 2001). In addition, desensitization of P2X receptors by α, β -meATP prevents the subsequent MO-induced central sensitization in Vo. Finally, the specific P2X₁ receptor agonist β, γ -methylene ATP produced no significant central sensitization and did not affect the subsequent MO-induced neuroplastic changes in Vo nociceptive neurons. These findings suggest that P2X₃ and possibly also the P2X_{2/3} receptor subtypes in Vc are involved in the MO-induced central sensitization of more rostral nociceptive neurons in Vo.

Technical considerations

Since no specific agonist and antagonist for individual subtypes are yet available (North and Surprenant 2000; Ralevic

and Burnstock 1998; Robertson et al. 2001), we had to use selective agonists and antagonists. The doses of P2X receptor agonists we used are within the range of doses used in previous studies (Cook et al. 1997; North and Surprenant 2000; Tsuda et al. 1999a,b; Ueno et al. 1999). The dose we used for TNP-ATP (2 μ g, equivalent to 3.84 nmol) is also comparable to that used with i.t. applications of this P2X receptor antagonist in previous behavioral studies in which an optimal inactivation of P2X receptors was obtained (Tsuda et al. 1999a), although it is higher than that used in *in vitro* studies (Lewis et al. 1998; Virginio et al. 1998). In recent cloned P2X receptor or smooth muscle cell studies, sensitivities (EC_{50}) of P2X₁, P2X₃, and P2X_{2/3} subtypes to TNP-ATP are in the nanomolar range, and those of P2X₂, P2X₄, and P2X₇ subtypes are in the micromolar range (for review, see North and Surprenant 2000), but in ATP agonist-induced arterial muscle contraction studies, higher concentrations of TNP-ATP (approximately 30 μ M) are often required to block P2X₁, P2X₃, and P2X_{2/3} subtypes, possibly because of metabolic breakdown by nucleotidases of TNP-ATP in the extracellular fluid (Lewis et al. 1998). In our preparation, the drug applied to Vc could be considerably diluted in the cerebrospinal fluid and then has to diffuse into the medullary to reach its target receptor. Therefore it is reasonable to assume that the concentration of TNP-ATP required to block P2X₁, P2X₃, and P2X_{2/3} subtypes when it is applied to the medullary surface is higher than those applied in *in vitro* studies.

Previous studies of central spinal P2X receptor processes

Although a few studies have indicated that i.t. administration of α, β -meATP may potentiate nociception in normal animals (Driessen et al. 1994; Tsuda et al. 1999a), most recent behavioral and electrophysiological *in vivo* studies have consistently shown that central P2X receptor activation is involved in acute inflammatory pain, in line with our findings. For instance, i.t. administration of suramin or pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), both nonselective ATP antagonists (Ralevic and Burnstock 1998), or the selective P2X₁, P2X₃, and P2X_{2/3} receptor antagonist TNP-ATP, can significantly attenuate formalin-induced nociceptive behavior in rats (Driessen et al. 1994; Tsuda et al. 1999b), particularly the phase 2 response that is generally assumed to reflect central sensitization; this has been further confirmed in P2X₃ null mice (Cockayne et al. 2000; Souslova et al. 2000). In addition, suramin administered i.t. to animals 3 h after the induction of carrageenan-induced inflammation has been reported to inhibit C fiber-evoked responses of dorsal horn neurons (Stanfa et al. 2000). In our experiment, the fact that TNP-ATP can only reversibly attenuate the increases in RF size and nociceptive responses after the MO-induced central sensitization, but cannot affect their baseline values (see Table 1), suggests that P2X receptors may not be involved in V nociceptive processing under normal conditions; this finding is consistent with several *in vivo* studies (Cockayne et al. 2000; Li et al. 1998; Souslova et al. 2000).

P2X receptor localization

Seven subtypes of P2X receptors have been cloned and P2X₁₋₆ receptor subtypes have been shown to be located in the

spinal dorsal horn and primary sensory neurons (for reviews see Brake and Julius 1996; Burnstock 2000, 2001; Ding et al. 2000; North and Surprenant 2000; Robertson et al. 2001). However, so far, our knowledge of their role in the CNS is very limited. In the case of nociceptive mechanisms, much attention has focused on the role of the P2X₃ receptors because they are selectively and uniquely expressed in a subset of predominantly small (presumed nociceptive) sensory neurons, including their central terminals, but not in dorsal horn neurons (Chen et al. 1995; Cook et al. 1997; Petruska et al. 2000a; Vulchanova et al. 1998; for review, see Brake and Julius 1996; Burnstock 2000, 2001; Ding et al. 2000; Khakh et al. 2001; MacDermott et al. 1999; North and Surprenant 2000). P2X₃ receptor expression has also been documented in many neurons supplying the rat and human tooth pulp (Alavi et al. 2001; Cook et al. 1997). P2X₃ receptors appear to be predominantly associated with those nonpeptidergic afferents that are sensitive to capsaicin and glial cell line–derived neurotrophic factor, contain the enzyme fluoride-resistant acid phosphatase, bind the isolectin B4 (IB4), and terminate in the inner part of dorsal horn lamina II (e.g., Bradbury et al. 1998; Guo et al. 1999; Petruska et al. 2000a,b; Ueno et al. 1999; Vulchanova et al. 1998; see Snider and McMahon 1998), and that also may be differentially involved compared with peptidergic afferents in certain pain conditions (e.g., inflammatory compared with acute and neuropathic pain) (see Burnstock 2000; Ding et al. 2000; Snider and McMahon 1998; Xu and Huang 2002). As described in greater detail below, the findings of this study indicate that P2X receptors, probably of the P2X₃ or P2X_{2/3} subtypes likely are involved in mediating the central sensitization induced by application of MO to the tooth pulp.

What receptor subtype is involved in V central sensitization?

In the spinal cord there is good evidence that ATP acts presynaptically to regulate transmitter release and to involve NMDA receptor mechanisms (Bardoni et al. 1997; Gu and MacDermott 1997; Nakatsuka and Gu 2001; Tsuda et al. 1999a,b; for review, see MacDermott et al. 1999), and this is likely to occur also in Vc due to its structural and functional similarities with the spinal dorsal horn. Given the fact that P2X₃ receptors are only found in the peripheral and central terminals of the small-diameter primary afferents, activation of these receptors could evoke presynaptic glutamate release onto Vc neurons in a manner analogous to that shown in spinal cord in vitro preparations (Gu and MacDermott. 1997) and in behavioral studies (Tsuda et al. 1999a). NMDA receptors would then be activated and cause central sensitization in Vc, the medullary dorsal horn (see Dubner and Bennett 1983; Sessle 2000), which has been shown to be blocked by the application of NMDA receptor antagonists (Chiang et al. 1998; Luccarini et al. 2001). Although ATP has been recently documented to be involved as an extracellular signaling molecule between neurons and glial cells mainly through P2Y receptors (Fam et al. 2000; Fields and Stevens 2000; Watkins et al. 2001), glial cells are unlikely involved in the MO-induced central sensitization in the present study since both α,β -meATP and TNP-ATP have their selective actions on P2X receptors. In this study, subtypes P2X₁, P2X₃, and P2X_{2/3} are potential candidates for our observed effects, because the antagonist TNP-ATP and the agonist α,β -meATP both have selective actions on these sub-

types (Lewis et al. 1998; North and Surprenant 2000; Virginio et al. 1998). TNP-ATP applied to Vc was shown to reversibly disrupt the MO-induced central sensitization in Vo. Moreover, application of α,β -meATP to Vc was found to induce central sensitization in Vo, suggesting that it can mimic the effect of MO application to the pulp in producing central sensitization by activation of P2X₁, P2X₃ and/or P2X_{2/3} receptors in Vc. However, since the application to Vc of the specific P2X₁ receptor agonist β,γ -meATP failed to affect the MO-induced central sensitization in Vo, this finding appears to rule out the possible involvement of the P2X₁ subtype. Therefore subtypes P2X₃ and P2X_{2/3} are the likely candidates in Vc for playing a role in the ascending modulation of nociceptive transmission in the V brain stem sensory complex. This result is consistent with most previous findings and also supports the notion that P2X₃-containing receptors (P2X₃ and P2X_{2/3}) in the spinal cord may be involved in nociception (Chen et al. 1995; Lewis et al. 1995; Petruska et al. 2000a,b; Tsuda et al. 1999a,b; Vulchanova et al. 1997, 1998; Xu and Huang 2002; for review, see Burnstock 2000, 2001; Ding et al. 2000; North and Surprenant 2000). Heteromeric P2X_{2/3} receptor subtypes are expressed mostly on capsaicin-insensitive medium-sized cells in the dorsal root ganglion (Petruska et al. 2000b; Tsuda et al. 2000; Ueno et al. 1999) and are characterized by slow desensitization kinetics, are less sensitive to α,β -meATP (EC₅₀, 63 μ M), and potentiated by low pH and readily distinguished from the P2X₃ receptor subtypes in in vitro studies. In particular, it has been shown that the ATP and α,β -meATP-evoked inward currents are dramatically decreased for a period of more than 10 min to a second application of the agonist in the case of P2X₁ or P2X₃ receptors, but little or no such decrease occurs in the case of P2X_{2/3} receptor subtypes (Lewis et al. 1995; Ueno et al. 1998, 1999). Since, in our studies, the initial effect produced by α,β -meATP could not be reproduced by a second application, this suggests that P2X₃ receptor subtypes rather than P2X_{2/3} receptor subtypes are involved in eliciting the central sensitization observed. This finding also suggests that the effects observed are due to a presynaptic action on capsaicin-sensitive nociceptive primary afferents since it is only the capsaicin-sensitive dorsal root ganglion neurons that display marked desensitization to repeated applications of α,β -meATP in in vitro studies (Ueno et al. 1999).

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