Changes in Gene Expression and Neuronal Phenotype in Brain Stem Pain Modulatory Circuitry After Inflammation

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Miki, Kenji, Q.-Q. Zhou, W. Guo, Y. Guan, R. Terayama, R. Dubner, and K. Ren. Changes in gene expression and neuronal phenotype in brain stem pain modulatory circuitry after inflammation. J Neurophysiol 87: 750–760, 2002; 10.1152/jn.00534.2001. Recent studies indicate that descending pain modulatory pathways undergo time-dependent changes in excitability following inflammation involving both facilitation and inhibition. The cellular and molecular mechanisms of these phenomena are unclear. In the present study, we examined N-methyl-D-aspartate (NMDA) receptor gene expression and neuronal activity in the rostral ventromedial medulla (RVM), a pivotal structure in pain modulatory circuitry, after complete Freund’s adjuvant (CFA)-induced hindpaw inflammation. The reverse transcription polymerase chain reaction analysis indicated that there was an upregulation of mRNAs encoding NMDA receptor subunits in the RVM after inflammation. The increase in the NR1, NR2A, and NR2B receptor mRNAs started at 5 h, maintained for 1–7 days (P < 0.05–0.001) and returned to the control level at 14 days after inflammation. Western blot analysis indicated that the protein translation products of the NR2A subunit were also increased (P < 0.01). In single-unit extracellular recordings, we correlated RVM neuronal activity with the paw withdrawal response in rats with inflammation. We describe these RVM cells as on-, off-, and neutral-like cells because of their similarity to previous studies in which neuronal responses were correlated with tail-flick nocifensive behavior in the absence of inflammation. In contrast to previous studies in the absence of inflammation, using tail flick as a behavioral correlate, fewer off-like cells in naïve animals exhibited a complete pause before the paw withdrawal to a noxious thermal stimulus. The percentage of cells showing a pause of activity after noxious stimulation was further reduced after inflammation (χ² P < 0.0001 vs. naïve rats). Continuous neuronal recordings (3–6.5 h) revealed a phenotypic switch of RVM neurons during the development of inflammation: 11/15 neutral-like cells initially unresponsive to noxious stimuli exhibited and maintained response profiles characteristic of pain modulatory neurons (became off-like: n = 5; became on-like: n = 6). Neutral-like cells recorded in noninflamed animals did not show response profile changes during continuous recordings (5–5.5 h, n = 7). A population study (n = 165) confirmed an increase in on- and off-like cells and a decrease in neutral-like cells at 24 h after inflammation as compared with naïve rats (P < 0.001). These results suggest that enhanced NMDA receptor activation mediates time-dependent changes in excitability of RVM pain modulatory circuitry. The functional phenotypic switch of RVM neurons provides a novel mechanism underlying activity-dependent plasticity and enhanced net descending inhibition after inflammation.

INTRODUCTION

Recent studies have addressed the role of descending brain stem pathways in the development of dorsal horn hyperexcitability and chronic or persistent pain (see Dubner and Ren 1999; Ren et al. 2000 for reviews). A key finding is that in response to a persistent tissue injury, the activation of pain modulatory pathways is enhanced (Hurley and Hammond 2000; Kauppila et al. 1998; Ren and Dubner 1996; Schaible et al. 1991; Tsuruoka and Willis 1996; Urban and Gebhart 1999). The descending pathways also undergo time-dependent changes in excitability after injury. There is an initial decrease and a subsequent increase in the excitability of descending pathways following inflammation (Terayama et al. 2000a). Further, the increased descending modulation involves both facilitation and inhibition (Urban and Gebhart 1999; Wei et al. 1999; also see Vanderah et al. 2001), and the interaction between these pathways will dictate the development of spinal hyperexcitability and hyperalgesia.

Behavioral evidence suggests that changes in excitability occur in neurons in the rostral ventromedial medulla (RVM), a pivotal structure in pain modulatory circuitry (Fields et al. 1991), after hindpaw inflammation (Terayama et al. 2000a). In lightly anesthetized rats, focal electrical stimulation of the RVM inhibited nociceptive paw withdrawal and tail flick in an intensity-dependent manner. The intensity of the electrical stimulus needed to produce inhibition decreased over time, suggesting changes in the excitability of brain stem pain modulatory circuitry. Microinjection of selective N-methyl-D-aspartate (NMDA) receptor antagonists prevented the inflammation-induced increase in RVM excitability (Terayama et al. 2000a). Thus the enhanced descending modulation appears to be mediated by changes in the activation of the NMDA excitatory amino acid (EAA) receptor in the RVM. However, the cellular and molecular mechanisms underlying these dynamic changes in excitability remain unclear.

The NMDA receptor is assembled with NR1 and NR2A-D subunits. NR1 and NR2 subunits have been shown to be distributed in the RVM region (Watanabe et al. 1994; Wenzel et al. 1995). Two types of neurons, on and off cells, have been identified as pain modulatory neurons in RVM because their response properties are correlated with the nocifensive tail-flick reflex (see Fields et al. 1991). On cells are characterized...
by a sudden increase in firing immediately before initiation of the tail-flick reflex, and off cells exhibit a pause in activity just prior to the tail flick reflex. The third class of RVM neurons, neutral cells, show no consistent change in activity associated with transient nociceptive reflexes. The activity of on and off cells has been associated with facilitation and inhibition of pain transmission, respectively. The role of neutral cells in pain modulation is unclear. It is generally believed that they are not involved in descending modulation, although recent studies suggest that neutral cells do play a role (Ellrich et al. 2000). Whether these different classes of neurons play a role in the endogenous mechanisms responsible for changes in excitability in the RVM after inflammation is not known. In the present study, we provide new evidence that time-dependent plasticity in the pain modulatory circuitry involves changes in NMDA receptor gene expression and a phenotypic switch of RVM neurons. Preliminary results have been reported (Miki et al. 2000; Terayama et al. 2000b).

**Methods**

**Inflammation**

Male Sprague-Dawley rats (200–400 g, Harlan, Indianapolis) were used. Inflammation was induced with complete Freund's adjuvant (CFA, Sigma, St. Louis) suspended in an oil/saline (1:1) emulsion and injected (0.1 mg Mycobacterium sc) into the hindpaw. The injection produces an intense tissue inflammation characterized by erythema, edema, and hyperalgesia. This animal model has been approved by the University of Maryland Dental School Animal Care and Use Committee.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

Rats were overdosed with pentobarbital (100 mg/kg). A brain stem block including the ventral half and middle 3-mm portion of the rostral medulla was dissected at 2 and 5 h and 1, 3, 7, and 14 days after inflammation. This block of tissue mainly included RVM structures, but excluded the facial nucleus and inferior olive. Total RNA was isolated using the TRIzol reagent (Gibco BRL Life Technologies). The mRNA samples were treated with DNase I to remove residual genomic DNA. The first-strand cDNA was synthesized with SuperScript RNase H⁻¹ reverse transcriptase (Gibco BRL) at 42°C for 1 h. The reaction product was used as the RT-PCR template. The PCR primers were designed according to published sequences of NMDA receptor gene expression and a phenotypic switch of RVM neurons. Preliminary results have been reported (Miki et al. 2000; Terayama et al. 2000b).
onset of a paw withdrawal, allowing the activity of the neuron to be correlated to the paw withdrawal. The neurons were then classified according to the temporal relationship of their responses to the paw withdrawal behavior (see RESULTS). Neuronal and EMG activity were displayed on an oscilloscope and stored on a computer for off-line analysis with Spike 2 (CED). During continuous recordings, we ensured that recordings were from the same neurons by isolating units with a window discriminator and monitoring spike configuration throughout the recording session (Spike 2, CED).

Rats were overdosed with pentobarbital sodium (100 mg/kg) at the conclusion of the experiment and perfused with 4% paraformaldehyde. The sections of brain stem tissue were stained with cresyl violet for verification of recording sites according to Paxinos and Watson (1998). The RVM refers to the region including nucleus raphe magnus, nucleus reticularis gigantocellularis pars alpha, and nucleus paragigantocellularis lateralis.

To determine whether changes in neuronal activity after a noxious stimulus were statistically significant, a cumulative-sum (cusum) technique (Davey et al. 1986; Ren et al. 1989) was employed on each neuron. The cusum integrates differences from a mean control level of counts in a peristimulus time histogram and can be used to detect and verify changes in neuronal activity. In the present study, cusums were derived from peristimulus time histograms of neuronal activity by a bin-by-bin analysis (binwidth = 10 ms) and represent the cumulative differences from the mean activity level averaged from a 10-s period immediately before a stimulus (Davey et al. 1986; Ren et al. 1989). To differentiate the deviation of a cusum from normal fluctuations in neuronal activity, the variance of the cusum was calculated according to Davey et al. (1986). The statistical limit for normal fluctuations of cusums is set at ±3 SD of the cusum and plotted (Davey et al. 1986). The deviation of the cusum beyond the 3 SD of the cusum indicates a significant change in activity.

RESULTS

NMDA receptor gene expression

We examined NR1, NR2A, and NR2B subunit mRNA expression in the RVM tissue in naïve and hindpaw-inflamed rats. The RT-PCR analysis indicated that there was an upregulation of mRNAs encoding NR1, NR2A, and NR2B receptor subunits in the RVM after induction of inflammation and hyperalgesia (Fig. 1). The increase in the NR1, NR2A, and NR2B receptor mRNAs started at 5 h after inflammation, although only the increase in the NR2B subunit mRNA reached statistical significance at this time point \((P < 0.05; \text{Fig. } 1B)\). At 1–7 days post-CFA time points, the three NMDA receptor subunits exhibited significant increases in mRNA expression \((P < 0.05–0.001; \text{Fig. } 1B)\). All mRNAs returned to the control level by 14 days after inflammation. The upregulation of NMDA receptor protein was verified by Western blot analysis. We chose to examine the protein levels of the NR2A subunit, which showed the largest increase in mRNA levels. Figure 2A shows an example of Western immunoblot using polyclonal antibodies against the NR2A subunit of the NMDA receptor. Compared to naïve controls, there was an increase in NR2A subunit proteins after inflammation. ANOVA revealed a significant effect over the time course of experiment \((P < 0.01)\). Post hoc analysis indicated that the increase in NR2A protein levels was significant at 1–7 days post-CFA (Fig. 2B). These results are consistent with the hypothesis that enhanced NMDA receptor activation mediates time-dependent changes in excitability of RVM pain modulatory circuitry (Terayama et al. 2000a). These time-dependent changes in excitability led us to determine if there were changes in the response properties of RVM neurons.

RVM neurons in inflamed rats

Extracellular single-unit recordings were made from the brain stem in rats lightly anesthetized with pentobarbital sodium. Histological staining confirmed that the recording sites were localized to the RVM (Fig. 3). Most neurons \((n = 188)\) were recorded from the nucleus raphe magnus and nucleus reticularis gigantocellularis pars alpha. A few neurons \((n = 7)\) were in the lateral paragigantocellular nucleus or in the area just dorsal to the RVM.

We have correlated RVM neuronal activity with the paw withdrawal response to thermal stimuli in rats after inflammation. We describe these RVM cells as on-, off-, and neutral-like cells because of their similarity to previous studies in which neuronal responses were correlated with tail-flick nociceptive behavior (Fields et al. 1991). On-like cells were characterized by a sudden increase in firing immediately before initiation of the paw withdrawal response (Fig. 4A), off-like cells exhibited...
a depression in activity just prior to the paw withdrawal response (Fig. 4C), and neutral-like cells showed no change in activity associated with the nocifensive paw withdrawal behavior (Fig. 5, A and C). Cusum analysis indicated that stimulus-induced changes in neuronal activity were statistically significant (Fig. 4, B and D). Neuronal activity was better correlated with the nocifensive behavior than the onset of the heat stimulus (Fig. 5, B and D), consistent with previous studies using tail flick as a behavioral correlate (Fields et al. 1983b).

In contrast to previous studies, in the absence of inflammation, fewer off-like cells exhibited a complete pause before the paw withdrawal response to a noxious thermal stimulus (Fig. 6A). The percentage of off-like cells showing a complete pause of activity after noxious stimulation was significantly lower after inflammation ($\chi^2 P < 0.0001$, vs. naive rats, Table 1). Most off-like cells (97%) recorded from the inflamed rats showed a sudden large reduction of activity prior to paw withdrawal (Figs. 4C, 5B, and 6B). Although not statistically significant, off-like cells recorded in inflamed animals tended to have a higher level of background firing (mean, 18.9 ± 2.4; range, 0.50–59.1; $n = 36$), when compared with that in naive rats (mean, 13.8 ± 1.8; range, 3.3–19.7; $n = 10$). The difference in background activity may have contributed to the lack of a complete pause of activity of off-like cells in inflamed rats because it would be more difficult to induce a complete pause in firing when background activity is high.

**Phenotypic switch of RVM neurons**

Our previous studies have identified a critical period within 24 h after inflammation that dynamic changes in descending
modulation occurred (Terayama et al. 2000a). There is an initial decrease and a subsequent increase in net descending modulation at 3 and 9–12 h after inflammation, respectively. The enhanced descending inhibition appears to peak at 24 h after inflammation, a time consistent with an increase in NMDA receptor gene expression in RVM (see preceding text). We chose to record during this time frame with the expectation that changes in neuronal activity would be detected. It is apparent that the NMDA receptor mRNA and protein levels peaked and plateaued at 24 h after inflammation. Although we followed the longer time course in our biochemical experiments, we did not pursue further time points in neuronal recordings. We determined whether the response profiles of RVM neurons correlated with the temporal changes in excit-

FIG. 4. Examples of on-like (A and B) and off-like (C and D) cell activity. Horizontal bars on top of each panel indicate the duration of the thermal stimulus. Downward arrows indicate the onset of paw withdrawal. B and D plot peristimulus cusums of the on-like (B) and the off-like (D) cell, respectively. The hyperbola (arrowhead) in B and D indicate the theoretical cusum that is 3 SD from the baseline (horizontal dashed line). The portion of a cusum curve outside the theoretical 3 SD line is considered statistically significant. The cusums of on-like (A) and off-like (C) cells reached and crossed the threshold 3 SD line just prior to the onset of paw withdrawals.

FIG. 5. Examples of phenotypic changes of RVM neurons following inflammation. A: neutral-like cell, 9 h post-complete Freund’s adjuvant (CFA). B: panels illustrate that at 14.5 h post-CFA, the same neuron shown in A exhibited a reduction of activity immediately before the paw withdrawal, characteristic of an off-like cell. C: neutral-like cell, 2 h post-CFA. D: the same cell shown in C became an on-like cell at 5.5 h post-CFA when the cell showed a burst of activity immediately before the paw withdrawal. All histograms were averaged from 3 repeated trials (200-ms binwidth). The histograms are time-locked to the thermal stimuli (top row) and the onset of paw withdrawal (middle row), respectively. Small arrowheads indicate the onset of paw withdrawal’s (top row) or onset of thermal stimuli (48°C, middle and bottom rows) for individual trials, respectively. Note that the changes in neuronal activity are best correlated with the paw withdrawal response. Bottom row: the cusums derived from the histograms of middle panels. The cusums of neutral-like cells were clustered around the baseline (A and C). The cusums of off-like (B) and on-like (D) cells were outside the threshold 3 SD line just prior to the onset of paw withdrawal (also see Fig. 4).
ability in the RVM after inflammation. In the first set of experiments, we recorded activity from single units for 3–6.5 h starting from 2 to 11 h and ending from 7 to 17 h after CFA injection. One neuron was recorded between 18 and 23 h after inflammation. The similarity of the action potential waveforms and the constant levels of background firing over the recording period assured us that the same neuron was being analyzed (Figs. 7 and 8). During the course of inflammation, some neutral-like cells changed their activity and exhibited response profiles of off- and on-like cells. As shown in Fig. 7, a neuron was classified as a neutral-like cell initially (Fig. 7A, 19 h post-CFA). The same neuron started to exhibit an on-like response profile to 48°C stimulus at 20 h (Fig. 7B) and maintained such a profile during the course of the recording (Fig. 7, C and D). As shown in Fig. 5A, at 9 h after CFA injection, another neuron was classified as a neutral-like cell because there was not a clear relationship between the firing pattern and the paw withdrawal response. This neuron started to show off-like activity from 10.5 h after CFA injection. Figure 5B shows that at 14.5 h after CFA near the end of the recording period, there was a large reduction of activity of this neuron after the start of the noxious heat stimulus and immediately prior to the onset of the paw withdrawal, characteristic of an off-like cell. The horizontal bars below the histograms indicate the duration of 48°C thermal stimuli. Arrows indicate the onset of a paw withdrawal response. Action potential waveforms of this cell during the 5-h recording are shown to the right of each panel. Each action potential trace represents 15 overlapping waveforms taken from digital files (Spike 2 Waveform configuration).

**Table 1.** Comparison of off-like cell activity between naïve and CFA-inflamed rats using paw withdrawal as a behavioral correlate

<table>
<thead>
<tr>
<th></th>
<th>Pause</th>
<th>Depressed</th>
<th>Total</th>
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<tbody>
<tr>
<td>Naïve</td>
<td>7 (70)</td>
<td>3 (30)</td>
<td>10</td>
</tr>
<tr>
<td>CFA 24 h</td>
<td>1 (3)</td>
<td>35 (97)</td>
<td>36</td>
</tr>
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χ² P < 0.0001. Parentheses enclose percentages. CFA, complete Freund’s adjuvant.
on-like neurons in inflamed rats were statistically significant (Fig. 5). Eleven of 15 cells that were initially neutral-like cells exhibited maintained profile changes during recordings (became off-like cells, \( n = 5 \); became on-like cells, \( n = 6 \)). The remaining four neutral-like cells recorded post-CFA exhibited neutral-like profiles at the end of the recording period.

The changes in response profiles of RVM neurons did not appear to correlate with a change in background firing. As shown in Fig. 8, C and D, most RVM neurons exhibited relatively constant levels of background activity. Among neurons showing phenotypic changes, only two neutral-to-on-like cells showed a relatively large reduction of background firing during or after the changes in response profile (Fig. 8D). The changes in response profiles were not transient. Neurons that were switched from neutral-like to on- or off-like cells did not reverse to neutral-like cells in the remaining recording session that lasted for 2.3 ± 0.4 h (range, 1–4.5 h).

In a second set of experiments, we recorded neurons from noninflamed (\( n = 72 \)) and 24 h post-CFA (\( n = 93 \)) animals for shorter time periods (Table 2). There was a significant increase in the percentage of on- and off-like cells and a decrease in the percentage of neutral-like cells recorded 24 h after CFA from heat stimulation of the inflamed paw as compared with naïve rats (\( P < 0.001, \chi^2 \)). The mean peak response frequency of on-like cells was significantly increased in inflamed as compared with naïve rats (\( P < 0.05 \); Fig. 9A). For off-like responses, the mean percent reduction of neuronal activity immediately before the onset of a withdrawal response was significantly less in inflamed rats (\( P < 0.001 \); Fig. 9B).

**DISCUSSION**

We have presented new evidence that persistent inflammation induces changes in NMDA receptor gene expression and a phenotype switch of neurons in the RVM pain-modulatory circuitry. The findings provide a cellular mechanism that underlies the activity-dependent plasticity contributing to modulation of persistent pain at supraspinal in addition to spinal levels. The response profile switch of brain stem neurons suggests a novel mechanism for enhanced descending inhibition after injury.

EAAs and their receptors play a prominent role in descending modulatory effects originating from brain stem sites (Aimone and Gebhart 1986; Beitz 1990; Jensen and Yaksh 1992; Jones and Gebhart 1988; Spinella et al. 1996). All three classes of RVM cells (on, off, and neutral) are activated by exogenous administration of glutamate (Heinricher and Roychowdhu 1997). These previous studies, however, did not address the possible changes in EAA receptor gene expression associated with activation of pain modulatory circuitry. Recently, it was shown that the administration of the NMDA

**TABLE 2.** Distribution of RVM neuronal types in rats with inflamed versus non-inflamed paws

<table>
<thead>
<tr>
<th>Treatment</th>
<th>On-like</th>
<th>Off-like</th>
<th>Neutral-like</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>11 (15)</td>
<td>10 (14)</td>
<td>51 (71)</td>
<td>72</td>
</tr>
<tr>
<td>24 h CFA</td>
<td>30 (32)</td>
<td>26 (28)</td>
<td>37 (40)</td>
<td>93</td>
</tr>
</tbody>
</table>

\( \chi^2 P < 0.001 \). Parentheses enclose percentages. RVM, rostral ventromedial medulla.
selective receptor antagonist, 2-amino-5-phosphonovaleric acid (AP-5), into the RVM, blocked the facilitation of the tail flick reflex for 1–3 h produced by mustard oil application to the hindleg (Urban et al. 1999), indicating the involvement of EAAs in endogenous mechanisms of persistent pain. It was further established that the time-dependent increase in excitability in brain stem circuitry associated with persistent nociceptive activity is NMDA receptor dependent (Terayama et al. 2000a). The present study demonstrates that there is a prolonged upregulation of NMDA receptor subunit gene expression and protein levels in the brain stem in response to persistent peripheral tissue injury. This upregulation occurred at a time when there were dramatic changes in descending modulation (Ren and Dubner 1996; Terayama et al. 2000a). These results complement our previous findings that increased EAA neurotransmission contributes to inflammation-induced hyperexcitability of RVM circuitry and enhanced descending inhibition of nociception.

The central terminals of peripheral nociceptors in the spinal dorsal horn release a number of chemical mediators including the EAA, glutamate, the major excitatory neurotransmitter in the dorsal horn in the brain stem in response to persistent peripheral tissue injury. This upregulation occurred at a time when there were dramatic changes in descending modulation (Ren and Dubner 1996; Terayama et al. 2000a). These results complement our previous findings that increased EAA neurotransmission contributes to inflammation-induced hyperexcitability of RVM circuitry and enhanced descending inhibition of nociception.

In previous studies, the response properties of three classes of neurons in the RVM, on, off, and neutral cells have mainly focused on animals without preexisting injury (Fields et al. 1983a, 1991; Heinricher et al. 1999; Mason 1997; Morgan and Fields 1994). We have used paw withdrawal as a behavioral correlate in lightly anesthetized rats to assess the relationship between increased hindpaw responsiveness and RVM neuronal activity. We describe these RVM neurons as “on-like,” “off-like,” and “neutral-like” cells, respectively, according to the relationship of their responses to the paw withdrawal behavior. Consistent with previous studies using tail flick as a behavioral correlate (Fields et al. 1983b; Heinricher et al. 1989), the changes in neuronal activity are best correlated with the paw withdrawal behavior.

**FIG. 9.** Mean changes in responses of RVM on- and off-like cells to the noxious thermal stimulus in noninflamed and inflamed rats. A: the peak response frequency of on-like cells immediately prior to the onset of a withdrawal response was compared between the naïve and CFA-inflamed rats. B: the percent reduction of neuronal activity of off-like cells before the onset of a withdrawal response. Note a nearly 90% reduction of activity in naïve rats and only a less than 60% reduction of activity in CFA-inflamed rats.
withdrawal response. We also introduced a cumulative sum analysis to verify statistically that the responses of on- and off-like cells were related to nociception. Our preliminary results showed that morphine inhibited on-like cell and facilitated off-like cell activity (unpublished observations), consistent with their role in pain modulation (also see Fields et al. 1991). One difference noted from previous studies using tail flick as a behavioral correlate was that off-like cells did not always exhibit a complete pause of activity after a noxious stimulus to the hindpaw. This was particularly obvious in inflamed rats, perhaps due to an increase in ascending input and a decrease in GABAergic inhibition of off cell activity (see Fields et al. 1991).

An important observation in the present study was that some initially classified neutral-like cells changed their response profile and were reclassified as on- or off-like cells during the continuous recordings when there was ongoing inflammation. The analysis of interspike intervals indicates that no spike intervals were within the range (approximately 0.5–1 ms) of the absolute refractory period of an action potential in our recording, suggesting that only one neuron was recorded. By monitoring the action potential waveforms and the levels of background activity, we were assured that the same neuron was being analyzed during long-term recordings. The possibility that the phenotype changes we found in 11 neurons is due to a shift in recording to a second neuron with the same waveform and level of background activity (note the wide range of background activity of RVM neurons) is unlikely. The apparent changes in response profiles of RVM neurons do not appear to be related to changes in background activity nor the anesthetic state. The background activity remained constant during the 3- to 6.5-h recording periods for almost all neurons. There also were no differences in the mean interspike interval and coefficient of variation of the interspike intervals between noninflamed and inflamed animals. In addition, we have been able to maintain relative constant levels of anesthesia in pentobarbital-anesthetized rats for a period >9 h (Terayama et al. 2000a). We maintained the recordings for 1–4 h after changes in neuronal profile to confirm that the changes were not temporary. One earlier study suggested that there might be an increase in the expression of off-like cells after inflammation (Montagne-Clavel and Oliveras 1994).

One caveat of the long-term extracellular single-unit recording is that, even with all precautions, one cannot be absolutely certain that the same one neuron is recorded since spike waveform may change over time and two neurons may have nearly identical waveforms. To verify the profile change of RVM neurons, we performed a population study. There was a significant increase in the percentage of on- and off-like cells and a decrease in the neutral-like cell population after inflammation, thus supporting the observations in the long-term recording experiments. It should be noted, however, that the population of neurons recorded in the present study in noninflamed and inflamed rats may not be represented equally because some RVM neurons may be overlooked due to their low levels of background activity and high mechanical thresholds. To avoid damage of the skin by repeated high-intensity stimuli, we did not use a high-intensity mechanical stimulus as a searching stimulus. If some neurons were to become spontaneously active with inflammation, they must be missed in the noninflamed group. Nevertheless, our combined population studies and long-term single-unit recordings are strongly suggestive of phenotypic changes in neutral cells after inflammation.

Our present findings do not rule out the possibility that the changes in excitability of RVM neurons may be secondary to inflammation-induced changes in the spinal cord as we focused on responses of RVM neurons to noxious stimulation of the inflamed paw in the present study. However, previous evidence indicates that this is unlikely. The enhanced descending modulation after inflammation occurred in inflamed as well as noninflamed paws. This has been demonstrated by electrical stimulation of the RVM (Terayama et al. 2000a), chemical stimulation of RVM with EAA receptor agonists (Guan et al. 2000), and microinjection of opioid receptor agonists into the RVM (Hurley and Hammond 2000). Further, direct stimulation of the dorsolateral funiculus that bypasses brain stem synaptic mechanisms does not produce a similar change in excitability in descending pathways (Terayama et al. 2000a).

The increased excitability in RVM circuitry after inflammation involves both facilitatory and inhibitory synaptic activity. Immediately before the onset of a withdrawal response there was a greater increase in on-like responses. The off-like responses, on the other hand, were reduced after inflammation as suggested by a less reduction of neuronal activity after a noxious stimulus and a lack of a complete pause. Because a pause of off-like cell firing is associated with disinhibition, a less reduction of neuronal activity and lack of a complete pause suggest an increase in inhibitory synaptic activity. These results confirm that the enhanced descending modulation after inflammation involves both facilitation and inhibition as there are changes in both on- and off-like cell activity. This effect of inflammation is different from that of morphine, which suppresses cell activity and increases off cell activity (Fields et al. 1991). However, it is difficult to predict the net effect of descending modulation from changes in single neuronal activity without recording from a large population of neurons (also see Heinricher and McGregor 1998). Our previous studies using a behavioral measure as an endpoint indicate an increased net inhibition arising from RVM during the later phase of inflammation (Terayama et al. 2000a). The mechanisms underlying the enhanced synaptic activation in RVM circuitry after inflammation will require further investigation.

The present finding suggests that neutral-like cells also play a role in the endogenous mechanisms responsible for changes in excitability in the RVM after inflammation. The role of the neutral cell in opioid and nonopioid descending inhibition in response to transient noxious stimuli is poorly understood, although a subgroup of neutral cells are serotonin-containing, project to the spinal dorsal horn, and express mu-opioid receptors (Gao et al. 1998; Mason 1997; Potrebic et al. 1994; Wang and Wessendorf 1999). Our previous studies suggest the involvement of serotonergic RVM neurons in increased descending inhibition after inflammation (Wei et al. 1999). However, we have analyzed interspike intervals separately for individual classes of neurons and found that the neurons recorded in the present study are largely not serotoninergic neurons according to Mason (1997) (data not shown). Thus the nonserotonergic population of RVM cells is also involved in enhanced descending inhibition after inflammation. The switch of the response profiles of RVM neurons correlated with the temporal changes in excitability in the RVM after inflammation (Terayama et al. 2000a) and changes in NMDA receptor...
gene expression. We propose that neutral-like cells in the RVM constitute a functional class that has the capacity to enhance net descending inhibition. The receptor mechanisms that account for the phenotypic changes in neutral-like cells after inflammation appear to be dependent on NMDA receptor activation. However, further studies are required to identify the subclasses of RVM neurons that exhibit profile change after inflammation and are also modulated by glutamatergic transmission. It is likely that opioid peptide activation (Hurley and Hammond 2001) or GABA disinhibition, or both, are also important in the initiation and maintenance of this RVM plasticity (see Fields and Basbaum 1999)

These findings emphasize that increased excitability in the spinal dorsal horn after inflammation leads to increased NMDA receptor gene expression and pain modulatory neuronal activity in the RVM and enhanced descending modulation that may include shifts in the balance between inhibitory and facilitatory components. This activity-dependent plasticity in descending pain modulatory circuitry complements the activity-dependent neuronal plasticity in ascending pain transmission pathways (Dubner and Ruda 1992).

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