Plasticity in Excitatory Amino Acid Receptor-Mediated Descending Pain Modulation after Inflammation

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ABSTRACT

The role for excitatory amino acids (EAAs) in the rostral ventromedial medulla (RVM) in descending pain modulation after persistent noxious input is unclear. In an animal model of inflammatory hyperalgesia, we examined the effects of intra-RVM microinjection of EAA receptor agonists and antagonists on paw withdrawal and tail-flick responses in lightly anesthetized rats. N-Methyl-D-aspartate (NMDA) produced effects that depended upon the postinflammatotary time period. At 3 h postinflammation, NMDA induced facilitation at a lower dose (10 pmol) and inhibition at a higher dose (1000 pmol). At 24 h postinflammation, NMDA (0.1–1000 pmol) produced a dose-dependent inhibition. The facilitation and inhibition, respectively, were attenuated significantly by the preadministration of an NMDA receptor antagonist, di-2-amino-5-phosphonovaleric acid (APV) (10 pmol, \( P < 0.05 \)), to the same site. Intra-RVM microinjection of \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (0.1–100 pmol) produced dose-dependent inhibition at both 3 and 24 h postinflammation that was blocked by the preadministration of an AMPA/kainate receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (100 pmol, \( P < 0.05 \)). Unexpectedly, AMPA-produced inhibition was also significantly attenuated by preadministration of APV (10 pmol, \( P < 0.05 \)). Compared with 3 h postinflammation, both NMDA and AMPA showed a leftward shift in their dose-response curves at 24 h postinflammation. These results demonstrate that NMDA and AMPA receptors in the RVM are involved in the descending modulation after inflammatory hyperalgesia. There is a time-dependent increase in EAA neurotransmission in the RVM after inflammation and NMDA receptors play an important role in AMPA-produced inhibition.

Descending pathways from the brain stem rostral ventromedial medulla (RVM) represent an important supraspinal mechanism in modulating spinal nociceptive transmission (Fields and Basbaum, 1978; Sandkühler and Gebhart, 1984; Willis, 1988; Urban and Gebhart, 1997; Gjerstad et al., 2001). Both inhibitory and facilitatory descending influences on nociceptive transmission can be simultaneously engaged throughout the RVM, a structure that includes the midline nucleus raphe magnus (NRM) and adjacent lateral reticular formation (Fields et al., 1983; Aimone and Gebhart, 1986). Although most earlier studies focused on responses to transient noxious stimuli (Fields and Basbaum, 1978; Fields et al., 1983; Sandkühler and Gebhart, 1984; Willis, 1988), recent evidence suggests that descending pathways from RVM also modulate spinal nociceptive transmission during inflammatory pain, and play a role in the development of persistent pain (Cervero et al., 1991; Montagne and Oliveras, 1994; Ren and Dubner, 1996; Wei et al., 1998; Danziger et al., 1999; Urban and Gebhart, 1999; Terayama et al., 2000a). A number of reports demonstrates that descending pain modulation is not fixed but exhibits changes in response to persistent noxious input under various conditions (Schaible et al., 1991; Ren and Dubner, 1996; Danziger et al., 1999; Dubner and Ren, 1999; Hurley and Hammond, 2000). The activity of the RVM pain modulatory circuitry increases during persistent inflammation and gives rise to enhanced descending pain inhibition (Schaible et al., 1991; Ren and Dubner, 1996; Wei et al., 1998; Hurley and Hammond, 2000; Terayama et al., 2000a) as well as facilitation (Urban et al., 1999; Wei et al., 1999; Terayama et al., 2000a). Collectively, these studies demonstrate an active modulation of spinal excitability and nocifensive behavior by the brainstem pain modulatory circuitry. However, the chemical mechanisms underlying this activity-induced plasticity in the RVM are unclear.

Although the inflammation-induced changes in neurotransmitter synthesis, receptor gene expression, and responses of dorsal horn neurons have been well studied at the spinal level (Iadarola et al., 1988; Dubner and Rudy, 1992), little is understood about the changes that may occur at the

ABBREVIATIONS: RVM, rostral ventromedial medulla; NRM, nucleus raphe magnus; EAA, excitatory amino acid; ES, electrical stimulation; NMDA, N-methyl-D-aspartate; APV, di-2-amino-5-phosphonovaleric acid; AMPA, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; PW, paw withdraw; TF, tail flick; CFA, complete Freund’s adjuvant; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline.
RVM level. Studies suggest that EAA and receptors play an important role in the descending pain modulation from the RVM (Aimone and Gebhart, 1986; Praag and Frenk, 1990; Jensen and Yaksh, 1992; Urban et al., 1999). In naïve animals, glutamate microinjected into the RVM induces biphasic descending modulatory effects on nociceptive transmission, showing facilitation at low doses and inhibition at higher doses (Zhuo and Gebhart, 1997); administration of EAA receptor antagonists into RVM increases the intensity of electrical stimulation (ES) necessary for inhibiting the nociceptive tail-flick in naïve animals (Aimone and Gebhart, 1986), suggesting that local interneurons in the RVM may also be involved in the release of EAA. However, little is known about roles of EAA in the RVM in descending modulation of persistent pain and hyperalgesia. Using a lightly anesthetized preparation of inflammatory hyperalgesia in rats, we investigated the effects of NMDA and AMPA subtype glutamate receptor agonists and antagonists microinjected in the RVM on paw withdrawal (PW) and tail-flick (TF) response latencies during the development of inflammatory hyperalgesia. Our results demonstrate that persistent hyperalgesia induces time-dependent enhancement of EAA neurotransmission in the RVM that involves both NMDA and AMPA receptors. The present study also revealed a novel type of interaction between NMDA and AMPA receptors in the RVM.

Materials and Methods

Animal Preparation. Adult male Sprague-Dawley rats (250–350 g; Harlan Bioproducts for Science, Indianapolis, IN) were on a 12:12-h light/dark cycle and received food and water ad libitum. For all behavioral and pharmacological experiments, rats were initially anesthetized with 45 mg/kg i.p. pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL). A catheter was inserted into a femoral vein and craniectomy was performed for intracerebral microinjection (see below). After surgery, wound margins were covered with a local anesthetic ointment and a light level of anesthesia was maintained thereafter throughout the course of the experiment (4–6 h) by an intravenous infusion of pentobarbital sodium (3–10 mg/kg/h). The level of anesthesia was judged by consistent PW and TF responses without other exaggerated nociceptive behaviors (Fields et al., 1983). The body temperature was maintained at 37–38°C through a water-circulated warm blanket.

Induction of Inflammation. Inflammation was induced with complete Freund’s adjuvant (CFA) suspended in an oil/saline (1:1) emulsion and injected s.c. (0.1 mg of Mycobacterium) into the plantar surface of one hindpaw. The injection produced an intense tissue inflammation of the hindpaw characterized by erythema, edema, and hyperalgesia that was confined to the injected hindpaw. The presence of thermal hyperalgesia in awake animals as indicated by a cumulative animal model has also been approved by the University of Maryland Dental School Animal Care and Use Committee. The International Association for the Study of Pain ethical guidelines for the treatment of animals were adhered to in these experiments (Zimmerman, 1983).

Behavioral Nociceptive Testing. Nociceptive behaviors of the rat were tested by a method modified from Hargreaves et al. (1988). Briefly, the rat was placed in a stereotaxic apparatus (David Kopf model 900) and placed on a glass platform that was maintained at 25°C. A noxious thermal stimulus was delivered by a radiant heat device from underneath the glass, and the light beam was positioned under either hindpaw or tail. The time for the rat to remove the paw or tail from the thermal stimulus was recorded to the nearest 0.1 s as the PW latency and TF latency, respectively. The intensity of the stimulus was set to produce a PW latency between 9 and 11 s in a naïve rat. The heat source was a high-intensity projector lamp bulb (Osram 58-8007; 8 V, 50 W). Noxious heat was applied at >2-min intervals and a 20-s cut-off value was used to prevent damage to the skin.

Intracerebral Microinjection. After a midline incision, an opening was made in the skull with a dental drill for lowering a guide cannula into the target area. A 26-gauge stainless steel guide cannula (C915 G; Plastics One, Roanoke, VA) was stereotaxically placed in the RVM. Drugs were microinjected into the RVM through a 33-gauge internal cannula (C915 I; Plastics One) inserted through and extending 1.0 mm beyond the tip of the guide cannula. The internal cannula was connected to a 10-μl Hamilton syringe by polyethylene-10 tubing. The stereotaxic coordinates for the RVM were 2.0 mm caudal to the interaural line (on the midline) and 9.0 mm beneath the surface of the cerebellum (Paxinos and Watson, 1998). All microinjections (0.5 μl) were performed by delivering drug or vehicle solution slowly over a 1-min period. The injection was continuously monitored by following the movement of an air bubble in the tubing. The behavioral tests were conducted immediately before and at 2 to 30 min after intra-RVM drug microinjections.

Experimental Design. Initial experiments were done to assess the effects of two glutaminergic receptor agonists, NMDA and AMPA, microinjected into the RVM on inflammatory hyperalgesia. Based on our preliminary studies, the experiments were conducted at 3 and 24 h postinflammation. Two key phenomena occur at these time points. At 3 h postinflammation, there is a reduced net descending inhibition, whereas at 24 h postinflammation, there is an enhanced net descending inhibition (Terayama et al., 2000a). At 3 and 24 h postinflammation, respectively, rats were maintained at a light level of anesthesia by an intravenous infusion of pentobarbital sodium (3–10 mg/kg/h). After the establishment of stable PW and TF latencies, cumulative dosing for either NMDA, AMPA, or the same volume of saline was microinjected into the RVM. The subsequent injection of a higher dose was given into the same brain stem site at >30-min interval after the previous injection (a time by which the PW and TF response latencies had returned to the preinjection level). The following doses were used: 0.1, 1.0, 10, 50, 100, and 1000 pmol of NMDA and 0.1, 1.0, 10, 50, and 100 pmol of AMPA. Saline (0.5 μl) was used as a drug control. In a second series of experiments, the effect of single doses of NMDA and AMPA receptor agonists on the PW and TF responses and the effects of their antagonists were determined. At 3 or 24 h postinflammation, either 10 pmol of APV, a selective NMDA receptor antagonist; 100 pmol of NBQX, an AMPA/kainate receptor antagonist; or saline was administered into the RVM at 10 min before either NMDA (3 h, 10 pmol; 24 h, 1000 pmol), AMPA (3 h, 10 pmol; 24 h, 100 pmol), or saline into the same site. Both PW and TF latencies were then determined after the second administration. A final series of experiments were conducted to explore the endogenous mechanisms of EAA transmission in descending modulation from the RVM by examining the effects of intra-RVM application of NMDA or AMPA receptor antagonists alone on the TF and PW latencies. Similar to the protocol used in the agonist study, in naïve animals and in inflamed animals at 24 h post-CFA, cumulative doses of APV (0.1, 1.0, and 10 pmol) or NBQX (0.1, 1.0, and 10 pmol) were microinjected into the RVM and PW and TF latencies were determined. Each experimental group consisted of six to eight rats. The drug doses examined in the present study were based on previous studies and verified in our pilot experiments (Hösli et al., 1983;
Histology. At the end of the experiment, rats were overdosed with pentobarbital sodium (100 mg/kg i.p.) and perfused with 4% paraformaldehyde. The coronal brain sections (40 μm) were stained with cresyl violet for verification of the sites of microinjection. The RVM refers to the region including the NRM, the adjacent nucleus reticularis gigantocellularis pars alpha and the nucleus paragigantocellularis lateralis. Only data from the RVM site were included in the analysis in all experiments.

Data Analysis and Statistics. Data are represented as means ± S.E.M. The effect of any given drug treatment was determined by monitoring the maximum response latency change during the 2- to 4-min period after drug administration. Unless indicated otherwise, data for PW and TF latencies are represented as percentage of predrug baseline latency (% baseline). Changes in the PW and TF response latencies after drug administration were analyzed by repeated measures analysis of variance with Fisher’s protected least significant difference as the post hoc test. Data for single dose experiments at 3 h postinflammation were analyzed by analysis of covariance. P < 0.05 was considered statistically significant in all tests.

Drugs. The drugs used in these experiments were NBQX disodium, APV, NMDA, and AMPA. All drugs were purchased from Sigma Chemical (St. Louis, MO). Stock solutions were freshly prepared by dissolving the drugs in sterile saline (0.9%) and then diluted as needed. All dosages reflect the salt form of the drugs. In pharmacological experiments, the investigator who performed the behavioral test was unaware of the drug treatment conditions.

Results

Throughout the course of experiments, consistent levels of PW and TF latencies were achieved in lightly anesthetized animal preparation in which nocifensive responses to hindpaw and tail noxious heat stimulation were intact and mimicked that in awake rats (Hargreaves et al., 1988, Iadarola et al., 1988, Terayama et al., 2000a). The time course of the development of inflammatory hyperalgesia has been established and described in our previous study (Terayama et al., 2000a). In the present study, the PW latency of the inflamed paw was significantly reduced from 10.6 ± 0.14 to 6.36 ± 0.28 s at 3 h after the injection of the inflammatory agent CFA (P < 0.001) and maintained at least for 24 h. There were no significant changes in response latencies in TF (10.58 ± 0.06 s) and the contralateral noninflamed paw (10.39 ± 0.13 s) after inflammation. The presence of increased sensitivity to noxious thermal stimuli of the inflamed hindpaw is similar to thermal hyperalgesia in awake animals (Terayama et al., 2000a). Thus, the reduction of PW latency in the noninflamed paw in the lightly anesthetized animal preparation can be considered a reliable measure of behavioral hyperalgesia. This preparation allows repeated intra-RVM microinjection that cannot be easily achieved in awake animals.

Effects of intra-RVM Microinjection of EAA Receptor Agonists on Descending Pain Modulation in Inflamed Rats. Initial experiments were conducted to assess the effects of two prototype ionotropic glutaminergic receptor agonists, NMDA and AMPA, on PW and TF latencies at different time periods of inflammatory hyperalgesia. Figure 1 illustrates the time course of increase in PW and TF latencies produced by microinjection of 1000 pmol of NMDA (n = 8) and 100 pmol of AMPA (n = 6) at 24 h postinflammation, respectively. The brain stem sites for NMDA and AMPA microinjection are illustrated on representative coronal brain sections (Paxinos and Watson, 1998) in Fig. 1, A and B, respectively. Both NMDA and AMPA produced a significant increase in withdrawal latencies on the inflamed hindpaw, contralateral noninflamed hindpaw, and tail. The drug effect was rapid in onset and short lasting. Typically, the peak effect occurred within 2 to 4 min of microinjection, persisted through 1 to 2 min, and was substantially diminished by 20 min after microinjection. Saline did not induce any significant effect. The dose-response curves for NMDA (Fig. 2) and AMPA (Fig. 3) were further established through cumulative dosing studies in the inflamed animals at 3 and 24 h postinflammation, respectively. NMDA produced effects that depended upon the postinflammatory time period. At 3 h postinflammation, NMDA produced a biphasic modulatory effect on PW and TF reflexes that was dose-dependent. Microinjection of low doses of NMDA (10 pmol) produced facilitation on both PW and TF responses that occurred at 2 to 4 min after administration, as indicated by a reduction in the response latencies (n = 8, P < 0.05). A higher dose of NMDA (1000 pmol) produced a significant inhibition of PW and TF responses (Fig. 2; n = 8, P < 0.01). AMPA produced only dose-dependent inhibition of the PW and TF latencies at the 3-h time point. No significant AMPA-produced facilitation was observed (Fig. 3). The facilitatory effect of 10 pmol of NMDA was further confirmed in single dosing experiments. Either 10 pmol of NMDA, 10 pmol of AMPA, or saline was
injected in the RVM at 3 h postinflammation and their effects on the PW and TF latencies were monitored. The 10-pmol dose of NMDA induced a significant facilitatory effect on both PW and TF latencies compared with that of the saline control (Fig. 4; n = 6, P < 0.05). Neither 10 pmol of AMPA (Fig. 4; n = 6) nor saline (data not shown) produced any significant modulatory effects on the PW and TF reflexes. The brainstem sites for NMDA microinjection are illustrated on a representative coronal brain section (Fig. 4) (Paxinos and Watson, 1998). At a later time period of inflammation (24 h postinflammation), both NMDA (Fig. 2) and AMPA (Fig. 3) increased PW and TF latencies in a dose-dependent manner.

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**Fig. 2.** Effects of intra-RVM microinjection of NMDA, on the PW and TF latencies in inflamed rats at 3 h (■) and 24 h postinflammation (●). Change of the PW and TF latencies are represented as a percentage of change of the predrug baseline latencies. Cumulative dosing was performed in these experiments. Intra-RVM microinjection of NMDA produced a biphasic descending modulation on the PW and TF latencies at 3 h postinflammation. NMDA produced a significant decrease of the PW and TF latencies with 10 pmol and a significant increase with 1000 pmol. At 24 h postinflammation, NMDA produced only dose-dependent inhibition of the PW and TF latencies. Symbols denote significant difference between 3- and 24-h groups (✦) and between drug treatment and saline control group (★). Each group consists of six to eight rats. ★, P < 0.05 to 0.01.

**Fig. 3.** Effects of intra-RVM microinjection of AMPA on the PW and TF latencies in inflamed rats at 3 (●) and 24 h postinflammation (■). Change of the PW and TF latencies are represented as a percentage change of the predrug baseline latencies. Cumulative dosing was performed in these experiments. Intra-RVM microinjection of AMPA produces a dose-dependent inhibition of the PW and TF latencies at both 3 h and 24 h postinflammation. Symbols denote significant difference between 3- and 24-h groups (✦) and between drug treatment and saline control group (★). Each group consists of six rats. ★, P < 0.05 to 0.01.
Effects of intra-RVM Microinjection of EAA Receptor Antagonists Alone on PW and TF Latencies. We determined the effects of intra-RVM administration of AMPA and NMDA receptor antagonists alone on the baseline PW and TF latencies in naive animals and in inflamed animals at 24 h postinflammation. APV produced no significant modulatory effects on PW and TF latencies in naive animals and in inflamed animals at the 0.1- to 10-pmol dose range examined in the present study. NBQX (10 pmol) induced a significant facilitation on both PW and TF latencies (n = 5, P < 0.05) in inflamed animals at 24 h postinflammation. The response latencies for PW and TF responses were reduced by 12.9 ± 6.4, 14.4 ± 4.4, and 9.3 ± 1.4% of the predrug baseline levels for the inflamed paw, the noninflammed paw, and the tail, respectively. NBQX (10 pmol) also induced a significant facilitation of PW and TF in naive animals (n = 5, P < 0.05). The drug effect was short-lived. The peak effect occurred within 2 to 4 min of microinjection, persisted through 1 to 2 min, and was diminished by 10 min after microinjection.

Effects of EAA Receptor Antagonists on NMDA- and AMPA-Produced Descending Modulation. In single dosing studies, the effects of the NMDA- and AMPA-selective antagonists in attenuating the observed NMDA- or AMPA-produced modulation were also studied. At 3 h postinflammation, the facilitation of the PW and TF produced by 10 pmol of NMDA was significant reversed by the preadministration of 10 pmol of APV, a competitive NMDA receptor antagonist (Fig. 4; n = 6, P < 0.05). At 24 h postinflammation, the effects of a single dose of 1000 pmol of NMDA or 100 pmol of AMPA on inflammatory hyperalgesia were first determined. Consistent with results from the cumulative dosing study, NMDA (Fig. 5) and AMPA (Fig. 6) produced significant inhibition of the PW and TF latencies (n = 6, P < 0.01). The inhibitory effects were significantly attenuated by predadministration of the respective selective antagonists, APV (10 pmol) (Fig. 5; n = 6, P < 0.05) and NBQX (100 pmol) (Fig. 6; n = 6, P < 0.01), at the same site. Unexpectedly, the AMPA-produced inhibition was significantly attenuated not only by the AMPA/kainate receptor antagonist NBQX but also by 10 pmol of APV, an NMDA receptor antagonist (Fig. 6; n = 6, P < 0.05), whereas NMDA-produced inhibition was not attenuated by the pretreatment of 100 pmol of NBQX (Fig. 5; n = 6, P > 0.05).

Effects of intra-RVM Microinjection of EAA Receptor Antagonists Alone on PW and TF Latencies.
The finding that NMDA produced descending facilitation suggests that the descending facilitatory effect after inflammation is dependent on NMDA receptor activation and occurs early after inflammation. This is consistent with results from other studies (Coutinho et al., 1998; Urban et al., 1999). Microinjection of NMDA into the supraspinal sites, including the RVM, produced nociceptive behaviors and facilitated the TF reflex in naive rats (Urban and Gebhart, 1999), whereas administration of APV blocked the facilitation of the TF reflex (Urban et al., 1999) and reversed visceral hyperalgesia (Coutinho et al., 1998). These studies and our present findings suggest that activation of the rostral medullary NMDA receptor after inflammation contributes to inflammatory hyperalgesia. The enhanced descending facilitation with low doses of NMDA at the early time period after inflammation may result in decreased net descending inhibition.

Over time, both the low doses and higher doses of NMDA significantly enhanced descending inhibition, resulting in a leftward shift of the NMDA dose-response curve. The switch from facilitation to inhibition at 24 h postinflammation with lower NMDA doses and the leftward shift of the NMDA dose-response curve mask any facilitatory effects and contribute to the progressively enhanced net descending inhibition. Therefore, our data support the conclusion that the time-dependent changes in descending modulation of inflammatory hyperalgesia involve NMDA receptor activation in the RVM. Indeed, our recent reverse transcription polymerase chain reaction study revealed a time-dependent up-regulation of NMDA receptor NR1 and NR2A subunit mRNA levels in the RVM after hindpaw inflammation (Terayama et al., 2000b), suggesting that increased NMDA receptor gene expression may contribute to the enhanced EAA neurotransmission.

AMPA microinjected into the RVM produced significant inhibition of the PW response of both inflamed and noninflamed sides as well as the TF at 3 and 24 h postinflammation. The AMPA-produced inhibition was significantly attenuated by the preadministration of an AMPA/kainate receptor antagonist, NBQX, indicating that activation of AMPA receptors in the RVM is also involved in mediating descending inhibition of spinal nociceptive transmission after inflammatory hyperalgesia. A role for AMPA receptors in RVM function is consistent with results from previous studies. For example, nonselective EAA receptor antagonists attenuate the short latency response of RVM neurons evoked by periaqueductal gray stimulation, whereas selective NMDA receptor antagonists are ineffective (Wiklund et al., 1988). In addition, non-NMDA receptors in the RVM have also been suggested to play a role in opiate-produced descending inhibition (Praag and Frenk, 1990). Administration of DNQX, an AMPA/kainate receptor antagonist, into the RVM enhanced nociceptive responses in models of secondary hyperalgesia (Urban et al., 1999) as well as visceral hyperalgesia (Coutinho et al., 1998). Further support for a role of endogenous activation of AMPA receptors in the RVM in inflammatory hyperalgesia comes from results of our antagonist study. Intra-RVM administration of the AMPA/kainate receptor antagonist NBQX produced a further reduction of PW latencies on both inflamed side and noninflamed sides, as well as TF latencies in inflamed animals at 24 h postinflammation. This AMPA receptor-mediated inhibitory control appears to be tonically active under normal conditions, as indicated by the finding that NBQX also produced a reduction of PW and TF latencies in naive animals.

Another important finding is the leftward shift of the dose-response curves of NMDA- and AMPA-produced inhibition at 24 h postinflammation compared with that at 3 h postinflammation. This enhanced descending inhibition was evident not only in the inflamed hindpaw but also in the noninflamed hindpaw and tail. Our recent study (Terayama et al., 2000a) demonstrates that an enhanced descending inhibition occurs at 24 h postinflammation compared with earlier time points. The leftward shift of the dose-response curve of EAA receptor agonist-produced inhibition parallels the time-dependent enhancement of net descending inhibition, suggesting that the functional changes in descending inhibition are mediated in
part by enhanced EAA neurotransmission. It is possible that the enhanced NMDA- and AMPA-produced inhibition is mediated exclusively by hyperexcitability at the spinal level after inflammation. However, this is unlikely because the enhanced inhibition on PW responses occurred not only on the inflamed side but also on the noninflamed side and the tail. Similar phenomena have been demonstrated by ES of the RVM (Terayama et al., 2000a) and by microinjection of opioid receptor agonists into the RVM (Hurley and Hammond, 2000). Furthermore, direct electrical stimulation of the spinal dorsolateral funiculus that bypasses brain stem synaptic mechanisms does not produce similar changes (Terayama et al., 2000a). Therefore, the present study supports the conclusion that tissue injury that leads to dorsal horn hyperexcitability (Woolf and Thompson, 1991) also induces supraspinal hyperexcitability and neuroplasticity in the RVM. There is now evidence that pain modulation may be somatotopic as revealed by site-specific placebo analgesia in humans (Benedetti et al., 1999). Our findings support the notion that descending pain modulation exhibits diffuse effects and weak somatotomy (Willis, 1988; Leung and Mason, 1998; Hurley and Hammond, 2000), because the descending effects occurred at multiple targets (inflamed hindpaw, non-inflamed hindpaw, and tail) but were most robust on the responses to stimulation of the inflamed hindpaw.

The increased potency of AMPA-produced inhibition could result from an increase in the presynaptic release of glutamate, or a modification in postsynaptic AMPA receptor function or number (or both) in the RVM. Our recent Western blot data indicate a time-dependent increase in the AMPA receptor GluR1 subunit levels in the RVM at 24-h, 3-day postinflammation, compared with that of naive animals (Guan et al., 2001). Using an antibody that recognizes the phospho-GluR1 subunits at the serine 831 residue, Western blots also demonstrated that the amount of phospho-GluR1 protein was increased at an early time point (2 h) after inflammation, suggesting that receptor phosphorylation may also contribute to the enhanced AMPA transmission (Guan et al., 2001).

An important finding in the present study is that the AMPA-produced descending inhibition was blocked by preadministration of a competitive NMDA receptor antagonist, APV, suggesting an interaction between NMDA and AMPA receptors in the RVM. It is unlikely that APV produced a nonselective, direct effect on AMPA receptors because APV at the dose used has been shown not to block AMPA-produced depolarization of brainstem neurons (Hosli et al., 1983). Several forms of interactions between NMDA and non-NMDA receptors exist in the central nervous system (Arias et al., 1999; Addae et al., 2000). Based on the present finding that the NMDA receptor antagonist APV also blocked the AMPA-produced descending inhibition, whereas NBQX failed to attenuate the NMDA-produced modulation, we hypothesize that a novel form of interaction between NMDA and AMPA receptors exists in the RVM in which non-NMDA ligands may exert their modulatory effects via activation of NMDA receptors. AMPA receptor activation may produce descending inhibition via interneurons that have excitatory synapses with downstream NMDA receptor-containing neurons (Fig. 7A). This notion is supported by our previous finding that ES-produced inhibition was antagonized by NMDA receptor antagonists, but not by AMPA receptor antagonists (Terayama et al., 2000a), because ES would have a dominant effect on the downstream NMDA receptor-containing neurons and their axons. Alternatively, because NMDA and AMPA receptors are colocalized in RVM neurons (Lai et al., 1996), AMPA receptors may affect neighboring NMDA receptors on the same neuron by removing the Mg2+ block of the NMDA channel and thereby produce descending inhibition. C, NMDA receptor-dependent modification of AMPA receptor function and subsynaptic localization may also be involved in contributing to the interaction between NMDA and AMPA receptors in the RVM. Phosphorylation of AMPA receptors and the delivery of AMPA receptors within the synaptic plasma membrane is important in modulating AMPA synaptic transmission and may contribute to AMPA produced descending inhibition. This process depends on the activation of protein kinases, primarily Ca2+ and calmodulin-dependent protein kinase II (CaMKII), subsequent to the Ca2+ influx after NMDA receptor activation. Therefore, blocking the upstream NMDA receptor activation by APV may result in a substantial reduction in the number of functional AMPA receptors available in the synaptic plasma membrane and a decrease in AMPA-produced inhibition.

Fig. 7. Schematic representation of the possible mechanisms underlying the interaction between NMDA and AMPA receptors in the RVM modulatory circuitry. A, AMPA receptor activation may produce descending inhibition via interneurons that have excitatory synapses with NMDA receptor-containing neurons that induce descending pain inhibition. B, AMPA receptors may also affect neighboring NMDA receptors on the same neuron by membrane depolarization and removal of the Mg2+ block of the NMDA channel and thereby produce descending inhibition. C, NMDA receptor-dependent modification of AMPA receptor function and subsynaptic localization may also be involved in contributing to the interaction between NMDA and AMPA receptors in the RVM. Phosphorylation of AMPA receptors and delivery of AMPA receptors within the synaptic plasma membrane is important in modulating AMPA synaptic transmission and may contribute to AMPA-produced descending inhibition. This process depends on the activation of protein kinases, primarily Ca2+ and calmodulin-dependent protein kinase II (CaMKII), subsequent to the Ca2+ influx after NMDA receptor activation. Therefore, blocking the upstream NMDA receptor activation by APV may result in a substantial reduction in the number of functional AMPA receptors available in the synaptic plasma membrane and a decrease in AMPA-produced inhibition.
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