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Activation of brainstem metabotropic glutamate receptors inhibits spinal nociception in adult rats

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Abstract

In this study, we provide evidence that focal electrical stimulation applied to the rostroventral medulla (RVM) at a high frequency (100 Hz) produced inhibition of the spinal nociceptive tail-flick (TF) reflex in lightly anesthetized adult rats. Chemical activation of metabotropic glutamate (mGlu) receptors by local injection of (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (tACPD), the mGlu receptor agonist, produced a dose-related inhibition of the TF reflex. Injection of the Group II mGlu receptor agonist (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl) glycine (DCGIV) produced strong inhibition, while injection of the Group III mGlu receptor agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4) did not produce any effect. (*RS*)-α-Methyl-4-carboxyphenylglycine (MCPG), a selective mGlu receptor antagonist, but not naloxone reversed the inhibitory effects of DCGIV. Our results provide physiological evidence in vivo that activation of Group II mGlu receptors in the brainstem is antinociceptive and drugs targetting these receptors may help to control pain in humans.

Keywords: Pain; Metabotropic glutamate receptors; Descending inhibition; Rostroventral medulla; Rats

1. Introduction

It is well known that spinal nociceptive transmission receives descending inhibitory modulation (see Gebhart and Randich, 1990; Fields et al., 1991; Sandkühler, 1996 for reviews). For example, in animal behavioral experiments, activation of descending inhibitory systems by electrical or chemical stimulation in the periaqueductal gray (PAG) or the rostroventral medulla (RVM), two major components of the endogenous analgesia systems, has been documented to inhibit a variety of nociceptive reflexes (e.g., the jaw-opening reflex, the tail-flick (TF) reflex, hot-plate (HP) response and visceral nociceptive reflexes). In support of the evidence provided in behavioral experiments, electrophysiological studies demonstrated that electrical stimulation in the PAG or RVM inhibits the responses of spinal dorsal horn neurons (including ascending tract neurons, such as spinothalamic tract and spinomesencephalic tract neurons) to noxious cutaneous stimulation of the skin (see Gebhart and Randich, 1990; Willis, 1988 for reviews). These results suggest that stimulation-produced antinociception from the PAG or RVM is due to an inhibitory effect on spinal nociceptive transmission.

Based on a large number of anatomical, pharmacological and electrophysiological studies, it has been proposed that inhibition of spinal nociceptive transmission produced by stimulation in the PAG has synaptic relays in the RVM, including the nucleus raphe magnus (NRM) and adjacent reticular formation (see Gebhart and Randich, 1990 for review). It has been documented that the PAG sends descending projecting axons to the ventromedial medulla, including the NRM, the nuclei reticularis gigantocellularis

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(NGC) and the gigantocellularis pars alpha (NGCα), the nucleus reticular paragigantocellularis (NPG) and the lateral reticular nucleus (LRN). Electrical or chemical stimulation at sites in the PAG has been reported to produce excitatory effects on neurons in the NGC, NGCα and NRM. Further support for relays in the RVM comes from work by Aimone and Gebhart (1986) and Gebhart et al. (1983). Using microinjection of the local anesthetic lidocaine, Gebhart et al. reported that descending inhibition produced by electrical stimulation in the PAG on responses of spinal dorsal horn neurons to noxious heating of the skin was affected only when lidocaine had been simultaneously microinjected into the medial and lateral medulla.

Glutamate is the major excitatory neurotransmitter in the central nervous system, including the RVM. Pharmacological studies showed that microinjection of glutamate into the RVM produced antinociceptive effects on behavioral reflexes as well as inhibiting responses of dorsal horn neurons to peripheral noxious stimuli (Zhuo and Gebhart, 1990, 1991, 1992, 1997). Aimone and Gebhart (1986) showed that microinjection of the excitatory amino acid receptor antagonist DL-2-amino-phosphonovalerate into the NRM significantly increased the stimulation threshold in the PAG for inhibition of the TF reflex in the rat. Spinella et al. (1996) showed that inhibition of NMDA receptors in the RVM significantly reduced antinociceptive effects produced by microinjection of morphine into the PAG. These data support an excitatory input from the PAG to the RVM. Less information is known about mGlu receptors within the RVM. In the

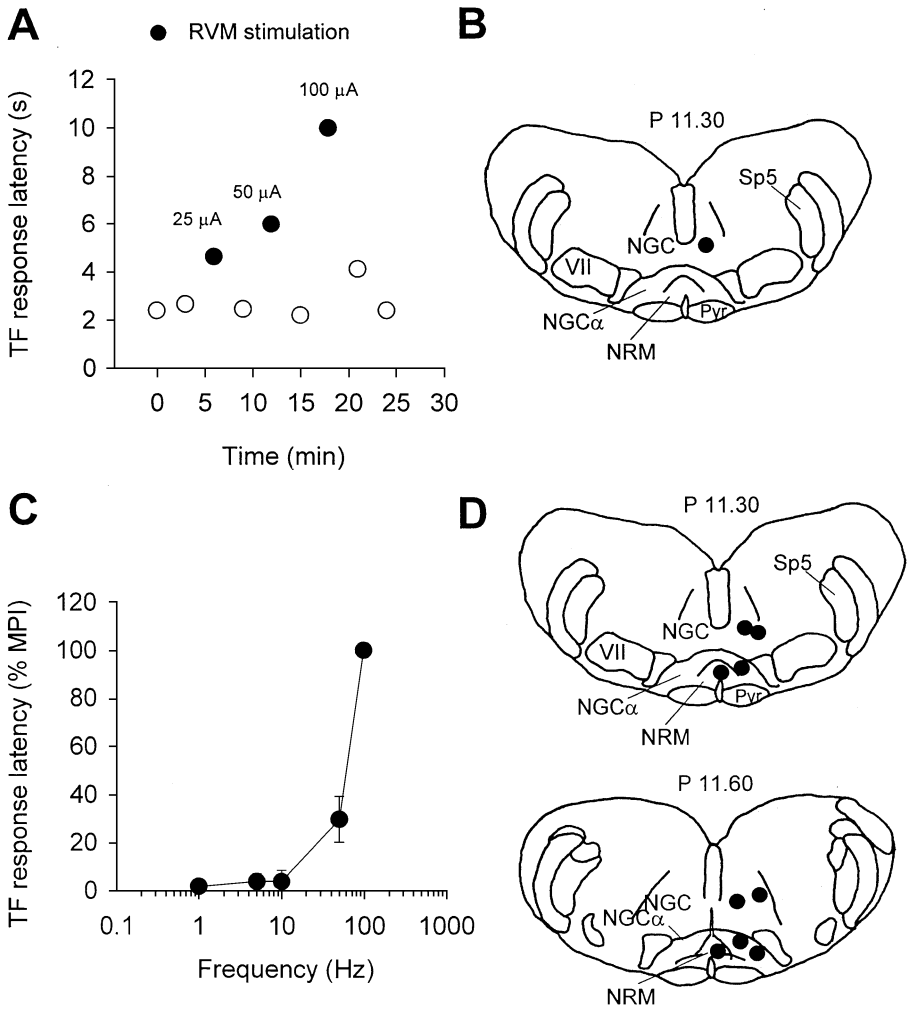

Fig. 1. Frequency-dependent inhibition of a spinal nociceptive TF reflex by electrical stimulation in the RVM. (A) An example showing electrical stimulation at three different stimulation intensities (25, 50 and 100 μA) produced intensity-related inhibition of TF response latency. TF response latencies measured during RVM electrical stimulation are shown as filled circles. Open circles indicate TF response latencies without RVM stimulation. (B) Brainstem site for stimulation in A illustrated on a representative coronal brain section. (C) Summarized data for stimulation-produced inhibition of the TF reflex at different frequencies of stimulation. In each experiment, stimulation intensities are the same for different frequencies and produce complete inhibition of the TF reflex at 100 Hz stimulation. (D) Brainstem sites for stimulation illustrated on representative coronal brain sections. NGC, nuclei reticularis gigantocellularis; NGCα, nuclei reticularis gigantocellularis pars alpha; NRM, nucleus raphe magnus; Pyr, pyramidal tract; Sp5, spinal trigeminal tract; VII, facial nucleus.

present study, we investigated the role of mGlu receptors in descending inhibitory modulation from the RVM. We showed that activation of Group II mGlu receptors within the RVM produced powerful inhibition of the spinal nociceptive TF reflex.

2. Materials and methods

2.1. Animals

Adult, male Sprague–Dawley albino rats (260–375 g, Charles River, Wilmington, MA, USA) were used in all experiments. Animals were housed in the AAALAC approved animal care facility and had free access to food and water. The Animal Studies Committee in Washington University approved the experimental protocols. Rats were anesthetized with 2–3% halothane (Halocarbon Laboratories, River Edge, NJ, USA) delivered via a specialized nose cone (Stoelting Instrument, IL, USA) (with 30% O_2 balanced with nitrogen; Puritan Bennett, KS). Body temperature was maintained at 37 ± 0.5 °C by a water-circulating, thermostatically controlled heating pad. The room temperature was always maintained at 20 °C. In some experiments, tail skin temperatures were measured with a digital thermometer. As reported previously, we found that rats can be

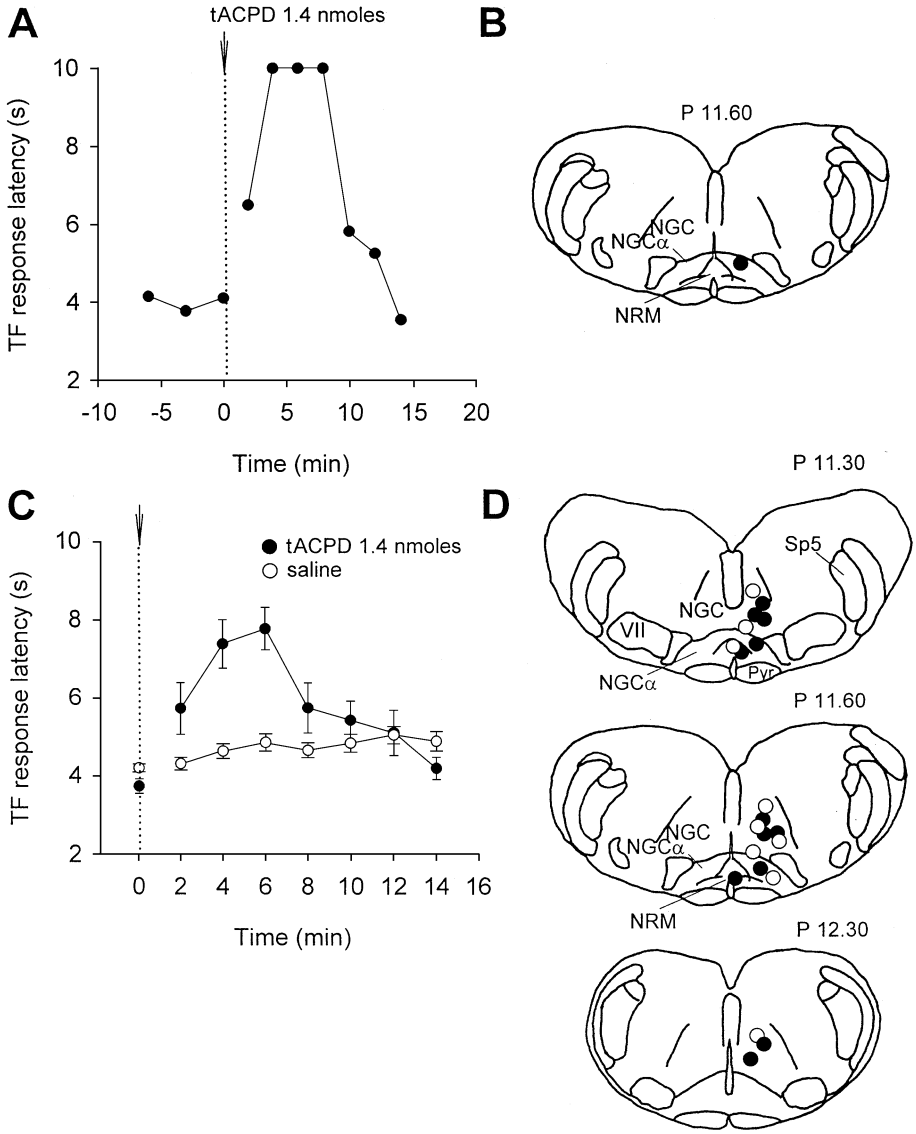

Fig. 2. Microinjection of an mGlu receptor agonist tACPD into the RVM produced inhibition of the TF reflex. (A) An example showing microinjection of tACPD into one site within the RVM produced inhibition of the TF reflex. TF response latency before and after tACPD microinjection was plotted against the time. (B) A brainstem site for microinjection in A illustrated on a representative coronal brain section. (C) Summarized data for tACPD microinjection-produced inhibition of the TF reflex ($n = 12$, filled circles). Results from control saline injection experiments are also shown ($n = 9$, open circles). (D) Brainstem sites for microinjection as shown in C illustrated on representative coronal brain sections. See Fig. 1 for abbreviations.

maintained at a light anesthetized state for a long period of time (2–3 h) with reliable TF reflexive responses (see Calejesan et al., 1998, 2000).

2.2. Spinal nociceptive TF reflex

The spinal nociceptive TF reflex was evoked by radiant heat applied to the tail. The underside of the tail 3,4, 5, 6 or 7 cm from its distal end was randomly heated at 2–3 min intervals to evoke the TF reflex. A photocell timer measured to the nearest 0.1 s the latency of reflexive removal of the tail from the heat. A cutoff time of 10 s was employed to minimize damage to the skin of the tail. Following stimulation of a site within the RVM, a control TF always followed at the next interval. The interval between consecutive heating of the tail (2–3 min) produced a stable reflex latency for the duration of the experiment (see Calejesan et al., 1998).

2.3. Electrical and chemical stimulation in the RVM

Monopolar stimulation electrodes, guided stereotaxically either 0, 0.5 or 1.0 mm lateral to the midline in the vertical plane (incisor bar at +3.3 mm), were inserted into the brainstem through a 26-gauge (0.45 mm O.D.) guide cannula (RVM: 10.30–12.30 mm posterior to the bregma, 0–1.0 mm lateral to the midline, Paxinos and Watson, 1986). The electrode was cut to extend 2 mm beyond the tip of the guide cannula. To determine stimulation thresholds for facilitation or inhibition of behavioral nociceptive reflexes, brain stimulation was started 10 s before and was

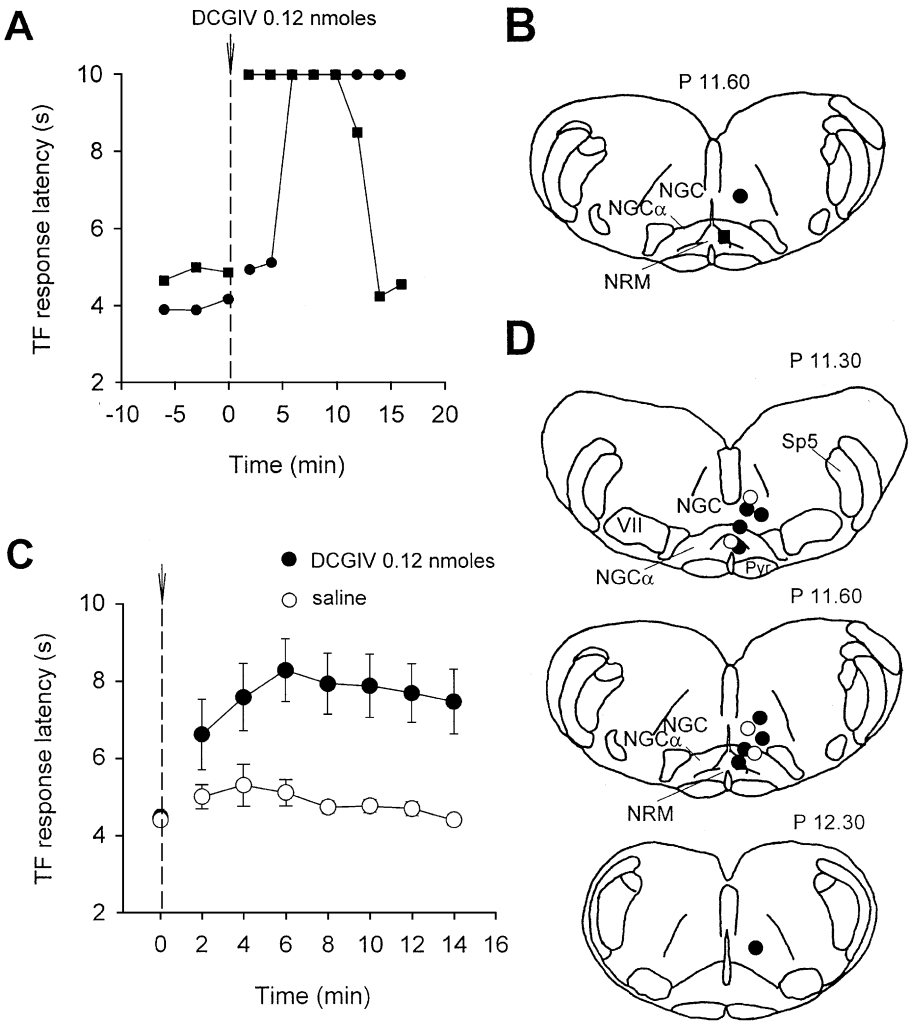

Fig. 3. Activation of Group I mGlu receptors within the RVM by DCGIV produced inhibition of the TF reflex. (A) Two separate examples showing microinjection of DCGIV (0.12 nmol, 0.5 μl) into two sites within the RVM produced inhibition of the TF reflex. TF response latencies before and after tACPD microinjection were plotted against the time. (B) Two brainstem sites for the microinjection experiments in A illustrated on a representative coronal brain section. (C) Summarized data for DCGIV microinjection-produced inhibition of the TF reflex ($n=9$, filled circles), including two experiments shown in A and B. Results from control saline injection experiments are also shown ($n=4$, open circles). (D) Brainstem sites for microinjection as shown in C illustrated on representative coronal brain sections. See Fig. 1 for abbreviations.

continued during noxious heating of the tail. This stimulation paradigm has been determined experimentally to require the lowest intensity of stimulation to inhibit the TF reflex (see Zhuo and Gebhart, 1990). At each site of stimulation in the RVM, stimulation at low intensity (5 or 10 μA) was tested first and the intensity was increased stepwise thereafter to a maximum of 100 or 200 μA. The threshold for inhibition of the behavioral reflex was defined as the intensity at which the latency or threshold was increased from baseline to cutoff.

A selective mGlu receptor agonist or antagonist was microinjected into the RVM in volumes of 0.5 μl via an injection cannula (33-gauge, 0.20 mm O.D.) inserted through the 26-gauge guide cannula and also extending 2 mm beyond its tip. Injection of a drug or saline was monitored by following the movement of an air bubble in a length of calibrated tubing between the syringe and the cannula (about 1 min duration). In some experiments, we performed a second injection of saline or another drug into the same sites by pulling out the injection cannula and refilling it with the solution. Drugs were dissolved in saline. For activation of mGlu receptors, (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (tACPD, 0.14 and 1.4 nmol) was used. (2*S*,2′*R*,3′*R*)-2-(2′,3′-Dicarboxycyclopropyl) glycine (DCGIV, 0.01 and 0.12 nmol) was used to activate Group II mGlu receptors. DCGIV at 1.2 nmol were also tested in preliminary experiments and it produced long-lasting inhibition of the TF reflex (greater then 20 min; $n=3$). L(+)-2-Amino-4-phosphonobutyric acid (L-AP4, 13.5 nmol) was used to activate Group III mGlu receptors, a dose at least 10 times higher than its effective dose in the nucleus of the solitary tract (see Matsumura et al., 1999). To confirm the involvement of mGlu receptors, we used (*RS*)-α-methyl-4-carboxyphenylglycine [(*RS*)-MCPG] at 2.4 nmol to test if the inhibitory effect of DCGIV can be reversed. Naloxone was used to test the involvement of opiate receptors. At the end of experiments, the same volume of dye (diluted India ink) was injected into the same site. The brain was removed and fixed in 10% formalin for at least 3–6 days. The stained sites in the RVM were verified under the microscope. tACPD, DCGIV, L-AP4 and (*RS*)-MCPG were purchased from Tocris Cookson (St. Louis, USA). Naloxone and other chemicals were purchased from Sigma (St. Louis, USA).

2.4. Data and analysis

Data were presented as mean values ± 1 standard error of mean (S.E.M). Inhibition of the TF reflex is presented as maximum possible inhibition (MPI)=(TF latency − baseline TF latency)/(cutoff − baseline TF latency) × 100. Facilitation of the TF reflex is presented as a percentage of the control TF response latency. Statistical comparisons were made with the use of one-way analyses of variance (ANOVAs; Dunnett or Newman–Keuls test for post hoc comparison). Student's *t* test was applied for comparisons between paired groups. In all cases, $P<.05$ is considered significant.

3. Results

Recent studies in different regions of the brain consistently suggest that mGlu receptors are activated by stimulation at higher frequencies (50–200 Hz) (for example, see Scanziani et al., 1997). To test the possible involvement of mGlu receptors in descending modulation, we first looked for the sites within the RVM producing inhibition of a spinal nociceptive TF reflex in lightly anesthetized adult rats. Consistent with our previous studies in pentobarbital- or halothane-anesthetized rats (Zhuo and Gebhart, 1990, 1992, 1997; Calejesan et al., 2000), electrical stimulation in the RVM produced biphasic modulation of the TF reflex. Because we focused on inhibitory modulation in this study, only inhibitory sites where electrical stimulation at different intensities induced only inhibitory effects were used. In a

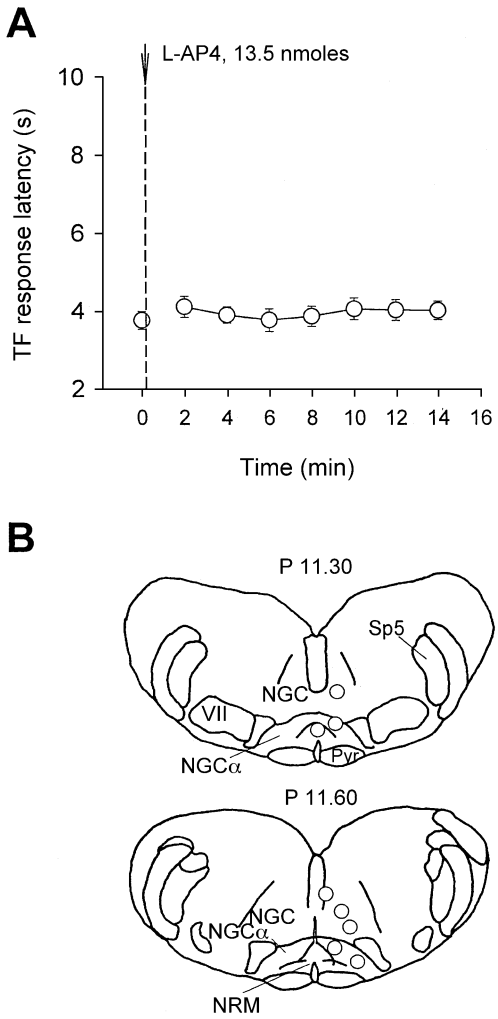

Fig. 4. Activation of Group III mGlu receptors within the RVM did not affect the TF reflex. (A) Summarized data for L-AP4 ($n=8$, 13.5 nmol, 0.5 μl) microinjection within the RVM. Averaged TF response latencies before and after L-AP4 were plotted against time. (B) Brainstem sites for microinjection as shown in A illustrated on representative coronal brain sections. See Fig. 1 for abbreviations.

total of nine inhibitory sites in the RVM (four in the NGC, two in the NRM, and three in the NGCα) tested, we found that stimulation at 100 Hz produced complete inhibition of the TF reflex at a mean 42.9 ± 12.8 μA ($n = 9$, ranged 25–100 μA). We then tested the frequency dependence by looking at the effects with the same intensity stimulation at different frequencies (5, 10, 25 and 50 Hz). Stimulation-produced inhibition was decreased with low stimulation frequencies, and at 5 Hz, no change in the TF reflex was found (3.4 ± 1.2% MPI, $n = 9$). Significant inhibition of the TF reflex was found at 50 Hz (29.8 ± 9.5% MPI) and complete inhibition at 100 Hz (Fig. 1).

If mGlu receptors are activated during high-frequency electrical stimulation in the RVM, we predict that activation of mGlu receptors in the RVM may be antinociceptive. To test it, we microinjected tACPD, an mGlu receptor agonist, into the inhibitory sites within the RVM. As shown in Fig. 2, tACPD (1.4 nmol, 0.5 μl) produced rapid inhibition of the TF reflex and the inhibition lasted for 5–10 min. At 15 min after the tACPD microinjection, the TF response latency returned to the baseline level (see Fig. 2). Summarized results from a total of 12 experiments were shown in Fig. 2C. The effects of tACPD were reproducible. In three experiments, we injected the same amount of tACPD into the same sites for a second time and similar inhibition of the TF reflex was observed. The effects of tACPD were also dose-related. At a dose of 0.14 nmol, no significant inhibition of the TF reflex was observed ($n = 3$). Injection of control saline solution did not produce any inhibition of the TF reflex ($n = 9$).

Three major groups of mGlu receptors are reported in the central nervous system. To test the involvement of individual groups of mGlu receptors in antinociception, we first injected a selective Group II mGlu receptor agonist, DCGIV (0.12 nmol, 0.5 μl). DCGIV produced rapid increases in the TF response latency and completely blocked the responses at about 5 min after the injection in some experiments (five of nine experiments; Fig. 3). The duration of inhibitory effects produced by DCGIV was longer than that induced by tACPD (1.4 nmol; see Fig. 2). At 14 min after the injection, TF response latencies remained increased (7.5 ± 0.8 s compared to baseline of 4.5 ± 0.2 s, $P < .05$). The inhibitory effect induced by DCGIV is dose-related. At a low dose of 0.01 nmol, DCGIV microinjection did not induce significant increases in TF latencies ($n = 4$; baseline 4.4 ± 0.1 s vs. 5.1 ± 0.3 s at 5 min after DCGIV microinjection).

We next tested the possible involvement of Group III mGlu receptors. Microinjection of L-AP4 (13.5 nmol, 0.5 μl) did not significantly affect the TF responses latency ($n = 8$), indicating that Group III mGlu receptors are unlikely involved (Fig. 4). To distinguish a possible difference in injection sites, we tested the effects of microinjection of

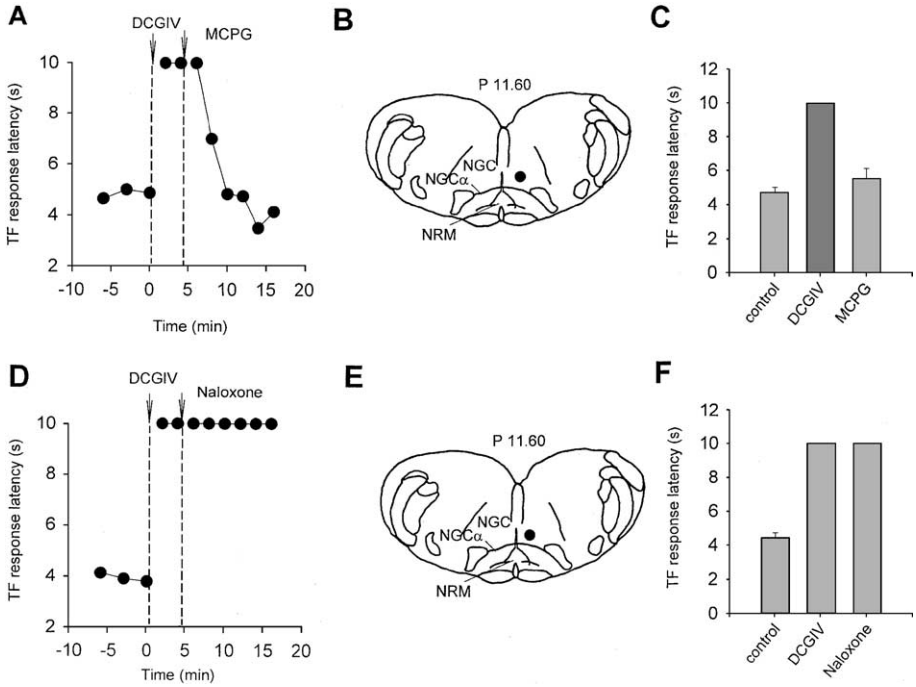

Fig. 5. Effects of mGlu or opiate receptor antagonists on the inhibitory effects produced by DCGIV microinjection in the RVM. (A) An example showed microinjection of MCPG reversed the inhibition of the TF reflex produced by DCGIV microinjection within the RVM. TF response latency before and after DCGIV and MCPG microinjection was plotted against the time. To insure the maximal inhibition produced by DCGIV, we only selected experiments with complete inhibition of the TF reflex after DCGIV injection. MCPG or naloxone was not administered if TF latencies were not greater than the cutoff time (i.e., 10 s). (B) A brainstem site for microinjection in A illustrated on a representative coronal brain section. (C) Summarized data for TF response latencies before and after the microinjection of DCGIV and MCPG ($n = 5$). (D) An example showed microinjection of naloxone did not affect the inhibition of the TF reflex produced by DCGIV microinjection within the RVM. (E) A brainstem site for microinjection in D illustrated on a representative coronal brain section. (F) Summarized data for TF response latencies before and after the microinjection of DCGIV and naloxone ($n = 3$). See Fig. 1 for abbreviations.

tACPD and L-AP4 on the TF reflex in the same animals at the same sites ($n=5$). As expected, tACPD (1.4 nmol) produced significant inhibition of the TF reflex, while L-AP4 did not.

To confirm the involvement of mGlu receptors in DCGIV-induced inhibition, we examined the effects two different receptor antagonists, an mGlu receptor antagonist, (*RS*)-MCPG, and an opiate receptor antagonist, naloxone. In five experiments, we injected (*RS*)-MCPG (2.4 nmol, 0.5 μl) at 4 min after DCGIV (0.12 nmol) microinjection. In all experiments, inhibitory effects were reversed (TF latency: 4.7 ± 0.3 s before, and to 10.0 ± 0 s after DCGIV, recovered to 5.5 ± 0.6 s after (*RS*)-MCPG injection). By contrast, microinjection of naloxone (1.4 nmol) did not significantly affect the inhibitory effect of DCGIV in the RVM ($n=3$) (Fig. 5). Microinjection of (*RS*)-MCPG (2.4 nmol) alone did not affect the baseline TF latency ($n=4$).

4. Discussion

Our results provide in vivo physiological evidence that activation of mGlu receptors in the brainstem produces antinociceptive effects. This is consistent with the results that electrical stimulation at high frequency is required to produce inhibitory effects. Both tACPD and DCGIV, mGlu receptor agonists, produced significant increases in TF response latencies. Our present findings suggest that the role of mGlu receptors in pain transmission and modulation are likely to be biphasic. Activation of mGlu receptors can lead to more pain or less pain, depending on their central locations. We would like to point out that the doses of DCGIV used in the current in vivo study is much higher than the EC_{50} value of DCGIV on Group II mGlu receptors, although it has been known that effective doses for a drug in in vivo experiments are usually 10–100 times higher than in in vitro experiments. Future studies using mice lacking certain types of mGlu receptors will help us confirm the involvement of selective subtypes receptors.

Eight known subtypes of mGlu receptors have been classified into three different groups (Nakanishi, 1992; Alagarsamy et al., 2001; De Blasi et al., 2001). Group I mGlu receptors include mGlu1 and mGlu5, which when expressed are coupled via Gq to phospholipase C. Group II (mGlu2 and mGlu3) and Group III (mGlu4, 6, 7 8) receptors are coupled to Gi and inhibit stimulated cAMP formation. Group I mGlu receptors are primarily located postsynaptically on neurons and contribute to biphasic regulation of glutamate synaptic transmission. The Group II and III mGlu receptors are found to contribute to presynaptic regulation of glutamate and GABA transmission (Schoepp, 2001). Unlike ionotropic glutamate receptors, these presynaptically located receptors serve as detectors for higher frequency activity. With single or low-frequency stimulation, they are unlikely to be activated. For example, in the hippocampus, Scanziani et al. (1997) reported that when transmitter (glutamate) release is enhanced at hippocampal mossy fiber synapses, the concentration of glutamate increases and its clearance is delayed, this allows it to spread away from the synapse and to activate presynaptic inhibitory mGlu receptors. In the present study, we found that tACPD or a selective Group II receptor agonist DCGIV caused strong antinociceptive effects on a spinally mediated TF reflex when injected into the RVM. Consistent with previous electrophysiological evidence from other central synapses, we found that activation of descending inhibition from the same areas required electrical stimulation at high frequencies (50–100 Hz).

It has been documented that neurons in the RVM receive both excitatory glutamate and inhibitory GABA innervations (Fields et al., 1991; Thomas et al., 1995). The inhibitory GABAergic tone in the RVM can be tonically active. Microinjection of $GABA_A$ receptor antagonists bicuculline or SR95531 produced antinociceptive effects in various behavioral nociceptive tests including the TF reflex (Drower and Hammond, 1988; Gilbert and Franklin, 2001). One possible synaptic mechanism for the effects of DCGIV is to inhibit inhibitory GABA presynaptic release in the RVM and lead to *disinhibition* of descending modulatory systems, in addition to direct activation of descending inhibitory systems by Group I mGlu receptors by tACPD. Future studies using modern electrophysiological approaches will help us to understand the exact synaptic mechanism for the involvement of subtypes of mGlu receptors in the RVM.

Cumulative evidence from numerous laboratories consistently indicates that mGlu receptors contribute to pain transmission, pain modulation as well as persistent pain. In the periphery, Group I mGlu receptors modulate nociception and pain in mice. Activation of Group I mGlu receptors caused increases in sensitivity to noxious heat, while inhibition of peripheral Group I mGlu receptors significant reduced inflammatory pain (Bhave et al., 2001). In the dorsal horn of the spinal cord, results using different approaches consistently suggest the importance of mGlu receptors in pain transmission and modulation, including Group I mGlu receptors. Activation of Group I mGlu receptors activates the extracellular signal-regulated kinases (ERKs)/mitogen-activated proteins kinases in dorsal horn neurons, enhances sensory dorsal horn neuronal responses to noxious stimuli and induces behavioral allodynia and hyperalgesia in animals (Cerne and Randic, 1992; Jones and Headley, 1995; Fundytus et al., 1998; Young et al., 1995; Fisher and Coderre, 1998; Neugebauer et al., 1999, 2000; Karim et al., 2001). In the thalamus, changes in mGlu3 receptors were reported after inflammation in rats and local injection of the mGluR2/3 receptor antagonists reduced inflammation-related persistent pain (Neto and Castro-Lopes, 2000; Neto et al., 2000). In summary, in sensory pathways for incoming and/or ascending sensory and pain transmission, activation of mGlu receptors seems to be predominantly excitatory or nociceptive. Activation of Group I mGlu receptors activates intracellular pathways

related to synaptic plasticity and enhances sensory responses at both single cell level and behavioral level. Furthermore, in pain-related forebrain areas such as the anterior cingulate cortex, activation of mGlu receptors facilitated spinal nociceptive TF reflex by activating the descending facilitatory system through the RVM (Calejesan et al., 2000).

The present results suggest that activation of mGlu receptors in the RVM activates descending inhibitory modulatory systems. Previous studies find that activation of NMDA and AMPA receptors produces descending inhibitory effects. Electrical stimulation and glutamate microinjection produced biphasic modulation of spinal TF reflex in previous studies (Zhuo and Gebhart, 1992, 1997). In the present studies, tACPD at different doses only produced inhibition of the TF reflex. It remains to be investigated if other selective mGlu receptor agonists may produce descending facilitation of the TF reflex. Recently, in the PAG, Maione et al. (1998, 2000) reported that activation of Group I mGlu receptors reduced behavioral responses to noxious heat and formalin-induced inflammatory pain responses. They proposed that Group I as well as Group II mGlu receptors may positively modulate antinociceptive descending pathways following a persistent noxious stimulation.

In summary, our results suggest that mGlu receptors can play dual roles in the central processing of pain-related information, enhancing pain transmission in ascending sensory pathways such as the periphery, spinal dorsal horn, thalamus and anterior cingulate cortex, and inhibiting pain transmission at central modulating nuclei such as the PAG and the RVM. Our understanding of the involvement of mGlu receptors in these transmission- and modulation-related central neurons might help us design better drugs and treatment for pain in humans.

Acknowledgments

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