Involvement of Glutamate Receptors in Strychnine- and Bicuculline-induced Alloodynia in Conscious Mice

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Background: Glycine and γ-aminobutyric acid (GABA) are inhibitory neurotransmitters that appear to be important in sensory processing in the spinal dorsal horn. Intrathecal administration of strychnine (strychnine-sensitive glycine receptor antagonist) or bicuculline (GABA_A antagonist) was reported to induce alloodynia. Although the strychnine-induced alloodynia was shown to be mediated through the N-methyl-d-aspartate (NMDA)-type glutamate receptor, it is not clear whether the bicuculline-evoked alloodynia is mediated through the glutamate receptor system or how different the alloodynia induced by strychnine and bicuculline are.

Methods: Male ddY mice weighing 20 ± 2 g were used in this study. A 27G stainless-steel needle attached to a microsyringe was inserted between the L5 and L6 vertebrae by a slight modification of the method of Hylden and Wilcox. Drugs in vehicle were injected slowly into the subarachnoid space to conscious mice at 22 ± 2°C. The volume of the intrathecal injection was 5 μl. Studies on alloodynia were carried out essentially according to the method of Yaksh and Harty.

Results: The intrathecal administration of strychnine or bicuculline in conscious mice resulted in alloodynia elicited by nonnoxious brushing of the flanks. The maximum alloodynia induced by strychnine was observed 5 min after intrathecal injection, but that induced by bicuculline was observed 10 min after intrathecal injection. Both responses gradually decreased over the experimental period of 50 min. The alloodynia induced by strychnine was dose-dependently relieved by NMDA receptor antagonists (d-AP5, ketamine, and 7-Cl-KYN) and non-NMDA receptor antagonists (GAMS and CNQX) but not by metabotropic receptor antagonists (I-AP3 and I-AP4). On the other hand, alloodynia induced by bicuculline was dose-dependently relieved by GAMS, I-AP3, and I-AP4, but not by d-AP5, ketamine, 7-Cl-KYN, and CNQX. Whereas the strychnine-evoked alloodynia was dose-dependently relieved by the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) and the soluble guanylate cyclase inhibitor methylene blue, the bicuculline-induced one was dose-dependently relieved by methylene blue but not by L-NAME.

Conclusions: These results demonstrate that both strychnine- and bicuculline-evoked alloodynia were mediated through pathways that include the glutamate receptor and nitric oxide systems but in a different manner. The current study suggests that GABA and glycine may modulate responses to an innocuous tactile stimulus as inhibitory neurotransmitters at presynaptic and postsynaptic sites in the spinal cord, respectively. (Key words: Antagonists, GABA_A, bicuculline, Antagonists, glycine, strychnine, Neurotransmitters, glutamate. Pain: alloodynia. Pharmacology: nitric oxide. Receptors: glutamate.)

Glycine and γ-aminobutyric acid (GABA) are both inhibitory neurotransmitters that mediate fast synaptic inhibition in the nervous system. Their actions are to bind specifically to glycine and GABA_A receptors, respectively. This is followed within milliseconds by the gating or opening of an integral chloride ion channel, which results, in general, in the hyperpolarization of the recipient neuronal cell. Pharmacologically, glycine receptors are defined by the antagonism by the convulsant alkaloid strychnine (strychnine-sensitive glycine receptor antagonist), in contrast to the strychnine-insensitive glycine-binding site that is associated with N-methyl-d-aspartate (NMDA) subclass of glutamate receptors. On the other hand, the convulsant alkaloid bicuculline blocks the hyperpolarizing actions of GABA and nerve stimulation. Molecular models show that GABA is isosteric with a GABA-like moiety in the bicuculline molecule, suggesting a competitive interaction on the GABA_A receptor.
Previous studies demonstrated that glycine and GABA are important in sensory processing in the spinal dorsal horn as inhibitory neurotransmitters. Intrathecal administration of strychnine or bicuculline to conscious mice was reported to induce allodynia, a state of discomfort and pain evoked by innocuous stimuli; the mice showed squawking, biting, and escaping in response to low-threshold stimuli. A growing body of evidence suggests that the pharmacology of the system activated in the pathologic state "allodynia" may differ from that activated under normal circumstances by high-threshold thermal, chemical, and mechanical stimuli. In fact, it was previously reported that intrathecal administration of the opioid and α₂-receptor agonists could produce a definitive inhibition of the spinal response to noxious stimuli but had little effect on the strychnine-induced allodynia. On the other hand, adenosine analogues showed the powerful effect on strychnine-induced allodynia at doses that have only a mild analgesic effect on hyperalgesia. Transmission at neural synapses is mediated by a variety of receptors that specify neurotransmitter interactions and transmit information to target cells in the spinal cord. In recent years, much attention has been directed toward the excitatory transmission mediated through the glutamate receptors in the central nervous system, and it has been suggested that there are mechanisms whereby interactions between excitatory and inhibitory neurotransmitter systems can modulate signal transmission in the spinal cord. The glutamate receptors are classified in three groups, NMDA, non-NMDA (AMPA-kainate), and metabotropic receptors. It has been reported that the NMDA receptor is a voltage-gated ion channel that, once activated, allows Ca²⁺ to enter the neuron. This increase in intracellular Ca²⁺ triggers a cascade of events that include activation of the constitutive form of nitric oxide synthase. Nitric oxide diffuses to its site of action, where it activates soluble guanylate cyclase and increases the intracellular content of cGMP. Although strychnine-induced allodynia was reported to be mediated through the NMDA-type glutamate receptor, it is not clear whether the bicuculline-evoked allodynia is mediated through the glutamate receptor system or how different the allodynia induced by strychnine and bicuculline are. The current study was designed to assign the involvement of glutamate receptors and nitric oxide system in strychnine- and bicuculline-evoked allodynia and seek the difference in the mechanisms of action between them by use of antagonists for glutamate receptors and inhibitors of nitric oxide system.

Materials and Methods

Intrathecal Administration and Studies on Allodynia

Male ddY mice weighing 20 ± 2 g were used in this study. The animals were housed under conditions of a 12-h light-dark cycle and a constant temperature of 22 ± 2°C and 60 ± 10% humidity. A 27-G stainless-steel needle (0.35 mm OD) attached to a microsyringe was inserted between the L5 and L6 vertebrae by a slight modification of the method of Hylden and Wilcox. Drugs in vehicle were injected slowly into the subarachnoid space of conscious mice at 22 ± 2°C. It was previously confirmed by use of Coomassie brilliant blue that the injected solution did not extend to the cervical segments.

Studies on allodynia were carried out according to the method reported previously. Control mice were given physiologic saline (5 µL). Drug-treatment groups were injected with 5 µL of vehicle containing various doses of test agents. After the intrathecal injection, each mouse was placed in an individual 14 × 10 × 12-cm Plexiglas enclosure with wood chips on the floor and observed. Allodynia was assessed once every 5 min over a 50-min period by light stroking of the flank of the mice with a paintbrush. The allodynic response was ranked as follows: 0 = no response; 1 = mild squawking with attempts to move away from the stroking probe; and 2 = vigorous squawking evoked by the stroking probe, biting at the probe, and severe efforts to escape. Each mouse was tested for 50 min following intrathecal injection. To evaluate the effects of various doses of blocking agents on strychnine- and bicuculline-induced allodynia, we assessed the scores at 5 min after intrathecal injection of strychnine for the former and the scores at 10 min after intrathecal injection of bicuculline for the latter.

The animals were used for only one measurement in each experiment. This study was conducted with the approval of the local ethics committee and in concordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain.

Drugs

Strychnine (mw 334.4; a strychnine-sensitive glycine receptor antagonist) and N²-nitro-l-arginine methyl ester (l-NAME; an inhibitor of nitric oxide synthase) were obtained from Sigma (Tokyo, Japan). Bicuculline (methylene bis-tetrahydroisoquinoline) and NMDA receptor antagonist (CNQX; mw 239.2) were obtained from Tocris (Ellisville, MO). Other drugs were purchased from Wako Pure Chemical Industries (Tokyo, Japan) or Sigma. For drug administration, the drugs were dissolved in sterile saline and injected at a rate of 5 µL into the subarachnoid space of each mouse. The saline used served as a control for the drug effect.

Statistics

The statistical test used was one-way analysis of variance. **P < 0.01**.
Fig. 1. Time courses (A) and dose-dependency (B) for the effect of intrathecal injection of strychnine and bicuculline on allodynia. Studies on allodynia were conducted as described in materials and methods. Mice were injected with 0.25 µg strychnine (○) and 1.25 µg bicuculline (●). Each column in A represents the percentage of the maximum possible cumulative score of six to eight mice evaluated every 5 min (mean ± SE). The values (mean ± SE, n = 6–8) of allodynia shown in B are expressed as a percentage of the maximum possible score over the 50-min observation period following different doses (12.5 ng–2.5 µg).

Results

Effect of intrathecal Strychnine or Bicuculline on Allodynia

Intrathecal administration of strychnine and bicuculline resulted in prominent agitation responses, such as vocalization, biting, and escape from the probe, to tactile stimuli applied to the flank. Brushing of the face or tactile stimulation of the forepaws did not elicit any response, indicating that allodynia appeared limited to the caudal dermatomes of the body.

Figure 1A presents the time courses evoked by strychnine (0.25 µg/mouse) and bicuculline (1.25 µg/mouse). Strychnine-induced allodynia showed the maximum effect at 5 min after intrathecal injection, gradually decreasing over the 50-min experimental period. On the other hand, the bicuculline-induced allodynia was evoked by the first stimulus at 5 min after intrathecal injection, but the maximum effect was observed at 10 min. The response was long-lasting and did not disappear by 50 min. Both strychnine- and bicuculline-induced allodynia showed the respective patterns of time courses similar to those shown in figure 1A, over a wide range of doses from 25 ng to 2.5 µg/mouse. When the scores of allodynia obtained for the overall 50 min were cumulated and expressed as a percent of the maximum possible score, both strychnine- and bicuculline-induced allodynia
showed a gradually increased pattern (25 ng–2.5 μg; fig. 1B), and mice displayed convulsions at a dose of 25 μg/mouse or more. The intrathecal administration of saline in conscious mice had no effect on allodynia.

Effects of NMDA Receptor Antagonists on Strychnine- and Bicuculline-evoked Allodynia

The effects of various antagonists for the glutamate receptor family on the allodynia were evaluated by the values obtained 5 min after injection of 0.25 μg strychnine or 10 min after injection of 1.25 μg bicuculline. The scores of allodynia induced by strychnine at 5 min and bicuculline at 10 min were 83.3% and 75.0% of the maximum possible score, respectively, and were taken as 100%.

We first investigated the involvement of the NMDA receptor in the strychnine- or bicuculline-induced allodynia by using d-AP5, ketamine, and 7-CI-KYNA. The allodynia evoked by strychnine was dose-dependently blocked by d-AP5, ketamine, and 7-CI-KYNA with IC₅₀ values of 389 ng, 147 ng, and 8.03 ng, respectively (fig. 2). On the other hand, the allodynia caused by bicuculline was not blocked by d-AP5, ketamine, or 7-CI-KYNA (fig. 2).

Effects of Non-NMDA Receptor Antagonists on Strychnine- and Bicuculline-evoked Allodynia

We investigated the involvement of non-NMDA receptors in allodynia caused by strychnine or bicuculline with GAMS and CNQX. The allodynia caused by strychnine was dose-dependently blocked by GAMS and CNQX with IC₅₀ values of 1.17 μg and 8.76 ng, respectively (fig. 3). The allodynia caused by bicuculline was dose-dependently blocked by GAMS with an IC₅₀ value of 214 ng (fig. 3A) but not blocked by CNQX (fig. 3B).

Effects of Metabotropic Glutamate Receptor Antagonists on Strychnine- and Bicuculline-evoked Allodynia

We further investigated the effect of l-AP3 and l-AP4 on allodynia caused by strychnine or bicuculline. The allodynia caused by bicuculline was dose-dependently antagonized by l-AP4 with an IC₅₀ value of 85.6 ng (fig. 4B) but was partially blocked by l-AP3 (fig. 4A). On the other hand, the allodynia caused by strychnine was not antagonized by l-AP3 or l-AP4 (fig. 4). These results demonstrated that NMDA and non-NMDA receptors in the spinal cord were involved in the strychnine-induced allodynia but that kainate and metabotropic

Fig. 2. Effects of NMDA receptor antagonists on strychnine- and bicuculline-induced allodynia. Strychnine (●, 0.25 μg) or bicuculline (●, 1.25 μg) was injected simultaneously with various doses of d-AP5 (▲), ketamine (□), or 7-CI-KYNA (○) into the subarachnoid space. Assessment of allodynia was made as described in materials and methods. The maximum score of strychnine (0.25 μg/mouse at 5 min) alone or bicuculline (1.25 μg/mouse at 10 min) alone is taken at 100% as the control. Statistical analyses were carried out by Duncan’s test. **P < 0.01, as compared with strychnine- or bicuculline-injected group.

Fig. 3. Effects of GAMS and CNQX on strychnine (○) or bicuculline (●) with various doses injected into the subarachnoid space as described in materials and methods. **P < 0.01, as compared with saline-injected group.
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Fig. 3. Effects of non-NMDA receptor antagonists on strychnine- and bicuculline-induced allodynia. Strychnine (C, 0.25 μg) or bicuculline (●, 1.25 μg) was injected simultaneously with various doses of GAMS (A) or CNQX (B) into the subarachnoid space. Assessment of allodynia was made as described in materials and methods.

receptors were involved in the bicuculline-induced allodynia.

Involvement of the Nitric Oxide System in Strychnine- or Bicuculline-evoked Allodynia

To examine whether the nitric oxide system is involved in inducing allodynia, we investigated the effects of l-NAME and methylene blue on allodynia caused by strychnine and bicuculline. The allodynia caused by strychnine was dose-dependently blocked by l-NAME and methylene blue with IC_{50} values of 68.8 pg and 38.6 μg, respectively (fig. 5). d-NAME, an inactive isomer of l-NAME, did not block the strychnine-induced allodynia (data not shown). On the other hand, the allodynia caused by bicuculline was blocked by methylene blue with an IC_{50} value of 120 pg (fig. 5B) but not altered by l-NAME (fig. 5A). These results demonstrate that the nitric oxide system in the spinal cord is involved in both strychnine- and bicuculline-induced allodynia.

Discussion

It was previously reported that intrathecal administration of strychnine or bicuculline to conscious mice...
induced allodynia and that strychnine-induced allodynia was dose-dependently relieved by NMDA antagonists. In the current study, we first demonstrated that the glutamate receptor system involves the bicuculline- and the strychnine-induced allodynia. However, whereas the latter was inhibited by NMDA receptor and non-NMDA receptor antagonists, the former was inhibited by the kainate receptor antagonist GAMS and metabotropic receptor antagonists (figs. 2–4), suggesting that the interactions of strychnine and bicuculline with the glutamate receptor system are different. This was supported by the difference in the blockade by l-NAME and methylene blue of allodynia evoked by strychnine and bicuculline (fig. 5). One of the mechanisms for touch-evoked allodynia was believed to result from removal of tonic or evoked inhibition from pathways relaying information about innocuous tactile stimuli. Yaksh suggested that the blockade by inhibition of spinal strychnine and bicuculline must either be presynaptic on the large primary afferent or postsynaptic on the second-order neuron and activated only by the large afferent input. Glycine binding is found throughout the spinal gray, with that in the dorsal horn being largely found in lamina II, III, and the lateral aspect of V. The glycineergic neurons in the laminae II and III receive a major monosynaptic input from myelinated low-threshold cutaneous primary afferents, and glycine is considered to act as a postsynaptic inhibitory transmitter. On the other hand, GABAergic neurons are present in laminae I–III of the rat spinal cord, and many of neurons with somata in lamina I–III are inhibitory interneurons containing GABA. These GABA-containing terminals frequently are present in the presynaptic axons at axoaxonal synapses and in presynaptic dendrites in the dorsal horn. Presynaptic inhibition depends on depolarization of excitatory axon terminals by a transmitter released from other axon terminals that form axoaxonal synapses with the excitatory terminals of the primary afferent neuron. Although GABA produces postsynaptic inhibition by hyperpolarizing the postsynaptic cell, it can act as a depolarizing transmitter on the presynaptic terminals of certain primary afferent neurons to produce presynaptic inhibition. L-Glutamate is a known neurotransmitter of primary afferents and descending projections from the brain and perhaps neurotransmitters of some intrinsic spinal neurons. Among the glutamate receptor family, the NMDA receptor and AMPA receptor were reported to be located mainly at postsynaptic site in the spinal cord, while the kainate receptor was likely to be located at presynaptic site in the spinal cord. On the basis of the selective depressant action of L-AP4 in spinal and certain hippocampal pathways, the metabotropic receptor was proposed to be located at a presynaptic site and possibly function as autoreceptors, controlling the release of neurotransmitters. Nitric oxide has been suggested to act as a retrograde transmitter. That is, activation of the NMDA receptor results in the production of nitric oxide by nitric oxide synthase in a postsynaptic neuron from which it rapidly diffuses to enter the presynaptic neuron. Thus, nitric oxide may modulate excitability and enhance synaptic connection through activation of guanylate cyclase, suggesting that the synaptic neurotransmitter recently discovered as novel endogenous potentiator, and nitric oxide may play a role in the control of the central nervous system. Moreover, nitric oxide itself may be a potent mediator of nociceptive signals and may influence the efficacy of neurotransmission from the spinal cord. In the current study, allodynia evoked by the large afferent input may be mediated by glycine, which was reduced by bicuculline. Furthermore, the nitric oxide synthesis inhibitor L-NAME was not effective in reducing or removing the nitric oxide-induced allodynia, suggesting that nitric oxide-mediated allodynia may not be mediated by nitric oxide. opioid-induced analgesia. Opioid-induced analgesia may be mediated by the endogenous opioid system, which is activated by the administration of opioids and may play a role in the control of pain perception.
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One of the hallmarks of allodynia was believed to be nociceptive or evoked inhibition. The blockade of 1-NAME and bicuculline in large primary afferent and second-order neuron and activated spinal gray. Glycine binding to axons at the dorsal laminae II, III, and the minor monosynaptic input to cutaneous primary afferents was considered to act as a positive feedback mechanism 

On the other hand, the dorsal laminae I–III of the rats with somatic pain. Intervening terminals frequently formed on axons at axoaxonic synapses. Dendrites in the dorsal horns are depolarized by a transmitter. Sites of the selective depolarization are hippocampal CA3 produces postsynaptic inhibition on the postsynaptic cell. Inhibitory afferents to primary nociceptive afferents. 1-NAME is a known GABA antagonist and descending GABAergic neurons can be involved in the modulation of incoming pain information through a number of local receptor systems at different sites. The disorder of the association may evoke allodynia.

Opioid-insensitive pain evoked by innocuous tactile stimulation is one of the most difficult problems in pain management. The features of strychnine- and bicuculline-induced allodynia apparently resemble those of patients suffering from postherpetic neuralgia or causalgia. The clinical allodynia may involve plastic changes in neural connectivity and synaptic strength in the spinal cord. Hao et al. developed an animal model which produces tonic and chronic states of allodynia in rats lasting several days and 1–3 months, respectively. After spinal cord injury induced photochemically by laser irradiation. They demonstrated that, although the NMDA receptor was involved in the development of allodynia through excitotoxicity, once the allodynia had developed, NMDA receptor antagonists were ineffective in relieving it. Furthermore, they showed that systemic 1-NNAME induced an analgesic effect on chronic allodynia-like behavior and suggested that the production of nitric oxide may be involved in the maintenance of this abnormal pain-related condition in rats with spinal cord injury. Similarly, the established allodynia induced by strychnine was not blocked by the glycine receptor agonist taurine and the NMDA receptor antagonist kainate.

We reported that intrathecal administration of prostaglandin (PG)-E2 or PGE2, to conscious mice induced allodynia. The time courses of allodynia evoked by PGE2 or PGE2, coincided with those by strychnine and bicuculline, respectively. Whereas the PGE2-induced allodynia was inhibited by NMDA and non-NMDA receptor antagonists similar to the strychnine-induced one, the PGE2-induced allodynia was inhibited by kainate and metabotropic receptor antagonists similar to the bicuculline-induced one. Furthermore, whereas the PGE2-induced allodynia was inhibited by 1-NNAME and methylene blue similar to the strychnine-induced one, the PGE2-induced allodynia was inhibited by methylene blue, but not by 1-NNAME, similar to bicuculline-induced one. The modes of inhibition of strychnine- and bicuculline-induced allodynia by glutamate receptor antagonists and nitric oxide synthase inhibitor were the same as those of the agents for PGE2 and PGE2, induced allodynia, respectively. Thus, many neurotransmitters are involved in the modulation of incoming pain information through a number of local receptor systems at different sites. The disorder of the association may evoke allodynia.

References


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