

Agmatine이 GABA_A 수용체 길항제로 유도한 촉각이질통에 미치는 효과

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Effects of Agmatine on GABA_A Receptor Antagonist-induced Tactile Allodynia

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Background: The intrathecal (IT) GABA_A receptor antagonist, bicuculline (BIC), results in tactile allodynia (TA) through disinhibition in the spinal cord. Such disinhibition is considered to be an important mechanism for neuropathic pain. Agmatine, an endogenous polyamine, has a neuro-protective effect in the central nervous system. We investigated the analgesic effects and mechanisms of agmatine action on BIC-induced TA.

Methods: Male Sprague-Dawley rats, weighting 250–300 g, were subjected to implantations of PE-10 into the lumbar subarachnoid space for IT drug injection. Five days after surgery, either 10 μ l of normal saline (NS) or agmatine (30 μ g or 10 μ g) in 10 μ l NS were injected 10 min prior to BIC (10 μ g) or NMDA (5 μ g). We assessed the degree of TA (graded 0: no response, 1: mild response, 2: moderate response, 3: strong response) every 5 min for 30 min. Areas under curves and degree of TA were expressed as mean \pm SEM. Results were analyzed using one-way ANOVA followed by a Tukey test for multiple comparisons. $P < 0.05$ was considered significant.

Results: IT BIC-induced strong TA reached its peak and plateaued between 10 to 15 min. IT NS-NMDA induced mild transient TA for up to 15 min. Preemptive IT AG attenuated IT BIC-induced TA dose dependently and preemptive IT AG10 completely abolished the IT NMDA-induced TA.

Conclusions: Preemptive IT AG attenuated the IT BIC-induced TA through inhibitory actions on postsynaptic NMDA receptor activation. AG might be a viable therapeutic option in the treatment of neuropathic pain. (Korean J Pain 2008; 21: 173-178)

Key Words: agmatine, GABA_A receptor antagonist, tactile allodynia.

INTRODUCTION

Peripheral nerve injury can produce a persistent pain state characterized by spontaneous pain, painful responses to normally innocuous stimuli (allodynia) and augmented pain states against normally nociceptive stimuli (hyperalgesia).

One of the physiological and biological mechanisms of neuropathic pain, that is pain initiated or caused by a primary lesion or dysfunction in the nervous system, is loss or dysfunction of the inhibitory system (disinhibition) in the spinal cord dorsal horn.¹⁻³⁾ Intrathecal (IT) administration of glycine or GABA_A receptor antagonists (strychnine: STR, bicuculline: BIC, respectively) resulted in disinhibition in the

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spinal cord and touch evoked allodynia which is an organized agitation response to light tactile stimulation.^{4,5)}

A β primary afferent fibers synapse to postsynaptic membranes and their transmissions are modulated by GABA releasing interneurons. IT BIC inhibits GABA_A receptors on the presynaptic and postsynaptic membrane and increases the responses to A β inputs of normally innocuous stimuli.^{5,6)} BIC and STR-induced allodynia were depressed by IT NMDA receptor antagonists. The order of activity was MK-801, AP-5, kynurenic acid, SKF10047 and ketamine.⁴⁾ IT NMDA also result in well defined hyperalgesia and allodynia.⁶⁾ While IT BIC and STR alone evoked a transient spinal release of glutamate, STR did not affect spinal neurotransmitter release at any time and both allodynic effects were blocked with adenosine A1 receptor agonist and NMDA receptor antagonist.^{7,8)} These findings suggest that IT BIC induces disinhibition of GABAergic inhibition of presynaptic glutamate release and postsynaptic NMDA receptor-nitric oxide synthase (NOS) pathway, both of which are crucial to inducing central sensitization and tactile allodynia.⁸⁾

Agmatine is an endogenous polyamine produced by decarboxylation of arginine, and has been shown to antagonize the NMDA receptor^{9,11)} and inhibit NOS.¹²⁻¹⁴⁾ Recent neuropharmacological studies have shown that exogenous agmatine protects against cell damage induced by glutamate and NMDA in cultured neurons.^{9,11)} It also prevents or reverses biological phenomena dependent on glutamatergic pathways in the spinal cord (central nervous system, CNS).¹⁵⁾ In the present study, we aimed to investigate the analgesic effects, and their mechanisms of agmatine action in a GABA_A receptor antagonist induced allodynia rat model.

MATERIALS AND METHODS

This study was performed with approval from the Institutional Animal Care and Use Committee and our study complied with the NIH Guide for the Care and Use of Laboratory Animals. The experiments were performed on male Sprague-Dawley rats weighting 250–300 g. Animals were housed in groups of 3 in plastic cages under a standard 12 h light/12 h dark cycle in a room maintained at 23 ± 3 °C. The rats were kept for at least 3 days under

these conditions before surgery.

1. IT Catheter Implantation

Rats were implanted with a PE-10 catheter for intrathecal (IT) drug injection using the method described by Yaksh and Rudy.¹⁶⁾ A PE-10 catheter was inserted through an incision in the cisternal membrane and the caudal end was passed into the lumbar enlargement whilst the rostral tip of the catheter was tunneled and fixed under the scalp for drug administration. Rats with neurological dysfunction were sacrificed. Drug experiments were initiated at least 5 days after the implantation operation.

2. IT Drug Injection

All the drugs were dissolved in 10 μ l of normal saline. Either BIC hydrochloride (Sigma, USA) 10 μ g or NMDA (Sigma, USA) 5 μ g were used for induction of tactile allodynia. Agmatine sulfate salt (Sigma, USA) was used to facilitate and antiallodynic effect. Normal saline (NS group, n = 7) and agmatine 10 μ g (AG30 group, n = 7) or 30 μ g (AG30 group, n = 7) were IT administered 10 min before BIC or NMDA IT injection. All drugs were flushed into the subarachnoid spaces by another 10 μ l of normal saline.

3. Measurement of Tactile Allodynia

The behavioral responses to touching hair on the lumbar dermatome were evaluated before and then every 5 min for 30 min after IT drug injection. A bundle of 3 pencils was briefly dragged along the flank of a rat in a rostral direction from the buttock 5 times per subject.^{4,6)} The responses of the rats (touch evoked agitation, TEA) were graded according to a 4 point score scale assessment as follows, 0: no response, I: less than 5 responses against 5 tactile stimuli, II: 5 mild responses against 5 tactile stimuli, III: 5 strong responses (such as body twisting, severe scratching, or attempted escape from the stimuli) against tactile stimuli.

4. Statistics

For statistical analyses, we employed a one-way ANOVA followed by Tukey test for multiple comparisons. All the data is presented as mean ± SEM.

RESULTS

The administration of a vehicle (normal saline) and agmatine alone had no effect on general behavior, and there was no TEA. In contrast, the NS group (IT NS-BIC 10 μg) showed prominent and comparable responses to tactile stimuli. The peak effect of BIC appeared at the 10 min mark and plateaued until 15 min after IT injection (Fig. 1). However, IT NS-NMDA only transiently induced mild TEA for 15 min after IT injection (data not shown).

Thirty μg of preemptive IT agmatine (AG30 group) significantly attenuated the BIC-induced TEA for 25 min after IT BIC injection ($P < 0.05$). Preemptive IT agmatine 10 μg (AG10 group) had no effect on BIC-induced TEA.

The mean (± SEM) areas under curves were 14.1 (± 0.5), 7.2 (± 0.4) and 11.4 (± 0.7) in the NS, AG30 and AG10 groups, respectively, and there were statistically significant differences between the NS group and AG30 group ($P < 0.01$), between the NS group and AG10 group ($P < 0.05$) and between the AG30 group and AG10 group ($P < 0.01$) (Fig. 2).

10 μg of preemptive IT agmatine completely abolished the TEA induced by 5 μg of NMDA. The areas under the curves were 9 and 1 in the IT NS-NMDA and preemptive

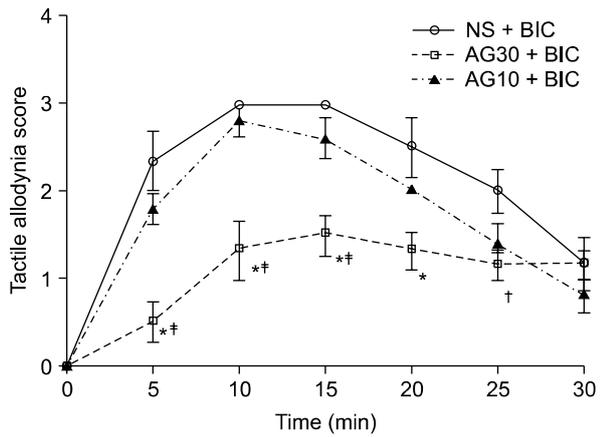


Fig. 1. Time course changes in the degree of touch evoked agitation (tactile allodynia score) assessed on a 4 point pain scale. Preemptive IT agmatine 10 μg (AG10-BIC) and 30 μg (AG30-BIC) dose dependently attenuated the bicuculline (NS-BIC) induced tactile allodynia. (* $P < 0.01$ vs NS-BIC, † $P < 0.05$ vs NS-BIC, ‡ $P < 0.01$ vs AG10-BIC,). Each curve represents mean ± SEM bar of $n = 6-7$ for each time point.

AG10 with NMDA groups, respectively (Fig. 3).

DISCUSSION

Over the past few decades, several mechanisms have been reported in the development and maintenance of devastating neuropathic pain. It has been generally accepted that neuropathic pain arises due to lesions or dysfunctions in either the peripheral nerve system (PNS) or CNS. The pivotal mechanisms in the CNS are central

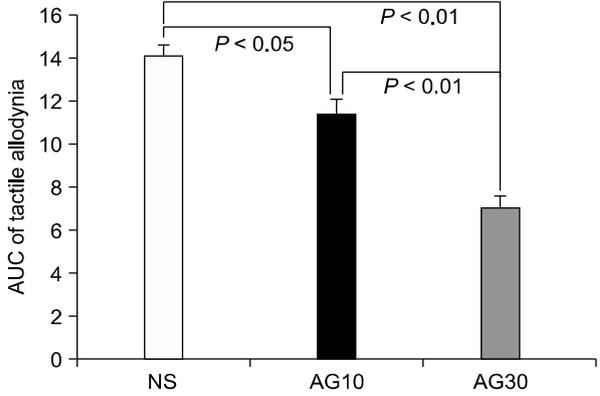


Fig. 2. Comparison of the areas under curves (AUC) of NS (NS-BIC), AG30 (agmatine 30 μg-BIC) and AG10 (agmatine 10 μg-BIC) groups. Agmatine significantly attenuated, in a dose dependent manner, the bicuculline (NS-BIC) induced tactile allodynia. Each bar is the mean ± SEM of $n = 6-7$ for each group.

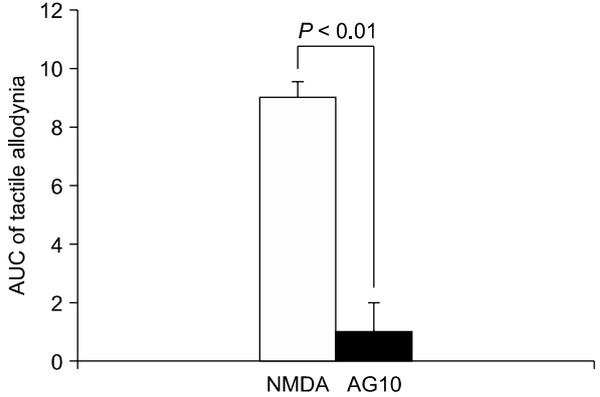


Fig. 3. Comparison of the areas under curves (AUC) of NMDA (NS-NMDA 5 μg) and AG10 (agmatine 10 μg-NMDA 5 μg) groups. Agmatine 10 μg significantly attenuated the NMDA 5 μg induced tactile allodynia. Each bar is the mean ± SEM of $n = 5$ for each group.

sensitization as well as a loss or dysfunction of the inhibitory control of spinal GABAergic and glycinergic interneurons.¹⁻⁴⁾ Central sensitization is initiated by excessive glutamate release and activation of the NMDA receptor. This is followed by a triggering of the NOS cascade and subsequent gene expression due to Ca^{2+} influx, resulting in long-term potentiation changes in synaptic transmission between nociceptive C-fibers and secondary projection neurons. An important sequela of nerve injury and other nervous system diseases is apoptosis of neurons in the PNS and CNS. Apoptotic neural death seems to induce neuronal sensitization and loss of inhibitory systems, and these irreversible processes and pathologic pain might be prevented by preemptive MK-801 in animal neuropathic pain models.^{1,17)}

Until now, there has been much controversy regarding the cause of neuropathic pain. Some workers believe significant loss of GABAergic boutonnières occurring at the denervated area after peripheral nerve injury are implicated,¹⁸⁻²⁰⁾ whilst others have speculated that decreased dorsal horn levels of the GABA synthesizing enzyme are responsible.^{17,21,22)} Although the above controversy focused on the morphological changes in the dorsal horn, the belief that functional synaptic disinhibition involved distinct mechanisms of both inflammatory pain and neuropathic pain has remained constant. The central component of inflammatory pain sensitization is caused by PGE2 mediated reduction in glycinergic neurotransmission. PGE2 synthesized by inflammation induced cyclooxygenase2 activates postsynaptic EP2 receptor. Once activated, EP2 receptor then down regulates the activation of G-protein that triggers cAMP and PKA which is responsible for the phosphorylation and inhibition of the alpha3 subunit of the glycine receptor (Gly R $\alpha 3$).³⁾ On the other hand, microglia induces the disinhibition of GABA/Glycine interneurons. That is, microglia releases BDNF which down-regulates KCC-2 expression which then diminishes the transmembrane Cl^- gradient. The net effect of this is that the GABAergic and glycinergic input is rendered less inhibitory i.e. more excitatory.^{3,23,24)}

In this our study, treatment with IT BIC (10 μg) resulted in a reproducible functional loss of GABA_A receptor activation and induced significant TEA which resembled

the clinical phenomenon observed in neuropathic pain patients suffering from tactile allodynia.⁴⁻⁸⁾ GABA_A may modulate responses to an innocuous tactile stimuli via their action as inhibitory neurotransmitters at pre- and postsynaptic sites in the dorsal horn of the spinal cord.^{6,7)} The disinhibition of presynaptic glutamate release, the postsynaptic NMDA receptor-NOS pathway^{4-8,25)} and activated microglia (which releases mediators such as cytokines, PGE₂, BDNF and NO) mediated modulation is crucial in inducing tactile allodynia.^{3,26)}

In CNS neurons, agmatine is packaged into synaptic vesicles which are released upon neuronal depolarization. Initially, agmatine was conceptualized as synaptic vesicles containing an endogenous substance that acted to displace clonidine on imidazoline receptors. However, agmatine has now been established to have affinity for several transmembrane receptors, such as alpha₂-adrenergic, imidazoline and NMDA receptors.⁹⁾ Our in vivo study established an antagonistic effect for agmatine on NMDA receptors which is in agreement with other in vitro studies.^{9,11)} In addition to activity at these receptors, agmatine irreversibly inhibits neuronal NOS and down-regulates inducible NOS.^{9,27)} The pharmacodynamic response to agmatine in NMDA-nociceptive behavior and thermal hyperalgesia tests showed that agmatine inhibits thermal hyperalgesia with significantly increased potency compared to nociceptive behavior, while MK-801 inhibits these 2 responses with equal potency. These results suggest that agmatine has 2 sites of action.²⁸⁾

Agmatine increased the neuropathic pain threshold through an effect that may involve the reduction of NO levels and noradrenergic activity in the brain.¹⁴⁾ An increased number of large stellate or elongated somata in deep lamina III-V of the L5 segment expressed high neuronal NOS immunoreactivity. These results indicated that marked alterations of neuronal NOS in the spinal cord may contribute to spinal sensory processing as well as to development of neuronal plasticity phenomena in the dorsal horn.²⁹⁾ Both systemically and spinally administered agmatine produced marked antinociception, and furthermore interacted with glutamate receptors, namely NMDA and metabotropic NMDA-ionic receptors.¹⁰⁾

However several reports found that NOS is not related

to allodynia in neuropathic pain models.^{30,31)} Lee et al.³⁰⁾ reported that a potent neuronal NOS inhibitor did not reduce mechanical sensitivity in spinal nerve ligated rats, and that neither the anti-allodynic nor the preemptive effects of L-NAME were mediated by NOS inhibition. Additionally, Karadag et al.³¹⁾ reported that agmatine reduced mechanical allodynia at high doses, but that MK-801 and the NOS inhibitors, such as N(G)-nitro-L-arginine methyl ester and 7-nitroindazole, did not influence the antiallodynic effects of agmatine. These results suggest that agmatine has a non-neuronal action site. Abe et al.³²⁾ reported that agmatine suppressed NO production in microglia. Neuronal death induced by microglia-derived NO was significantly attenuated in the presence of agmatine, which suggests that agmatine works to protect neurons by inhibiting production of NO in microglia.^{32,33)} Agmatine also has neuroprotective effects against hypoxia-induced retinal ganglion cell damage through the JNK and NF- κ B signaling pathways.³⁴⁾ This result suggests that agmatine could inactivate intra-microglial mitogen-activated protein kinase which activates microglial secretion of potentially neurotoxic mediators including proinflammatory cytokines, NO, BDNF and PGE2.

In contrast to the pharmacokinetic results, agmatine action on the pain behaviors had a duration of only 10 to 30 min. Such results suggested the existence of a currently undefined agmatinergetic extracellular clearance process in the spinal cord.¹⁵⁾ In the present study, agmatine significantly attenuated BIC-induced tactile allodynia for 25 min which showed similar pharmacokinetic effects to the above mentioned reports.

We suggest that BIC induces the functional loss of GABA_A receptors which results in excessive pre-synaptic glutamate release and activation of post-synaptic NMDA receptors. Agmatine, exogenously administered to the BIC-induced allodynia rat model, decreased TEA significantly, an effect mediated through its inhibitory actions on either neuronal NMDA receptor or NOS pathway. In addition, spinal microglia might facilitate the development of BIC-induced tactile allodynia. However, further studies are needed to reveal the neuroglial interactions involved in the mechanisms of BIC-induced allodynia. Agmatine might represent a viable treatment choice for neuropathic pain In

a clinical situation where there is no commercially available NMDA receptor antagonist or microglial inhibitor.

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