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Antinociceptive effect of intracerebroventricular administration of
D-serine on formalin-induced pain

伊藤 美保

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I got permissions for the submission of the doctoral thesis to the institutional repository of Tokai University, in addition to rescue of part of my article in my doctoral thesis.

Introduction

Pathway for pain

Pain perception depends on the activation of specialized peripheral neurons called primary afferent nociceptors (PANs) that are free nerve ending of primary afferent nerves called A δ and C fibers. Different PANs are tuned to respond to a variety of intense, potentially tissue damaging, thermal, mechanical and chemical stimuli. PANs arise from unipolar cell bodies in the dorsal root ganglion (DRG). This axon bifurcates to send a peripheral process out to innervate somatic and visceral tissues and a central process into the spinal cord where it terminates in the gray matter of the superficial dorsal horn (Fig.1 and Fig.2). A δ nociceptors are activated by mechanical and thermal stimuli. A δ fibers are the smallest of the myelinated nerves. They are 2-5 μ m in diameter and are fast with a conduction velocity of 6-30 m/s. Activation of A δ fibers results in shortlasting, pricking-type pain. C nociceptors are polymodal (mechanical, thermal, chemical). C-fibers are unmyelinated, less than 2 μ m in diameter and have a slow conduction velocity of 0.5-2 μ m/s. Activation of C fibers results in dull, poorly localized, burning type pain. Stimulation of these nociceptors occurs when the pain threshold is reached. This results in propagation of impulses along the afferent fibers toward the dorsal horn of the spinal cord or to the medulla. Stimulation of the primary afferent nociceptors that result in pain can be from physical stimuli such as mechanical injury, noxious heat, radiation, chemical mediators released from the afferent nociceptors include substance P, calcitonin-gene-related peptid(CGRP), neurokinins, chemical mediators released from damaged cells include bradykinin, potassium, serotonin, histamine,

cytokines, nitric oxide, hydrogen ions, prostaglandins, leukotrienes, slow reacting substance of anaphylaxis.

Primary afferent nerves form synapse upon spinal dorsal horn (DH), which then contact the projection cells. Nociceptive projection cells terminate in the brainstem and thalamus. In the brainstem, the projection cells target the reticular formation in the medulla and pons, and then the dorsolateral region of the pons and midbrain. There are several distinct thalamic regions that receive nociceptive input and each thalamic region has a different cortical projection target. Cortical areas receiving nociceptive input include the primary and secondary somatosensory cortex, the anterior cingulate and insular cortex. Human functional image studies have demonstrated that activity in each of these areas correlates with perceive pain intensity and/or unpleasantness [1]. Descending pain modulation systems originating in the brainstem can inhibit or facilitate peripheral nociceptive input in the dorsal horn (Fig.1). Brainstem descending pathways linking the periaqueductalgray (PAG), the rostral ventromedial medulla (RVM), and the spinal cord constitute a major mechanism in the modulation of pain transmission [2-5]. The PAG-RVM-spinal cord descending pathways are involved in pain modulation. The PAG in the midbrain has efferent projections to the RVM, and RVM neurons directly project to DH where nociceptive input is initially processed [6]. Primary afferent dorsal root ganglion (DRG) neurons transmit peripheral noxious input to the spinal DH(dorsal horn) (Fig.2). Supraspinal projecting DH neurons also issue collaterals to reach terminating in pain modulatory structures and the PAG also receives input from forebrain structures including hypothalamus, cerebral cortex and amygdala. Inflammation induces peripheral sensitization associated with an increased barrage of primary afferent activity and increased activation of spinal DH neurons, followed by central sensitization. Descending pain inhibitory or facilitatory pathways from RVM act in DH in acute and chronic pain states through activation of 5-HT_{1 or 7} serotonin receptor and 5-HT₃ serotonin

receptor, respectively [7, 8]. Additionally, descending pain inhibitory pathway from locus coeruleus act in DH through α_2 adrenaline receptors in primary nociceptive terminals [9].

NMDA receptor

N-methyl-D-aspartate receptor (NMDAr) have critical roles in excitatory synaptic transmission, plasticity and excitotoxicity in the CNS. The involvement of NMDAr in these diverse processes reflects their unique features, which include voltage-sensitive block by extracellular Mg^{2+} and Ca^{2+} , unusually 'activation/deactivation' kinetics. NMDAr also display sensitivity to an array of endogenous ligands and modulators present in the vicinity of the synapse: the co-agonist glycine must bind before the receptors can be activated, whereas physiological levels of protons suppress NMDAr activation. Extracellular Zn^{2+} and polyamines also act on the receptor to modify its behavior. Furthermore, NMDAr subunits interact with various intracellular scaffolding, anchoring and signaling molecules associated with the postsynaptic density. Several NMDAr subtypes have now been identified in central neurons, differing in their sensitivity to endogenous and exogenous ligands. Biophysical, pharmacological and molecular methods are all providing a clearer picture of the key features defined by particular subunits and are furnishing tools that can be used to determine the involvement of specific subunits in synaptic transmission. NMDAr subunit variety can be useful for therapeutic advantage. Recent investigators have reviewed that role of NMDAr is developmental plasticity, learning and memory [10]. In their early studies of excitatory and depressant amino acids in brain, noted that the d-isomer of serine (but not the natural l-isomer) excited preparations of toad spinal cord, while glycine was a depressant. These investigators later showed that the depressant effect of glycine was due to its action at inhibitory receptors that could be blocked by the poison strychnine [11], but they did not refer to the excitatory effects of D-serine. For the receptor binding methods, it was confirmed that glycine and strychnine bound to the same receptor

complex, an inhibitory chloride channel, located mainly in the brainstem and spinal cord. However, it was noticed that glycine and strychnine binding sites were not always the same [12]. A number of investigators described a 'strychnine-insensitive' binding site for glycine in brain that was displaced by D-serine and was concentrated in the forebrain [13]. These early reports are not trusted until demonstrated that the NMDAr subtypes of glutamate receptors required the presence of a small, heat stable factor from their conditioned culture media in order to operate. They concluded that this factor was probably glycine. Subsequent work has demonstrated that the NMDA receptor channel is unusual among ligand-gated ion channels in that the channel opens to allow calcium and sodium influx only when two different ligands bind the receptor simultaneously [14]. The channel complex itself is almost certainly a tetramer [15], consisting of two NMDAr 1 (NR1) subunits and two NMDAr 2(NR2) subunits. Two molecules of the neurotransmitter glutamate bind to the NR2 subunits and two molecules of a different agonist bind to the NR1 subunit at the called 'glycine site'. The glycine site can be activated not only by glycine but also by the d-isomer of serine, and this explains the early investigators. At some glycine sites, D-serine is in fact more efficacious than glycine itself [17, 18]. D-Serine is often used in experiments as a glycine mimic because it does not bind to strychnine sites nor is it rapidly taken up into cells and metabolized. Despite glycine's higher affinity, D-serine is often more effective at gating expressed channels [17]. This is due to the relative lack of uptake and metabolism systems for D-serine compared with those for glycine [16]. The concentrations of glycine in the cerebrospinal fluid are in the low micro-molar range [19, 20] similarly, micro-dialysis experiments of the extracellular fluid from rat frontal cortex report the concentrations of both glycine and D-serine to be ca. 7 μ M [17]. Based on the reasoning that micro-molar concentrations of ligand would saturate the glycine sites in vivo, it was suggested that the glycine site might be constantly occupied and therefore physiologically silent [21], and

at least one study has reported that exogenous glycine has no enhancing effect on NMDAr activity in vivo [22]. However, this view is untenable in light of the more than 40 studies in the literature demonstrating that this site is not saturated in vivo [16, 23-32] for references to the many excellent earlier studies and a more detailed discussion.

Recently, it was reported that ligand binding at the glycine site leads to a priming of the endocytic machinery that internalizes the NMDAr following activation this points to an exciting new physiological role for the glycine site. The trafficking of non-NMDA glutamate receptors regulates some forms of synaptic plasticity [33], and similar mechanisms might also regulate surface expression or turnover of NMDAr. Because the glutamate and glycine sites reside on different subunits, one can envision situations where different ligands could influence the trafficking of their cognate receptors. Whatever the reason for the existence of the glycine site, the NMDA subtype of the glutamate receptor is a key component of many developmental, learning and memory processes in mammals and endogenous agonists acting at its glycine site participate in the modulation of these processes. However, the physiological role of the glycine site agonists is less studied compared with glutamate because many physiologists routinely supplement their recording buffers with micro-molar concentrations of glycine. Under these conditions the glycine site can be conveniently ignored.

The surprising discovery that the 'wrong isomer' of serine occurs in brain grew out of advances in techniques to assay small quantities of d-amino acid isomers against the high background of l-isomers present in biological samples [29, 34, 35]. The high correlation between D-serine levels and NMDAr binding in brain suggested that D-serine might be an endogenous glycine site agonist [36]. Confident evidence for this proposal has reported since 1993. Immunohistochemistry revealed that endogenous D-serine is localized in astrocytes of the forebrain grey matter, near or ensheathing NMDAr synapses. Agonists of the non-NMDA

subtypes of the glutamate receptor promote the release of preloaded D-serine from cultured astrocytes [37]. Treatment of brain slices with d-amino acid oxidase (DAO), which selectively destroys endogenous D-serine but not glycine, reduces NMDAr responses, demonstrating that some glycine sites are occupied by D-serine in vivo [38]. A case for D-serine NMDAr has also been made for the retina, where D-serine is localized in the Muller glia, and DAO treatment reduces the NMDA response [39]. Perhaps the most compelling evidence for a physiological role for D-serine was the discovery and characterization of the brain enzyme serine racemase, which converts L-serine into D-serine [40-42].

D-serine

D-serine, a co-agonist of the glycine site of the NMDAr, have been involved in many neurodegenerative disorders, in which pathological over activation of the receptor results in neurotoxicity, as revealed by studies performed in cell cultures and observed in the Alzheimer disease. Activation of NMDAr and metabotropic glutamate receptors contribute to Ca^{2+} overload, the latter via phospholipase C signaling. As a result of glutamate mediated overactivation, Na^+ and Cl^- enter the neurons via channels for monovalent ions (for example, the AMPA receptor channel). The overactivation of the NMDAr contributes also to acute disorders as ischemia [43]. In the in vitro studies, D-serine induced cytotoxicity in hippocampal slice cultures, since the removal of endogenous D-serine completely attenuate NMDA neurotoxicity. In serine racemase KO mice (SR-KO), around 90% decrease in forebrain D-serine content has been observed, and in parallel, a reduced neurotoxicity induced by both NMDA and β -Amyloid1-42 forebrain SR-KO mice injection was found. The former strongly suggest that SR is the responsible for the major D-serine production (Ran Inoue). Microglia culture cells exposed to amyloid β -peptide as an inflammatory stimuli elicited a release of glutamate. This activated microglia express serine racemase (SR), and thereby release D-serine, contributing to the neurotoxicity found in

inflammatory conditions in the brain, and also in the Alzheimer's disease where SR RNA was elevated. Regarding D-serine degradation, it is performed by the D-amino acid oxidase, thus contributing to the D-serine homeostasis [44]. Another mechanism proposed to be responsible of D-serine modulation is gated by NMDAr activation, promoting the translocation of SR to the plasma membrane, thus reducing enzyme activity [37]. In brain areas where D-amino acid oxidase is poorly expressed or absent, (forebrain areas) degradation of D-serine is accomplished by a self-degradation mechanism by the α , β -elimination of water [44]. A cell line (HEK293) expressing mouse serine racemase was used to test L-serine-O-sulfate as an alternative substrate. The authors found that the degradation of L-serine-O-sulfate (to pyruvate and ammonia) was two or three orders of magnitude faster than the L-serine racemization. Also, they demonstrated the pharmacological inhibition of D-serine synthesis in cultured astrocytes, thus providing a strategy to selectively decrease NMDAr activation [45]. NMDARs are key glutamatergic receptors in the CNS. Their permeability to voltage-dependent Mg^{2+} block make the messential for synaptic transmission, synaptic plasticity, gene express ion and excite toxicity. One very unique characteristics is that the inactivation requires the binding of both glutamate and a co-agonist like glycine or D-serine. D-serine is synthesized main lying glial cells and it is released upon activation of glutamate receptors. Its concentration in the synaptic cleft controls the number of NMDAr available for activation by glutamate. Consequently, the glia of neurons has critical impact on the direction and magnitude of NMDAr dependent synaptic plasticity [46].

D-serine acts as an obligatory ligand for the glycine sites of NMDAr [16, 17, 29]. D-serine is confined to the forebrain, where NMDAr are abundantly expressed [29], and has been proposed as an endogenous co-agonist at NMDAr in mammalian brain [36, 41]. A large body of evidence has shown that D-serine has several physiological and/or pathological functions.

D-serine and schizophrenia

At present, all medications for schizophrenia function primarily by blocking dopamine D2 receptors. Over 50 years ago, the first observations were made that subsequently led to development of alternative, glutamatergic conceptualizations. This special issue traces the historic development of the phencyclidine (PCP) model of schizophrenia from the initial description of the psychotomimetic effects of PCP in the early 1960s, through discovery of the link to NMDAr in the 1980s, and finally to the development of NMDA-based treatment strategies starting in the 1990s. PCP had been used as an anesthetic, and it was never used for the side effect (schizophrenia like symptom). NMDAr antagonists uniquely reproduce both positive and negative symptoms of schizophrenia, and induce schizophrenia-like cognitive deficits and neurophysiological dysfunction. At present, there remain several hypotheses concerning mechanisms by which NMDAR dysfunction leads to symptoms/deficits, and several theories regarding ideal NMDAR-based treatment approaches as outlined in the issue. Several classes of agent, including metabotropic glutamate agonists, glycine transport inhibitors, and D-serine-based compounds are currently in late-stage clinical development and may provide long-sought treatments for persistent positive and negative symptoms and cognitive dysfunction in schizophrenia. [47] Schizophrenia is a chronic, pervasive, and complex neuropsychiatric disorder that is ranked as a leading cause of disability worldwide. The disease afflicts nearly 1% of the world's population, affecting both men and women equally, and striking all ethnic and socioeconomic groups with a similar level of prevalence. The illness is characterized by a constellation of overlapping symptoms that are categorized into three clusters known as positive symptoms (hallucinations and delusional behaviors), negative symptoms (anhedonia, social withdrawal and apathy), and cognitive dysfunction.

Activation of the NMDAr relies on two synergistic processes; simultaneous binding of glutamate at GluN2 and an obligatory co-agonist at GluN1 (glycine or D-serine) to initially open

the channel followed by membrane depolarization required to alleviate the magnesium block from the channel pore. Once these two events have occurred, calcium and sodium ions permeate into and potassium ions out of the post-synaptic neuron triggering signaling cascades that promote long term potentiation (LTP), and neuronal plasticity. These two processes required for NMDAr activation present multiple points of intervention for enhancing function. Several recent and comprehensive reviews have been written about the current approaches that focus on glutamatergic targets for treating schizophrenia. This digest will present a concise overview of the most recent developments in these areas [48].

D-serine and amyotrophic lateral sclerosis

A potential role for D-amino acids in motor neuron disease/amyotrophic lateral sclerosis (ALS) is emerging. D-Serine, which is a co-agonist at the NMDAr subtype, is elevated both in spinal cord from cases of ALS and in an animal model of ALS. Furthermore, recent investigator said hat a mutation in D-amino acid oxidase (DAO), an enzyme strongly localized to spinal cord motor neurons and brain stem motor nuclei, is associated with familial ALS. DAO plays an important role in regulating levels of D-serine, and its function is impaired by the presence of this mutation and this may contribute to the pathogenic process in ALS. In sporadic ALS cases, elevated D-serine may arise from induction of serine racemase, its synthetic enzyme, caused by cell stress and inflammatory processes thought to contribute to disease progression. Both these abnormalities in D-serine metabolism lead to an increase in synaptic D-serine that may contribute to disease pathogenesis [49].

D-serine induced nociceptive effect (spinal level)

During nociceptive transmission, the output of the spinal cord is dependent on various spinal mechanisms which can either increase or decrease the activity of dorsal horn neurons. Such mechanisms include local excitatory interneurons, activation of glutamate receptors NMDAr,

α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. Neuropathic pain following nerve injury is a chronic pain by allodynia and hyperalgesia of either mechanical or thermal origin. The mechanism underlying this disease is poorly understood leading to pharmacologic and physiotherapeutic control that is often insufficient.

Allodynia is a popular symptom of the neuropathic pain. It is condition to recognize tactile stimulus as a pain. Allodynia expression relies on NMDAr, and it has been shown that astrocytes can regulate their activation through the release of the NMDAR co-agonist D-serine. Selective degradation of D-serine with D-amino acid oxidase applied in vivo prevented allodynia. Conversely, allodynia blockade by the glia inhibitor, fluorocitrate, was reversed by exogenous D-serine. These results suggest that activated astrocytes may provide D-serine to enable NMDAR activation and thus allodynia [50].

D-serine induced antinociceptive effect (supraspinal level)

Although there is a variety of information concerning the effects of the NMDAr on opioid-induced antinociception at the spinal level, little is known about the effects at the supraspinal level. To clarify the role of the NMDA receptor on the morphine-induced antinociception at the supraspinal level, we investigated the effects of the intracerebroventricular (i.c.v.) administration of D-serine, a selective agonist for the glycine site of the NMDAr, alone or in combination with morphine using the tail-flick test [51]. The previous study has demonstrated following four results. 1) The i.c.v. administration of D-serine, but not L-serine, alone produced a dose-dependent antinociception. 2) D-Serine also dose-dependently potentiated the antinociceptive effect induced by the i.c.v. administration of morphine and the simultaneous administration produced an additive effect. 3) The potentiation of the antinociception produced by both D-serine alone or in combination with morphine was dose-dependently attenuated by the i.c.v. administration of L-701,324, a selective antagonist for the glycine site of the NMDA

receptors. 4) the potentiation of the D-serine-induced antinociception was antagonized by the i.c.v. administration of naloxone, a nonselective opioid receptor antagonist. These observations, together with the fact that D-serine is an endogenous and selective co-agonist for the glycine site of the NMDAr, strongly suggested that the activation of the supraspinal NMDAr by D-serine leads to the potentiation of the antinociception in the tail-flick test and that endogenous D-serine could modulate the μ -opioid receptor mediated antinociception via the glycine site of the NMDAr at the supraspinal level. These results elucidated by using tail flick test [51].

Formalin test and tail-flick test

The formalin test is used to measure acute pain induced by chemical or physical damage or tonic pain induced by inflammatory stimulation [52]. The formalin test in mice is a popular model of nociception and is sensitive for various classes of analgesic drugs. The noxious stimulus is an injection of dilute formalin (1~5% in saline) under the skin of the dorsal surface of the hindpaw. The response is the amount of time the animals take action, for example licking, flinching, lifting, biting, guarding, shaking. Two distinct periods of high activity can be identified, an early phase lasting the first 6 min and a late phase lasting from 10 min after the injection of formalin. It is suggested that the early phase is due to a direct effect on nociceptors and that prostaglandins do not play an important role during this phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [53].

Whereas most other models, such as the tail-flick test, only allow the threshold for an escape response to be measured, the formalin test permits assessment of the way animals respond to moderate and continuous pain generated by injury to tissue [54]. Thus, it mimics some of the features of the type of pain that naturally occurs following damage to or injury of tissue. This, then, provides a more accurate model of clinical pain than that achieved with the

more typically used analgesic tests such as the tail-flick test [55]. As the type of pain and analgesic response mechanism involved is different to that with the tail-flick test [54], it is necessary to investigate the role of supraspinal NMDAr in elucidating antinociception against formalin-induced pain.

Summary

Objective: In a previous study using the tail-flick test, we found that intracerebroventricular administration of D-serine, an endogenous co-agonist at the glycine sites of N-methyl-D-aspartate (NMDA) receptors, elicited an antinociceptive effect on thermal nociception. The purpose of the present study was to evaluate the effect of intracerebroventricular administration of D-serine on nociception induced by tissue damage or inflammation using the formalin test.

Methods: Infusion of drugs into the third ventricle in rat was performed via indwelling cannulae. Drugs were infused at a volume of 10 µl over 2 min and the infusion cannula left in place for 2 min before removal. The formalin test was performed 10 min after drug administration.

Results: Intracerebroventricular administration of D-serine significantly and dose-dependently decreased the number of flinches in both the early and late phases in the formalin test. This antinociceptive effect was antagonized by intracerebroventricular administration of L-701,324, a selective antagonist at the glycine sites of NMDA receptors.

Conclusion: The present data suggest that activation of NMDA receptors via glycine sites at the supraspinal level induces an antinociceptive effect on both acute and tonic pain.

Discussion

The results of the present study demonstrated that intracerebroventricular administration of D-serine, but not L-serine, produced a dose-dependent antinociceptive effect on both acute and tonic formalin-induced pain. The effective doses (0.5-10 μmol) induced no behavioral side effects. The antinociceptive effect of D-serine, but not L-serine, may be related to stimulation of the glycine sites of NMDA receptors, as this stereoselectivity corresponds well with the potency of these stereoisomers as agonists at glycine sites. In fact, D-serine has a 100-times greater potency in displacing [^3H] glycine binding to glycine sites compared with L-serine [16]. Further support for this comes from the fact that a selective antagonist at glycine sites on NMDA receptors, L-701,324, antagonized the antinociceptive effect induced by D-serine in a dose-dependent manner.

These observations suggest that activation of NMDA receptors at the supraspinal level may lead to potentiation of the antinociceptive effect in the formalin test. Taken together with the present results, several lines of evidence also support this possibility: first, administration of NMDA into the cerebroventricular region or PAG induced an antinociceptive effect in the formalin test [62, 63]; second, in the present study, intracerebroventricular administration of L-701,324 alone increased paw flinch latencies in phase 1 and phase 2; third, again, in this study, intracerebroventricular administration of D-serine induced the antinociceptive effect of a selective antagonist for glycine sites on NMDA receptors in an L-701,324-reversible manner; and fourth, intracerebroventricular administration of D-serine induced the antinociceptive effect in the tail-flick test [51]. Interestingly, it has also been reported that intracerebroventricular administration of D-serine induced the antinociceptive effect produced by morphine in both the formalin [64] and tail-flick test [51]. These studies indicate functional interactions between NMDA and μ -opioid receptors in the regulation of the antinociceptive effect at the supraspinal

level. The mechanisms underlying these interactions between supraspinal NMDA and μ -opioid receptors remain to be determined. However, activation of supraspinal NMDAr may lead to opioid-induced activation of the descending inhibitory system and produce an antinociceptive effect, as off-cell activation is known to be necessary for the descending inhibitory effects of supraspinal administration of morphine; and because administration of AP5 into the rostral ventromedial medulla blocked the morphine-induced activation of off-cells, significantly reducing the antinociceptive effect of this drug [40, 65, 66]. Alternatively, the antinociceptive effect of D-serine could be due to the release of endogenous opioid peptides at the supraspinal level, as administration of NMDA and L-glutamate into the hypothalamus or striatum leads to the release of methionine-enkephalin and β -endorphin, respectively, in an NMDA receptor-antagonist-reversible manner [67-69].

In previous studies, administration of AP5 and MK-801, NMDA receptor antagonists, into the cerebroventricular region and PAG, respectively, induced an antinociceptive effect in the formalin test [70, 71]. In the present study, however, intracerebroventricular administration of L-701,324 (137 nmol) significantly induced nociception in phase 1 of the formalin test. L-701,324 (137 nmol) caused an increase, but not significantly so, in the number of flinches in phase 2 of the formalin test. One reason for this inconsistency in the results may lie in differences in the recognition sites for these antagonists. Both competitive antagonists at glutamate recognition sites, such as AP5, and non-competitive antagonists at phencyclidine sites on NMDAr, such as MK-801, are reflected in neurotoxic, psychotomimetic and other functional actions. Compared to these two types of antagonist, antagonists at the glycine sites of NMDAr, such as L-701,324, display a more benign side-effect profile in terms of a relative lack of neurotoxic action, including motor disco-ordination and psychotomimetic properties, while retaining neuroprotective and anticonvulsive properties [72]. The safety profile of

antagonists at the glycine sites of NMDAr may be superior to those of both competitive and non-competitive antagonists [73]. In fact, a previous study using the tail-flick test demonstrated that intracerebroventricular administration of L-701,324 induced nociception [51].

In an earlier study using the tail-flick test, we showed that intracerebroventricular administration of D-serine elicited an antinociceptive effect on acute thermal nociception, and the ED₅₀ (7.660 μ mol) was higher than that on formalin-induced nociception in the present study (0.255 μ mol and 3.242 μ mol in phase 1 and 2, respectively)[51]. These data suggest that different pain tests reveal different pain modulation systems or different characteristics of a single analgesic system. Indeed, the antinociceptive effect produced by stimulation of PAG requires significantly less current intensity in the formalin test than in the tail-flick test [74]. The results of the present study suggest that different neural systems are involved in the antinociceptive effect of D-serine in tests of thermal and formalin-induced pain. Further study is necessary, however, to determine the reason for these different potencies.

Conclusion

Intracerebroventricular administration of D-serine elicited an antinociceptive effect on formalin-induced nociception in an L-701,324-reversible manner. Taken together with those of our earlier study [51], these findings suggest that activation of the glycine sites of NMDA receptors at the supraspinal level by the endogenous ligand D-serine induces an antinociceptive effect on both acute and tonic pain.