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# Intrathecally Administered D-Cycloserine Produces Nociceptive Behavior Through the Activation of *N*-Methyl-D-aspartate Receptor Ion-Channel Complex Acting on the Glycine Recognition Site

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**Abstract.** Intrathecal (i.t.) administration of D-cycloserine (100 and 300 fmol), a partial agonist of the glycine recognition site on the *N*-methyl-D-aspartate (NMDA) receptor ion-channel complex, produced a behavioral response mainly consisting of biting and/or licking of the hindpaw and the tail along with slight hindlimb scratching directed toward the flank in mice, which peaked at 5 – 10 min and almost disappeared at 15 min after the injection. The behavior induced by D-cycloserine (300 fmol) was dose-dependently inhibited by an intraperitoneal injection of morphine (0.5 – 2 mg/kg), suggesting that the behavioral response is related to nociception. The nociceptive behavior was also dose-dependently inhibited by i.t. co-administration of 7-chlorokynurenic acid (0.25 – 4 nmol), a competitive antagonist of the glycine recognition site on the NMDA receptor ion-channel complex; D-(−)-2-amino-5-phosphonovaleric acid (62.5 – 500 pmol), a competitive NMDA receptor antagonist; MK-801 (62.5 – 500 pmol), an NMDA ion-channel blocker; ifenprodil (0.5 – 8 nmol); arcaine (31 – 125 pmol); and agmatine (0.1 – 10 pmol), all being antagonists of the polyamine recognition site on the NMDA receptor ion-channel complex. However, [D-Phe$^7$,D-His$^9$]-substance P(6 – 11), a specific antagonist for substance P (NK1) receptors, and MEN-10,376, a tachykinin NK2-receptor antagonist, had no effect on D-cycloserine-induced nociceptive behavior. These results in the mouse spinal cord suggest that D-cycloserine-induced nociceptive behavior is mediated through the activation of the NMDA receptor ion-channel complex by acting on the glycine recognition site and that it does not involve the tachykinin receptor mechanism.

***Keywords***: D-cycloserine, *N*-methyl-D-aspartate (NMDA) receptor ion-channel complex, nociceptive behavior (mice), intrathecal administration

## Introduction

The *N*-methyl-D-aspartate (NMDA) receptor ion-channel complex has been demonstrated to play an important role in spinal nociceptive transmission (1, 2). In addition to possessing the NMDA receptor, to which glutamate binds, the NMDA receptor ion-channel complex contains several allosteric sites such as polyamine and glycine recognition sites that modulate the receptor function [for a review, see Ref. 3]. We have previously reported that extremely low doses of intrathecally (i.t.)-administered spermine (0.1 – 10000 fmol), an endogenous polyamine, produces nociceptive behavior in mice that is inhibited by several NMDA-receptor antagonists including ifenprodil, an antagonist of the polyamine recognition site (4). This observation suggests that the polyamine recognition site plays a crucial role in spinal nociceptive transmission. On the other hand, it has been reported that i.t.-administered D-serine, a putative endogenous agonist of the glycine

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recognition site, significantly enhances the C-fiber response of wide dynamic range neurons in the rat spinal dorsal horn, which can be blocked by 7-chlorokynurenic acid (7-Cl-Kyn), a selective antagonist of the glycine recognition site (5). In addition, i.t. administration of D-serine induces 7-Cl-Kyn-reversible hyperalgesia as measured by the tail-flick test in rats (6). However, there is no available report on the role of the glycine recognition site in nociceptive transmission in the mouse spinal cord. Therefore, in order to elucidate the role of the glycine recognition site on nociceptive transmission in the mouse spinal cord, the main purpose of the present study was to examine whether or not i.t. administration into mice of D-cycloserine, a partial agonist of the glycine recognition site, induces nociceptive behavior.

## Materials and Methods

*Animals*

Experiments were performed in male ddY-strain mice (Japan SLC, Hamamatsu) weighing 20 – 22 g and maintained on a 12-h light/dark cycle (light on from 8:00 a.m. to 8:00 p.m.) with a constant temperature (23 ± 1°C) and relative humidity of 55 ± 5%. Groups of 10 mice were used only once in each experiment.

*Intrathecal injections*

Intrathecal (i.t.) injections were made in unanesthetized mice in the L5, L6 intervertebral space as described by Hylden and Wilcox (7). Briefly, a volume of 5 $\mu$l was administered i.t. using a 28-gauge needle connected to a 50 $\mu$l Hamilton microsyringe with the animal lightly restrained to maintain the position of the needle. Puncture of the dura was indicated behaviorally by a slight flick of the tail.

*Behavioral observation*

Approximately 60 min before the i.t. injection, the mice were adapted to an individual transparent cage (22.0 × 15.0 × 12.5 cm), which was also used as the observation chamber after injection. Immediately after the i.t. injection, the mice were returned to their cage and the accumulated response time of hindlimb scratching directed toward the flank, biting, and/or licking of the hindpaw and the tail was measured for 15 min except in the time course experiment. The measurement of the behavioral responses was performed blind; the observer had no information as to group designation.

*Drugs*

The following drugs and chemicals were used: D-cycloserine, 7-Cl-Kyn, ifenprodil tartrate, arcain sulfate (Sigma Chemical Co., St. Louis, MO, USA); D(−)-2-amino-5-phosphonovaleric acid (D-APV), (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) (Research Biochemical, Inc., Natick, MA, USA); agmatine sulfate (Tocris Cookson, Bristol, UK); morphine hydrochloride (Sankyo, Tokyo); and MEN-10,376 (Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Lys-$NH_2$) (Peninsula Labs., Belmont, CA, USA). [D-Phe$^7$,D-His$^9$]-substance P(6 – 11) was a gift from Dr. Masataka Ohba (Asahi Glass Co., Yokohama). For i.t. injections, most compounds were dissolved in Ringer's solution, whereas 7-Cl-Kyn, ifenprodil, and MEN-10,376 were dissolved in Ringer's solution containing 8%, 20%, and 25% dimethylsulfoxide (DMSO), respectively. NMDA receptor-related and tachykinin-receptor antagonists or the corresponding vehicle were co-administered i.t. with D-cycloserine (300 fmol), and the effect of each antagonist was compared with the corresponding vehicle-treated group. Morphine was dissolved in saline and administered intraperitoneally (i.p.) 5 min prior to injection of D-cycloserine.

*Statistics*

The results are presented as the means and S.E.M. The $ID_{50}$ values with 95% confidence limits were calculated for reduction in the D-cycloserine-induced scratching, biting, and licking responses by a computer-associated curve-fitting program (GraphPad Prism; GraphPad Software, Inc., San Diego, CA, USA). Significant differences between groups were determined by Fisher's PLSD post hoc test for multiple comparisons after analysis of variance (ANOVA) except in the time course experiment, which was tested with the Mann-Whitney U-test (two-tailed). In all statistical comparisons, $P<0.05$ was used as the criterion for statistical significance.

## Results

*Behavioral response induced by i.t.-administered D-cycloserine*

The i.t. administration of D-cycloserine (300 fmol) produced a characteristic behavioral response mainly consisting of biting and/or licking of the hindpaw and the tail along with slight hindlimb scratching directed toward the flank, which peaked at 5 – 10 min and had almost disappeared by 15 min after injection (Fig. 1a). As seen in Fig. 1b, a dose-dependent increase in the total time of scratching, biting, and licking was observed following i.t. administration of D-cycloserine in doses ranging from 30 to 300 fmol. However, no further increase in the behavioral response was produced by injection of D-cycloserine at higher doses of 1000 and

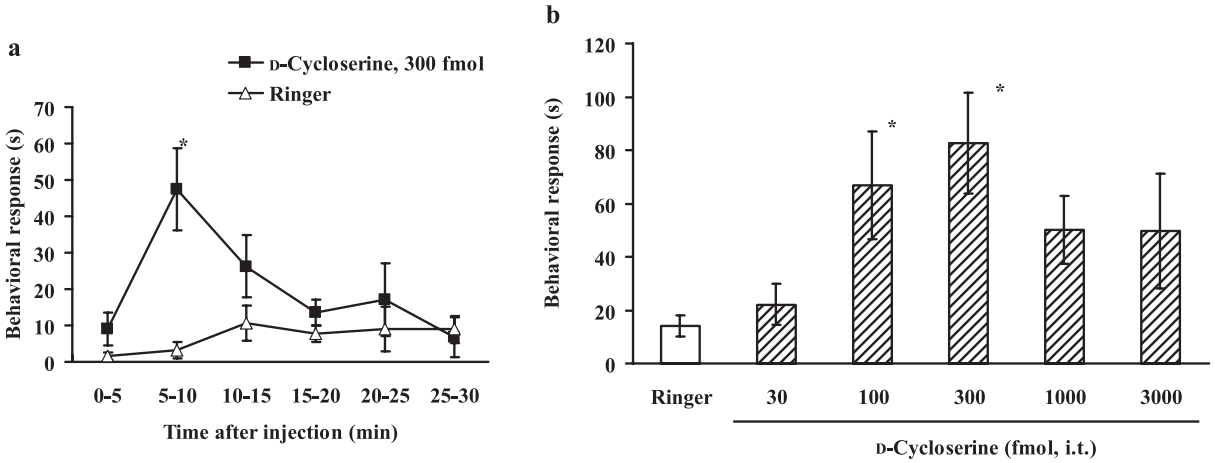


**Fig. 1.** Scratching, biting, and licking responses induced by i.t.-administered D-cycloserine in mice. a: Time-courses of the behavioral response induced by D-cycloserine (300 fmol) or Ringer's solution alone. The ordinate shows the total time of scratching, biting, and licking response that occurred during each 5 min of measurement. b: Effects of varying doses of D-cycloserine. The duration of scratching, biting, and licking induced by D-cycloserine or Ringer's solution was determined during a 15-min period starting immediately after the i.t. injection. These data are given as the means and S.E.M. for groups of 10 mice. **P*<0.05, when compared with the Ringer's solution controls.

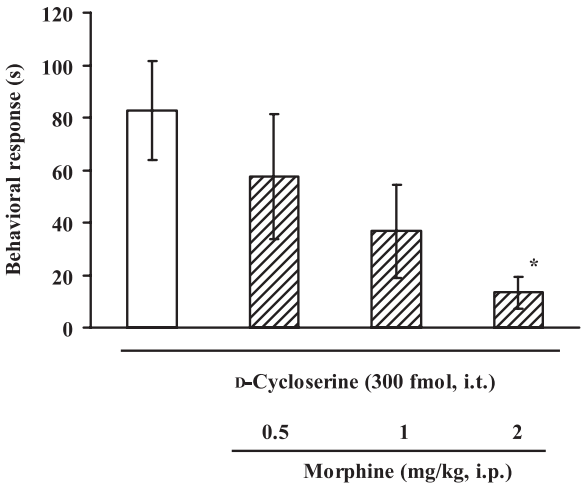


**Fig. 2.** Effect of morphine on D-cycloserine-induced scratching, biting, and licking responses in mice. Morphine was administered i.p. 5 min prior to injection of D-cycloserine (300 fmol). The duration of scratching, biting, and licking responses induced by D-cycloserine was determined during a 15-min period starting immediately after i.t. injection. These data are given as the means and S.E.M. for groups of 10 mice. **P*<0.05, when compared with D-cycloserine alone.

3000 fmol. In further experiments, 300 fmol of D-cycloserine and a 15-min observation time were therefore used in combination with various drugs to test their inhibitory actions. It was then examined whether or not D-cycloserine-induced behavior is reversed by morphine. As shown in Fig. 2, pretreatment with morphine (0.5 – 2 mg/kg, i.p.) inhibited D-cycloserine-induced behavior in a dose-dependent manner with an $ID_{50}$ value of 0.8 (0.5 – 1.4) mg/kg, suggesting that the behavioral response is related to nociception.

*Effects of NMDA receptor-related antagonists and tachykinin-receptor antagonists on D-cycloserine-induced nociceptive behavior*

7-Cl-Kyn (0.25 – 4 nmol), a competitive antagonist of the glycine recognition site on the NMDA receptor ion-channel complex, co-administered i.t. with D-cycloserine caused dose-dependent inhibition of D-cycloserine-induced nociceptive behavior with an $ID_{50}$ value of 1.8 (0.5 – 6.1) nmol (Fig. 3a). D-APV (62.5 – 500 pmol), a competitive NMDA-receptor antagonist; MK-801(62.5 – 500 pmol), an NMDA ion-channel blocker; ifenprodil (0.5 – 8 nmol); arcaine (31 – 125 pmol); and agmatine (0.1 – 10 pmol), all being antagonists of the polyamine recognition site on the NMDA receptor ion-channel complex, yielded results similar to that observed for 7-Cl-Kyn (Fig. 3: b – f). The $ID_{50}$ values for D-APV, MK-801, ifenprodil, arcaine, and agmatine were 220 (58 – 834) pmol, 145 (60 – 348) pmol, 1.9 (0.8 – 4.5) nmol, 87 (84 – 90) pmol, and 0.6 (0.5 – 0.7) pmol, respectively. On the other hand, both [D-Phe$^7$,D-His$^9$]-substance P(6 – 11) (2 nmol), a specific antagonist for substance P (NK1) receptors, and MEN-10,376 (2 nmol), a tachykinin NK2-receptor antagonist, failed to inhibit D-cycloserine-induced nociceptive behavior at doses that significantly reduce the nociceptive behavior evoked by substance P and neurokinin A, respectively (8 – 10) (Table 1). The antagonists in doses used in the present study caused no motor impairment or hindlimb paralysis.

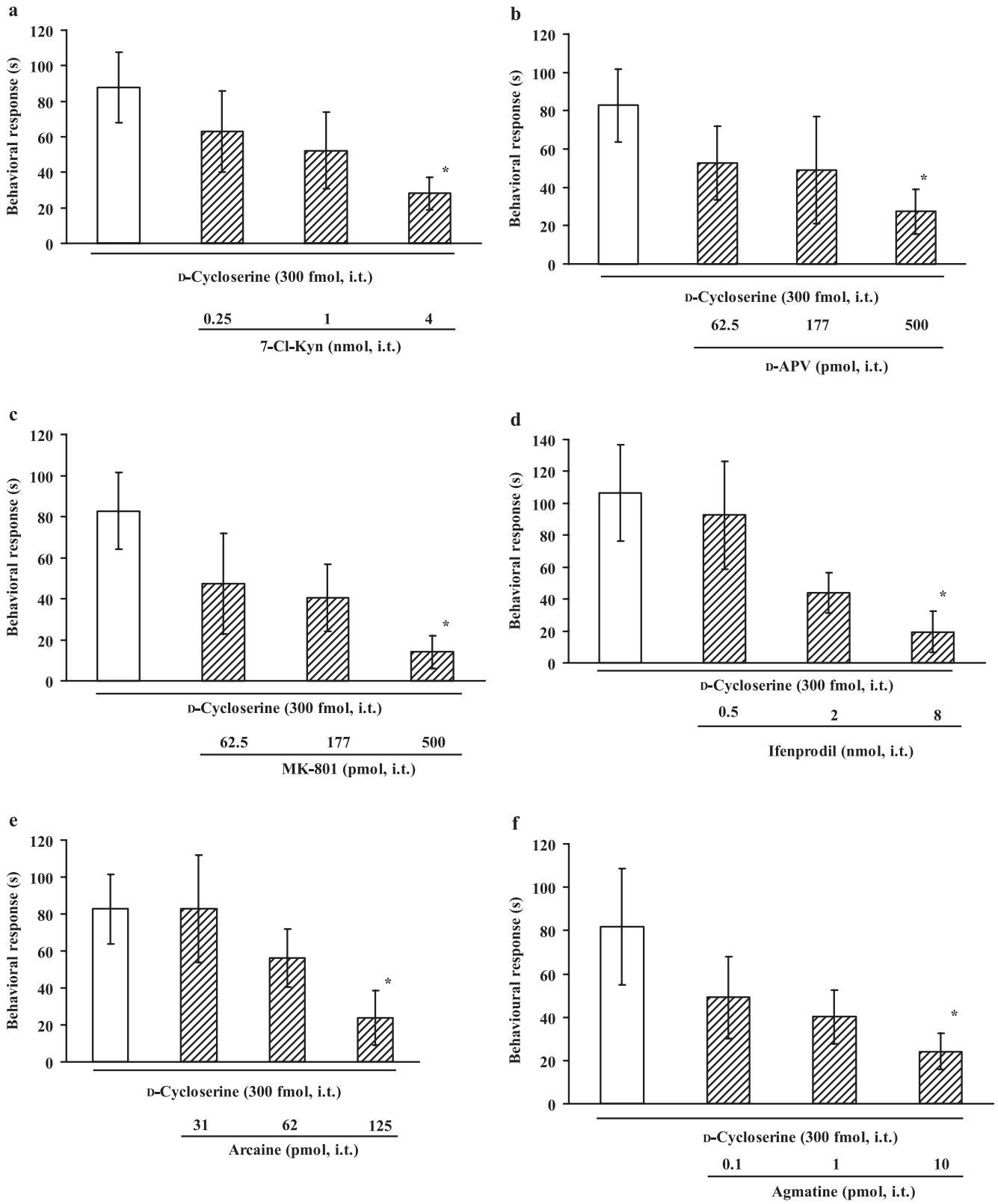


**Fig. 3.** Effects of 7-Cl-Kyn (a), D-APV (b), MK-801 (c), ifenprodil (d), arcaine (e), and agmatine (f) on D-cycloserine-induced nociceptive behavior in mice. Each agent or the corresponding vehicle was co-administered i.t. with D-cycloserine (300 fmol). The duration of scratching, biting, and licking responses induced by D-cycloserine was determined during a 15-min period starting immediately after the i.t. injection. These data are given as the means and S.E.M. for groups of 10 mice. *$P$<0.05, when compared with D-cycloserine alone.

**Table 1.** Effects of [D-Phe$^7$,D-His$^9$]-substance P(6 – 11) and MEN-10,376 on D-cycloserine-induced nociceptive behavior in mice

| Agents | Behavioral response (s/15 min) |
|---|---|
| Ringer | 82.7 ± 18.8 |
| [D-Phe$^7$,D-His$^9$]-substance P(6 – 11) (2 nmol) | 74.6 ± 14.7 |
| Vehicle (25% DMSO) | 78.8 ± 26.2 |
| MEN-10,376 (2 nmol) | 73.1 ± 17.1 |

Each agent was co-administered i.t. with D-cycloserine (300 fmol). The duration of scratching, biting, and licking responses induced by D-cycloserine was determined during a 15-min period starting immediately after i.t. injection. These data are given as the means ± S.E.M. for groups of 10 mice.

## Discussion

In the present study, we found that i.t.-administered D-cycloserine at low doses (100 and 300 fmol) produced a characteristic behavioral response mainly consisting of biting and/or licking of the hindpaw along with slight hindlimb scratching directed toward the flank, which was similar to that observed with NMDA (11 – 13) or spermine (4). It is noteworthy that the onset of the behavioral response induced by D-cycloserine as well as spermine (4) was much later than that of NMDA-induced behavior, which occurred within 10 s following i.t. injection (11 – 13). D-Cycloserine and spermine act on glycine and polyamine recognition sites as the allosteric agonists, respectively. Therefore, the difference in latency may be explained by the speculation that the onset of response mediated through the allosteric sites that modulate the NMDA-receptor function is much later than that of NMDA. Pretreatment with morphine (0.5 – 2 mg/kg) given i.p. reduced the behavior induced by D-cycloserine at 300 fmol in a dose-dependent manner. Therefore, D-cycloserine-induced behavior seems to be related to nociception. The present study also showed that the dose-response for D-cycloserine-induced nociceptive behavior had a bell-shaped pattern. D-Cycloserine enhances the binding of [$^3$H]-1-[1-(2-thienyl) cyclohexyl] piperidine ([$^3$H]-TCP), an NMDA ion-channel ligand, to synaptic plasma membranes, although the maximal stimulation of [$^3$H]-TCP binding induced by D-cycloserine is lower than that produced by glycine or D-serine, which also acts at the glycine recognition site (14). Moreover, the stimulation of [$^3$H]-TCP binding induced by D-cycloserine in the presence of various fixed concentrations of glycine results in a family of dose-response curves that asymptotically converge to 40% – 50% of the maximal stimulation induced by glycine alone (14). These facts indicate that D-cycloserine is a partial agonist of the glycine recognition site on the NMDA receptor ion-channel complex. In addition, subcutaneous injection into mice of D-cycloserine produces a biphasic effect on the level of c-GMP in the cerebellum that suggests partial agonism; D-cycloserine at low doses (1.25 – 10 mg/kg) elicits an increase in c-GMP, whereas at higher doses, it decreases c-GMP to the basal level and eventually to below the basal level (15). Therefore, the reason why D-cycloserine-induced nociceptive behavior had a bell-shaped pattern may be explained by the fact that D-cycloserine is a partial agonist of the glycine recognition site.

Glycine has been shown to enhance the binding of [$^3$H]-MK-801 (16, 17) and [$^3$H]-TCP (18 – 20) through the glycine recognition site. This enhancement is blocked by competitive NMDA-receptor antagonists such as D-APV. These facts indicate that the glycine recognition site positively regulates the functions of the NMDA receptor ion-channel complex. In the present study, D-cycloserine-induced nociceptive behavior was inhibited by not only 7-Cl-Kyn but also by D-APV and MK-801. Therefore, these results lead us to suggest that D-cycloserine-induced nociceptive behavior may be mediated through the activation of the NMDA receptor ion-channel complex by acting on the glycine recognition site.

It is surprising that ifenprodil, arcaine, and agmatine also inhibited D-cycloserine-induced nociceptive behavior. Spermine produces an approximate 3-fold increase in affinity without a significant change in $B_{max}$ in [$^3$H]-glycine binding to rat cortical membranes (21). It has also been reported that spermidine further elevates the enhancement of [$^3$H]-MK-801 binding to rat cortical membranes by glycine (22). On the other hand, spermine, spermidine, and poly-L-lysine, the polycationic compounds, displace the specific binding of [$^3$H]-ifenprodil to rat cortical membranes (23). We have previously reported that i.t. administration into mice of spermine (4), poly-L-lysine (24), and big dynorphin (25), a prodynorphin-derived peptide consisting of dynorphins A and B and a strong cationic compound with 10 out of 32 amino acids being basic, induces nociceptive behavior. Moreover, *N*-ethylmaleimide, a cysteine protease inhibitor, induces nociceptive behavior after i.t. administration by blocking the degradation of prodynorphin-derived peptides, presumably big dynorphin (26). The antagonists of the polyamine recognition site, including ifenprodil, inhibit the nociceptive behavior produced by these polycationic compounds and *N*-ethylmaleimide, whereas 7-Cl-Kyn has no effect (4, 24 – 26). These findings lead us to speculate that the glycine recognition site-mediated NMDA response is positively regulated by the activation of the polyamine recognition

site, although the polyamine recognition site-mediated response is not regulated through the glycine recognition site. Taken together with the speculation, it seems that D-cycloserine-induced nociceptive behavior was inhibited by the polyamine recognition site antagonists, since the antagonists negatively regulate the glycine recognition site-mediated NMDA response.

Spinal tachykinin NK1 and NK2 receptors have been shown to be involved in nociception [for a review, see Ref. 27]. Behavioral studies in mice have indicated that i.t. administration of substance P, an endogenous NK1-receptor agonist, and neurokinin A, an endogenous NK2-receptor agonist, induces nociceptive behavior (8, 9, 13) which is similar to that observed with D-cycloserine. These facts suggest involvement of the spinal tachykinin receptors in D-cycloserine-induced nociceptive behavior. To clarify the suggestion, the effect of tachykinin-receptor antagonists on D-cycloserine-induced nociceptive behavior was therefore examined. Both [D-Phe$^7$,D-His$^9$]-substance P(6 – 11) and MEN-10,376 failed to inhibit D-cycloserine-induced nociceptive behavior, suggesting that D-cycloserine-induced nociceptive behavior is not mediated through the tachykinin-receptor mechanism in the spinal cord.

In conclusion, evidence is presented that i.t.-administered D-cycloserine induces nociceptive behavior. The mechanism underlying D-cycloserine-induced nociceptive behavior may be mediated through the glycine recognition site on the NMDA receptor ion-channel complex without the involvement of the tachykinin receptors in the mouse spinal cord. It has recently been reported that i.t.-administered D-serine enhances the C-fiber response of wide dynamic range neurons in the rat spinal dorsal horn by activating the glycine recognition site (5). Collectively, these results indicate that the spinal glycine recognition site of the NMDA receptor ion-channel complex plays a crucial role in nociceptive transmission.

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