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Competitive NMDA receptor antagonists disrupt prepulse inhibition without reduction of startle amplitude in a dopamine receptor-independent manner in mice

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Abstract

Prepulse inhibition is thought to reflect the operation of the sensorimotor gating system in the brain, and is reduced in schizophrenic patients and in animals treated with non-competitive NMDA receptor antagonists such as phencyclidine and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-MK-801). Previously, we reported that a competitive NMDA receptor antagonist, *cis*-4-phosphonomethyl-2-piperidine-carboxylate hydrochloride (CGS 19755), also disrupts prepulse inhibition concomitantly with a marked reduction of startle amplitude elicited by pulse alone in rats. In the present study, the effect of NMDA receptor antagonists on prepulse inhibition was tested in mice. In addition, involvement of the dopaminergic system in CGS 19755-induced disruption of prepulse inhibition was examined. When CGS 19755 was subcutaneously administered at 40 and 80 mg/kg, prepulse inhibition was disrupted without any change in the startle amplitude elicited by pulse alone. Intracerebroventricularly administered CGS 19755 disrupted prepulse inhibition at dosages of 0.1 and 0.2 μg/mouse. The same dosages of *R*-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (*R*-CPP), another competitive NMDA receptor antagonist, also decreased prepulse inhibition, while its less active enantiomer, *S*-CPP, did not affect prepulse inhibition at 0.2 μg/mouse (i.c.v.). A typical neuroleptic, haloperidol, did not significantly improve CGS 19755 (40 mg/kg s.c.)-induced disruption of prepulse inhibition. These results suggest that the disruption of prepulse inhibition by CGS 19755 and *R*-CPP is NMDA receptor-mediated and dopamine receptor-independent.

Keywords: Prepulse inhibition; NMDA receptor; CGS 19755; *R*-CPP; Schizophrenia; Haloperidol

1. Introduction

The acoustic startle reflex is inhibited by a preceding weak stimulation, which itself does not elicit the startle reflex. This phenomenon is named prepulse inhibition and is thought to reflect the operation of a sensorimotor gating system in the brain (Braff and Geyer, 1990; Swerdlow et al., 1992; al-Amin and Schwarzkopf, 1996). It is well known that prepulse inhibition is disrupted in schizophrenic patients (Braff et al., 1978, 1992; Karper et al., 1996; Schall et al., 1997). The prepulse inhibition model is useful to study the neuropharmacology of schizophrenia since an identical method is applicable to experimental animals and humans, and data obtained from animal models are predictive of the deficit in sensorimotor gating in schizophrenic patients (Swerdlow et al., 1994).

There are many reports that non-competitive NMDA receptor antagonists such as phencyclidine and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-MK-801) disrupt prepulse inhibition in experimental animals (Mansbach and Geyer, 1989; Keith et al., 1991; Johansson et al., 1997; Zhang et al., 1997). Recently, we reported that, in rats, prepulse inhibition is disrupted by a competitive NMDA receptor antagonist (Furuya and Ogura, 1997), as well as by a non-competitive NMDA receptor antagonist. We also reported that the deficit in prepulse inhibition elicited by *cis*-4-phosphonomethyl-2-piperidine-carboxylate hydrochloride (CGS

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19755) was accompanied by a marked reduction of startle amplitude elicited by pulse alone. It was also suggested that the disruption of prepulse inhibition did not result from the reduced amplitude, since there was no correlation between prepulse inhibition and startle amplitude, implying that the competitive NMDA receptor antagonist induces sensorimotor gating deficits in rats.

In a preliminary study with mice, we found that CGS 19755 disrupted prepulse inhibition without reducing the pulse alone-elicited startle amplitude, which was different from our previous results in rats. This unexpected finding led us to examine in detail the effect of some competitive NMDA receptor antagonists on prepulse inhibition and startle amplitude in mice, since we thought it might be possible to demonstrate more clearly that competitive NMDA receptor antagonists induce sensorimotor gating deficits in mice. For this purpose, we measured the effects of CGS 19755, as well as of another competitive NMDA receptor antagonist, *R*-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (*R*-CPP), and its less active enantiomer, *S*-CPP, on prepulse inhibition in mice. The possible contribution of dopaminergic systems to the CGS 19755-induced deficit in prepulse inhibition was also investigated.

2. Materials and methods

2.1. Subjects

Male ddY mice (SLC, Shizuoka, Japan), 4–5 weeks old, were housed at 23 ± 1°C in a humidity-controlled ($55 \pm 10\%$) animal facility under a 12 h light/dark cycle (lights on at 0700 h), and allowed access to tap water and food ad libitum. Each animal was used only once.

2.2. Drugs

For the experiment involving peripheral administration, *cis*-4-phosphonomethyl-2-piperidine-carboxylate hydrochloride (CGS 19755, synthesized at Eisai) and 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone (haloperidol, Sigma) were dissolved in distilled water and equimolar tartaric acid solution, respectively. *R*(−)-10,11-Dihydroxyaporphine hydrochloride (apomorphine, Sigma) was dissolved in 0.1% ascorbate solution and the solution was kept on ice in the dark to prevent oxidative degeneration. The drugs were administered subcutaneously to animals in a volume of 0.1 ml/10 g. The same volume of vehicle solution was administered to control animals. For the experiment involving intracerebroventricular administration, CGS 19755 and *R*- or *S*-CPP (Research Biochemicals International, Natick, MA) were both dissolved in PBS (phosphate-buffered saline, pH 7.4) and 5 μl of the solution was injected into the cerebroventricle by the method of Haley and McCormick (1957). Control animals received the same volume of PBS.

2.3. Prepulse inhibition

Four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used. Each chamber consisted of a Plexiglas cylinder 3.8 cm in diameter, resting on a Plexiglas frame in a sound-attenuated, ventilated enclosure. Acoustic noise bursts were presented via a loudspeaker mounted 29 cm above the cylinder. A piezoelectric transducer mounted below the frame detected motion of the animal in the cylinder. Stabilimeter readings were rectified and recorded by a microcomputer and interface ensemble (San Diego Instruments).

One mouse was placed in each chamber and allowed to become accustomed to it for 10 min, then the experimental session was started. Background noise was set at 65 dB of white noise throughout both the preliminary period and the session. In a session, three types of 10 trials (total of 30 trials) were given in pseudo-random order after three initial startle-stimuli (20-ms burst of 120 dB white noise), which were given to avoid the effect of high responses to initial stimulations in the experiments. One of the types was a pulse-alone (P alone) trial, which involved a 20-ms burst of 120 dB white noise, and the other two were prepulse and pulse (PP70 & P and PP80 & P) trials, which involved a 20-ms burst of 70 or 80 dB white noise, respectively, followed by the same pulse as in the P alone trial 100 ms later. The intertrial intervals averaged 40 s (20–60 s) and were pseudo-randomized. The startle response was measured for 100 ms from the start of pulse presentation, and the largest value was defined as the startle amplitude. The animals' startle amplitudes in response to repetitions of each trial type were averaged across the session. The experimental schedule was controlled by a microcomputer.

The startle response was measured 15 and 30 min after subcutaneous administration of apomorphine and haloperidol, respectively, and 30 or 120 min after administration of CGS 19755. In the intracerebroventricular study, measurement was started 15 min after administration.

2.4. Data analysis

The three initial startle-stimuli were excluded from the statistical analysis. Prepulse inhibition in the PP70 & P or PP80 & P trials is presented as percent inhibition of the startle amplitude in the P alone trial according to the following formula [% prepulse inhibition = (1 − SA in PP & P/SA in P alone) × 100]. SA in PP & P and SA in P alone are the averaged startle amplitudes in the PP70 & P or PP80 & P and P alone trial, respectively.

A repeated measures analysis of variance (ANOVA) with doses as a between-subjects factor and trial type as a within-subject factor was used. Whenever the dose factor or the drug × trial type interaction was significant, post-hoc individual comparison was carried out. The statistical significance of differences between two groups was analyzed

with Student's *t*-test. Dose–effect relationships of haloperidol, CGS 19755 and *R*-CPP were analyzed with Dunnett's test. A P value of less than 0.05 was considered to represent a significant difference.

3. Results

3.1. Effects of CGS 19755 (s.c.) on prepulse inhibition

Fig. 1A shows the effects of CGS 19755 on prepulse inhibition 30 min after its subcutaneous administration. A repeated measures ANOVA using the dose of CGS 19755 as a between-subjects factor and trial type as a within-subject factor revealed a significant effect of dose ($F(4,35) = 3.03$, $P < 0.05$), a significant effect of trial type ($F(1,35) = 117.24$, $P < 0.01$), and no significant dose × trial type interaction ($F(4,35) = 2.32$, NS). Dunnett's test revealed that CGS 19755 (40 and 80 mg/kg s.c.) significantly reduced prepulse inhibition in the PP80 & P trial. Fig. 1B shows the effects of CGS 19755 on prepulse inhibition at 120 min after administration. A repeated measures ANOVA revealed a significant effect of dose ($F(4,35) = 3.80$, $P < 0.05$), a significant effect of trial type ($F(1,35) = 65.71$, $P < 0.01$), and no significant dose × trial type interaction ($F(4,35) = 2.42$, NS). Dunnett's test revealed that CGS 19755 (40 and 80 mg/kg s.c.) significantly reduced prepulse inhibition in the PP80 & P trial.

Table 1 shows the effects of CGS 19755 on startle amplitude. A repeated measures ANOVA using dose of CGS 19755 as a between-subjects factor and trial type as a within-subject factor revealed no significant effect of dose ($F(4,35) = 1.64$, NS), a significant effect of trial type ($F(2,70) = 106.31$, $P < 0.01$), and a significant dose × trial type interaction ($F(8,70) = 5.28$, $P < 0.01$). Dunnett's test showed that there was no difference in startle amplitude between the control and each CGS 19755-treated group in each trial type. When measurement was started 120 min after administration of CGS 19755, no significant effect of dose ($F(4,35) = 2.38$, NS), a significant effect of trial type ($F(2,70) = 66.94$, $P < 0.01$) and a significant dose × trial type interaction ($F(8,70) = 2.09$, $P < 0.05$) were revealed by a repeated measures ANOVA. Dunnett's test showed that CGS 19755 (40 mg/kg s.c.) significantly increased startle amplitude in the PP80 & P trial.

Table 1
Effects of subcutaneously administered CGS 19755 on acoustic startle amplitude in mice

Drug	Dose (mg/kg)	Startle amplitude		
		P alone	PP70 & P	PP80 & P
Control	Vehicle	213.5 ± 35.4	165.4 ± 32.7	95.4 ± 26.7
CGS 19755 (30 min)	10	310.8 ± 35.1	220.5 ± 27.2	147.8 ± 24.0
	20	258.7 ± 28.9	199.6 ± 12.3	151.7 ± 11.4
	40	154.7 ± 38.6	130.5 ± 32.2	111.5 ± 31.9
	80	197.8 ± 32.6	175.5 ± 35.8	138.3 ± 34.3
Control	Vehicle	157.0 ± 12.8	107.4 ± 15.1	66.8 ± 8.8
CGS 19755 (120 min)	10	237.1 ± 22.1	171.1 ± 25.1	121.8 ± 23.0
	20	238.5 ± 43.6	192.1 ± 34.3	137.0 ± 26.5
	40	218.0 ± 22.4	208.1 ± 35.7	174.4 ± 24.8[a]
	80	185.8 ± 26.7	146.1 ± 13.8	128.8 ± 10.2

CGS 19755 was subcutaneously administered 30 or 120 min before the measurement. The three measures of startle amplitude are P alone (120 dB pulse alone), PP70 & P (70 dB prepulse followed by 120 dB pulse) and PP80 & P (80 dB prepulse followed by 120 dB pulse). Data represent means ± S.E.M. for eight animals per group. [a] $P < 0.01$ compared with the control group by Dunnett's post-hoc test.

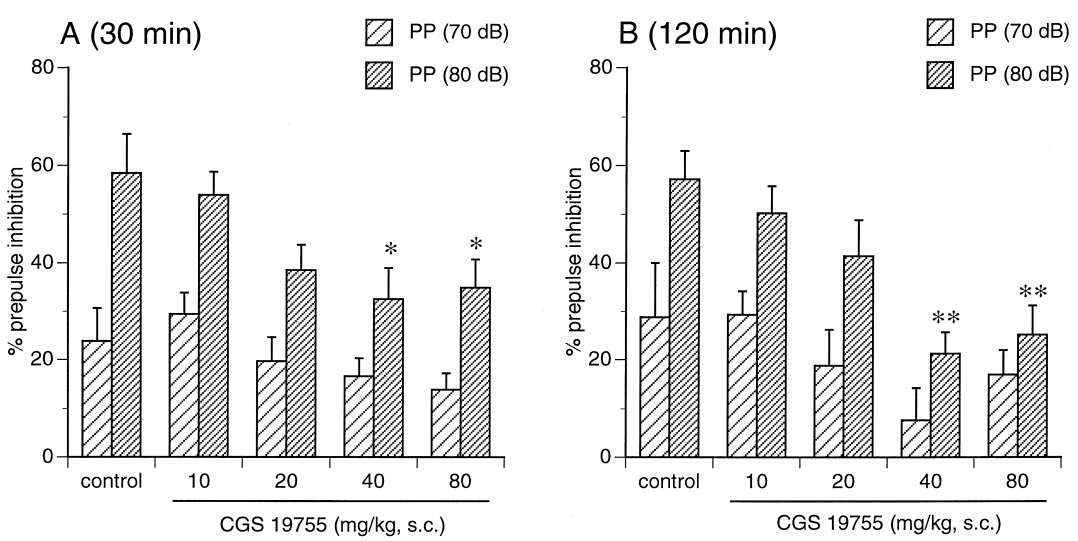

Fig. 1. Effects of subcutaneously administered CGS 19755 on prepulse inhibition in mice. Bars represent percent prepulse inhibition in the PP70 & P (70 dB prepulse followed by 120 dB pulse) (left) and PP80 & P (80 dB prepulse followed by 120 dB pulse) (right) trials. CGS 19755 was subcutaneously administered 30 or 120 min before the measurement (A or B). Data represent means ± S.E.M. for eight animals per group. * $P < 0.05$, ** $P < 0.01$ compared with the control group by Dunnett's post-hoc test.

3.2. Effects of CGS 19755, R-CPP and S-CPP (i.c.v.) on prepulse inhibition

Fig. 2 shows the effects of intracerebroventricularly administered CGS 19755, as well as *R*-CPP and its less active enantiomer, *S*-CPP, on prepulse inhibition. A repeated measures ANOVA using the dose of CGS 19755 as a between-subjects factor and trial type as a within-subject factor revealed a significant effect of dose ($F(3,60) = 3.71$, $P < 0.05$), a significant effect of trial type ($F(1,60) = 87.80$, $P < 0.01$), and no significant dose × trial type interaction ($F(3,60) = 1.34$, NS). Dunnett's test revealed a significant reduction of prepulse inhibition in the PP80 & P trial by CGS 19755 (0.1 and 0.2 μg/mouse i.c.v.). In the *R*-CPP study, repeated measures ANOVA revealed a significant effect of dose ($F(3,60) = 4.86$, $P < 0.01$), a significant effect of trial type ($F(1,60) = 106.86$, $P < 0.01$), and no significant dose × trial type interaction ($F(3,60) = 1.13$, NS). Dunnett's test showed that CGS 19755 significantly reduced prepulse inhibition in the PP70 & P trial at 0.2 μg/mouse i.c.v. and that in the PP80 & P trial at 0.1 and 0.2 μg/mouse i.c.v. In the *S*-CPP study, a repeated measures ANOVA revealed no significant effect of *S*-CPP treatment ($F(1,30) = 0.10$, NS), a significant effect of trial type ($F(1,30) = 36.96$, $P < 0.01$), and no significant effect of *S*-CPP treatment × trial type interaction ($F(1,30) = 0.01$, NS).

Table 2 shows the effects on startle amplitude. A repeated measures ANOVA using the dose of CGS 19755 as a between-subjects factor and trial type as a within-subject factor revealed a significant effect of dose ($F(3,60) = 4.45$, $P < 0.01$), a significant effect of trial type ($F(2,120) = 169.02$, $P < 0.01$), and no significant dose × trial type interaction ($F(6,120) = 1.80$, NS). Dunnett's test showed that CGS 19755 increased all startle amplitudes at 0.05 and 0.1 μg/mouse i.c.v. and also that in the PP80 & P trial at 0.2 μg/mouse i.c.v. In the *R*-CPP study, a repeated measures ANOVA revealed no significant effect of dose ($F(3,60) = 1.47$, NS), a significant effect of trial type ($F(2,120) = 86.82$, $P < 0.01$), and no significant dose × trial type interaction ($F(6,120) = 1.39$, NS). In the *S*-CPP study, a repeated measures ANOVA showed no significant effect of dose ($F(1,30) = 1.25$, NS), a significant effect of trial type ($F(2,60) = 60.81$, $P < 0.01$), and no significant dose × trial type interaction ($F(2,60) = 0.24$, NS).

Table 2
Effects of intracerebroventricularly administered CGS 19755 and *R*- or *S*-CPP on acoustic startle amplitude in mice

Drug	Dose (μg/mouse)	Startle amplitude		
		P alone	PP70 & P	PP80 & P
Control	Vehicle	201.0 ± 17.8	131.0 ± 14.2	82.2 ± 14.5
CGS 19755	0.05	350.5 ± 33.3[b]	263.8 ± 28.6[b]	202.2 ± 25.7[b]
	0.1	316.6 ± 41.0[a]	249.0 ± 37.0[a]	198.0 ± 34.9[b]
	0.2	266.7 ± 31.0	218.5 ± 30.2	177.8 ± 27.6[a]
Control	Vehicle	216.1 ± 24.6	157.2 ± 23.7	110.8 ± 17.8
R-CPP	0.05	236.1 ± 32.0	194.2 ± 28.1	149.6 ± 26.1
	0.1	276.1 ± 49.1	233.4 ± 46.2	198.6 ± 45.7
	0.2	272.7 ± 26.7	260.8 ± 26.9	195.5 ± 25.3
S-CPP	0.2	259.3 ± 28.8	193.1 ± 27.2	139.9 ± 22.2

CGS 19755 and *R*- or *S*-CPP were intracerebroventricularly administered 15 min before measurement. The three measures of startle amplitude are P alone (120 dB pulse alone), PP70 & P (70 dB prepulse followed by 120 dB pulse) and PP80 & P (80 dB prepulse followed by 120 dB pulse). Data represent means ± S.E.M. for 16 animals per group. [a]$P < 0.05$ and [b]$P < 0.01$ compared with the control group by Dunnett's post-hoc test.

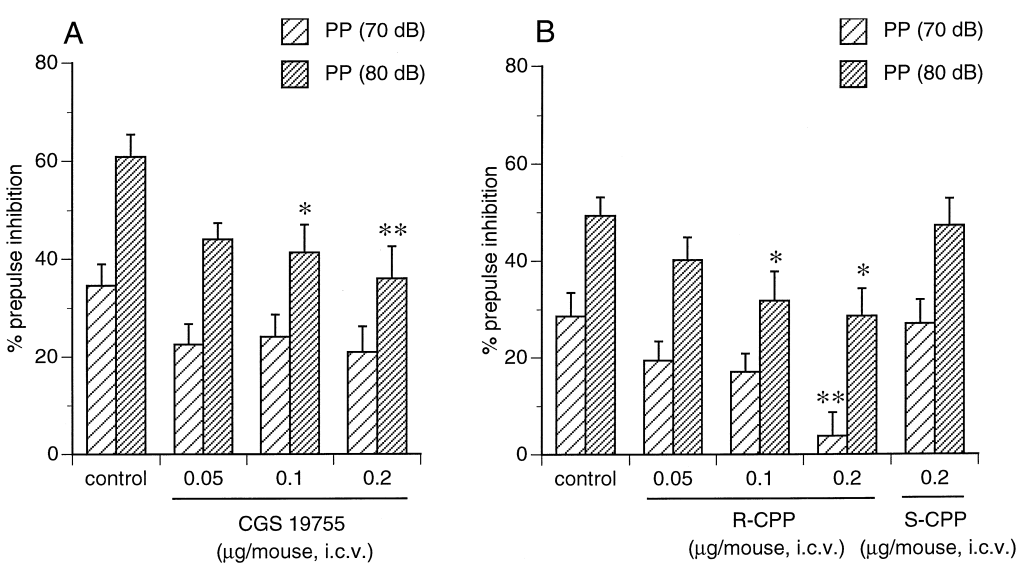

Fig. 2. Effects of intracerebroventricularly administered CGS 19755 and *R*- or *S*-CPP on prepulse inhibition in mice. Bars represent percent prepulse inhibition in the PP70 & P (70 dB prepulse followed by 120 dB pulse) (left) and PP80 & P (80 dB prepulse followed by 120 dB pulse) (right) trials. CGS 19755 and *R*- or *S*-CPP were intracerebroventricularly administered 15 min before measurement (A and B). Data represent means ± S.E.M. for 16 animals per group. * $P < 0.05$, ** $P < 0.01$ compared with the control group by Dunnett's post-hoc test.

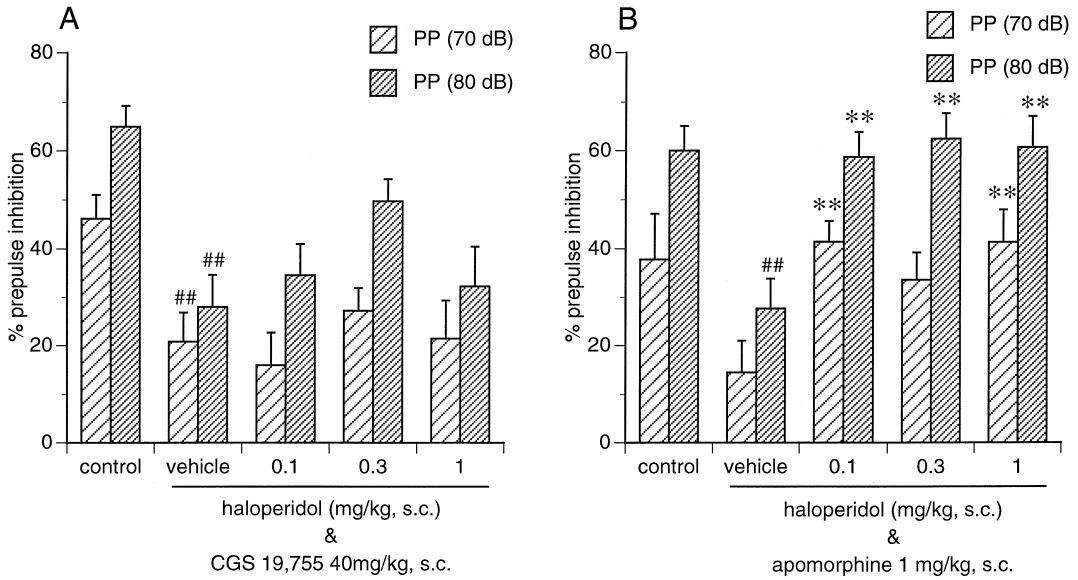

Fig. 3. Effects of haloperidol on CGS 19755 (A)- or apomorphine (B)-induced disruption of prepulse inhibition in mice. Bars represent percent prepulse inhibition in the PP70 & P (70 dB prepulse followed by 120 dB pulse) (left) and PP80 & P (80 dB prepulse followed by 120 dB pulse) (right) trials. Haloperidol, CGS 19755 and apomorphine were subcutaneously administered 30, 120 and 15 min before the measurement, respectively. Data represent means ± S.E.M. for eight animals per group. $\#\#P < 0.01$ compared with the control group by Student's *t*-test. $**P < 0.01$ compared with CGS 19755 or apomorphine alone group by Dunnett's post-hoc test.

3.3. Effects of haloperidol on the disruption of prepulse inhibition induced by CGS 19755 and apomorphine

Fig. 3A shows the effects of the typical neuroleptic, haloperidol, on CGS 19755-induced disruption of prepulse inhibition. As shown in Fig. 1B, the dosage of CGS 19755 used (40 mg/kg s.c.) was the minimum able to disrupt prepulse inhibition. A repeated measures ANOVA for the control and CGS 19755 alone groups revealed a significant effect of CGS 19755 treatment ($F(1,14) = 17.57$, $P < 0.01$), a significant effect of trial type ($F(1,14) = 28.72$, $P < 0.01$) and a significant dose × trial type interaction ($F(1,14) = 5.60$, $P < 0.05$). Student's *t*-test revealed a significant reduction of prepulse inhibition by CGS 19755 in both trial types. A repeated measures ANOVA for all CGS 19755-treated groups (four groups) revealed no significant dose effect of haloperidol ($F(3,28) = 1.17$, NS), a significant effect of trial type ($F(1,28) = 41.08$, $P < 0.01$) and no significant dose × trial type interaction ($F(3,28) = 2.29$, NS).

Table 3
Effects of co-administration of CGS 19755 or apomorphine and haloperidol on acoustic startle amplitude in mice

Drug	Dose (mg/kg)	Startle amplitude		
		P alone	PP70 & P	PP80 & P
Control	Vehicle	161.6 ± 25.1	87.9 ± 15.7	52.6 ± 8.7
CGS 19755 (40 mg/kg) and haloperidol	Vehicle	165.3 ± 32.7	131.1 ± 25.1	120.5 ± 24.5[a]
	0.1	141.3 ± 22.0	120.8 ± 21.3	92.4 ± 17.8
	0.3	191.9 ± 18.9	139.7 ± 16.0	98.3 ± 14.6
	1	64.9 ± 16.5[b]	48.7 ± 13.3[b]	41.0 ± 12.0[b]
Control	Vehicle	212.6 ± 13.1	127.6 ± 15.3	82.3 ± 8.2
Apomorphine (1 mg/kg) and haloperidol	Vehicle	201.3 ± 25.4	169.5 ± 21.5	142.0 ± 19.8[a]
	0.1	251.2 ± 33.3	152.0 ± 29.2	111.4 ± 26.6
	0.3	225.6 ± 30.1	146.5 ± 20.2	82.6 ± 15.6
	1	262.9 ± 47.4	149.9 ± 30.2	93.8 ± 17.0

CGS 19755, apomorphine and haloperidol were subcutaneously administered 120, 15 and 30 min before the measurement, respectively. The three measures of startle amplitude are P alone (120 dB pulse alone), PP70 & P (70 dB prepulse followed by 120 dB pulse) and PP80 & P (80 dB prepulse followed by 120 dB pulse). Data represent mean ± S.E.M. of eight animals per group. [a]$P < 0.05$ compared with the corresponding control group by Student's *t*-test, and [b]$P < 0.05$ compared with the CGS 19755 or apomorphine alone group by Dunnett's post-hoc test.

Table 3 (upper) shows the effects on startle amplitude. No significant effect of CGS 19755 treatment ($F(1,14) = 1.54$, NS), a significant effect of trial type ($F(2,28) = 31.13$, $P < 0.01$) and a significant treatment × trial type interaction ($F(2,28) = 5.24$, $P < 0.05$) were revealed by a repeated measures ANOVA for the control and CGS 19755 alone groups. Student's *t*-test revealed a significant increase of startle amplitude by CGS 19755 in the PP80 & P trial. To evaluate the effects of haloperidol treatment, two-way ANOVA was applied to all CGS 19755-treated groups (four groups). A significant dose effect of haloperidol ($F(3,28) = 5.95$, $P < 0.01$), a significant effect of trial type ($F(2,56) = 57.95$, $P < 0.01$) and a significant dose × trial type interaction ($F(6,56) = 3.61$, $P < 0.01$) were revealed. Dunnett's test showed that 1 mg/kg s.c. of haloperidol significantly reduced all startle amplitudes.

Fig. 3B shows the effect of haloperidol on disruption of prepulse inhibition induced by a direct dopamine receptor agonist, apomorphine. In a preliminary study, apomorphine showed a tendency to disrupt prepulse inhibition at the dosage of 0.25 mg/kg s.c. (data not shown), but sufficient and reproducible disruption of prepulse inhibition was observed only at the dosage of 1 mg/kg s.c. or more. Based on this result, a dosage of 1 mg/kg s.c. was chosen for the next test. A repeated measures ANOVA for the control and apomorphine alone-treated groups revealed a significant effect of apomorphine treatment ($F(1,14) = 9.49$, $P < 0.01$), a significant effect of trial type ($F(1,14) = 21.92$, $P < 0.01$) and no significant apomorphine treatment × trial type interaction ($F(1,14) = 1.48$, NS). Student's *t*-test revealed a significant reduction of prepulse inhibition by apomorphine in the PP80 & P trial. A repeated measures ANOVA for all apomorphine-treated groups (four groups) revealed a significant dose effect ($F(3,28) = 6.70$, $P < 0.01$), a significant effect of trial type ($F(1,28) = 142.74$, $P < 0.01$) and a significant dose × trial type interaction ($F(3,28) = 4.16$, $P < 0.05$). Dunnett's *t*-test showed that haloperidol (0.3 mg/kg s.c.) antagonized the reduction in prepulse inhibition in all cases except in the PP70 & P trial.

Table 3 (lower) shows the effects on startle amplitude. A repeated measures ANOVA for the control and apomorphine alone-treated groups revealed no significant effect of apomorphine treatment ($F(1,14) = 1.81$, NS), a significant effect of trial type ($F(2,28) = 38.65$, $P < 0.01$) and a significant apomorphine treatment × trial type interaction ($F(2,28) = 5.77$, $P < 0.01$). Student's *t*-test revealed a significant increase of startle amplitude by apomorphine in the PP80 & P trial. To examine the effects of haloperidol on the apomorphine-induced change, a repeated measures ANOVA was done for all apomorphine-treated groups (four groups). No significant effect of haloperidol treatment ($F(3,28) = 0.14$, NS), a significant effect of trial type ($F(2,56) = 102.47$, $P < 0.01$) and a significant apomorphine treatment × trial type interaction ($F(6,56) = 3.87$, $P < 0.01$) were revealed. Dunnett's test showed that there was no significant difference in startle amplitude between the control and each CGS 19755-treated group in each trial type.

4. Discussion

The results in this study clearly demonstrated that a competitive NMDA receptor antagonist affects the sensorimotor gating system in mice. We found that the competitive NMDA receptor antagonist, CGS 19755, disrupted prepulse inhibition without reducing the startle amplitude in the P alone trial in mice. This finding was confirmed by the observation that CPP stereoselectively disrupted prepulse inhibition. Furthermore, it also became apparent that haloperidol hardly improves the disruption of prepulse inhibition induced by CGS 19755, suggesting that the disruptive effect of the competitive NMDA receptor antagonist on prepulse inhibition is mainly elicited by a dopamine receptor-independent mechanism.

CGS 19755 disrupted prepulse inhibition in mice, 120 min after its subcutaneous administration. In contrast to our previous results with rats, prepulse inhibition was disrupted even when the test was started 30 min after administration of CGS 19755. These results show that CGS 19755 may more easily penetrate the blood–brain barrier in mice than in rats, and that its effect persists for at least 120 min. Surprisingly, the startle amplitude in the P alone trial was not decreased at all by CGS 19755 treatment. The absence of a reduction of startle amplitude was quite different from our previous result with rats (Furuya and Ogura, 1997). It is not apparent why the effect of the NMDA receptor antagonist on startle amplitude differs between species, but the involvement of NMDA receptors in the neural circuit connecting the auditory nerve, cochlea, lateral lemniscus and nucleus reticularis pontis caudalis with the spinal motor neuron to elicit the audiogenic startle response (Davis et al., 1982; Ison and Hoffman, 1983) may be different in mice and in rats. The results support the idea that the sensorimotor gating system is modulated by the NMDA receptor.

There are reports that competitive NMDA receptor antagonists had no effect on prepulse inhibition in rats (Mansbach, 1991; Wedzony et al., 1994), in contrast to our present results with mice and previous result with rats (Furuya and Ogura, 1997). The main reason for the discrepancy is thought to be the difference in dosages of the compound administered. Mansbach (1991) reported that CGS 19755 did not disrupt prepulse inhibition in rats at dosages up to 10 mg/kg; on the other hand, we observed CGS 19755-induced disruption of prepulse inhibition at the dosage of 40 mg/kg or more in both rats and mice.

Low dosages of CGS 19755 disrupted prepulse inhibition when administered intracerebroventricularly, whereas high dosages were required when the drug was administered subcutaneously. These results are supposed to reflect

poor penetration of the blood–brain barrier by CGS 19755 (Olverman and Watkins, 1989). Similar results were reported for suppression of the clonic phase of sound-induced seizures in DBA/2 mice. Compared to non-competitive NMDA receptor antagonists, (+)-MK-801 and phencyclidine, the ratio of dosages (i.p./i.c.v.) for CGS 19755 to suppress the seizures was high (Lehmann et al., 1988; Chapman and Meldrum, 1989). Another NMDA receptor antagonist, *R*-CPP, also disrupted prepulse inhibition. On the other hand, its less active enantiomer *S*-CPP, which has a 20 times lower affinity for the NMDA receptor than does *R*-CPP (Aebischer et al., 1989), did not affect prepulse inhibition at the dosage tested. The stereoselective effect of CPP implies that the disruptive effect of CPP in prepulse inhibition results from antagonism of the NMDA receptor, but not from non-specific effects. Intracerebroventricularly administered low and moderate dosages of CGS 19755 significantly increased the startle amplitude in the P alone trial. The increase may not have been related to antagonism of the NMDA receptor, because *R*-CPP (i.c.v) and CGS 19755 (s.c.) did not affect startle amplitude. However, the possibility that antagonism of the NMDA receptor increases the startle amplitude cannot be entirely excluded, since there are reports that non-competitive NMDA receptor antagonists, (+)-MK-801 and phencyclidine, cause an inverted-U type increase in the startle amplitude in rats (Johansson et al., 1994, 1995).

Disruption of prepulse inhibition is thought to reflect sensorimotor gating abnormalities and some psychoses; prepulse inhibition is disrupted by various psychotomimetics, such as 2,5-dimethoxy-4-iodoamphetamine (DOI) (Sipes and Geyer, 1994; Padich et al., 1996), amphetamine (Mansbach et al., 1988) and phencyclidine (Mansbach and Geyer, 1989; Keith et al., 1991). Our finding that CGS 19755 disrupted prepulse inhibition seems plausible, considering that this competitive NMDA receptor antagonist produces psychotomimetic effects, such as agitation, hallucination, confusion, paranoia and delirium in patients with ischemic stroke (Gorotta et al., 1995). In addition, a role of glutamatergic deficiency in schizophrenia has been proposed (Kornhuber and Kornhuber, 1986; Carlsson and Carlsson, 1990; Coyle, 1996; Hirsch et al., 1997). Therefore, abnormal behavior induced by NMDA receptor antagonists is proposed to reflect some aspects of schizophrenic symptoms. The results in this study, combined with the clinical evidence that prepulse inhibition is disrupted in schizophrenic patients (Braff et al., 1978, 1992; Karper et al., 1996; Schall et al., 1997), provide further support for this idea.

Dopamine receptors also play an important role in the operation of the sensorimotor gating system. It is reported that activation of the receptors by agents such as apomorphine (Mansbach et al., 1988; Davis et al., 1990; Wan et al., 1996) and amphetamine (Mansbach et al., 1988) elicits disruption of prepulse inhibition. In the present study, haloperidol antagonized the disruption of prepulse inhibition by apomorphine at all dosages used (0.1–1 mg/kg s.c.), in agreement with previous findings with rats (Mansbach et al., 1988; Swerdlow et al., 1996; Furuya et al., 1998). On the other hand, haloperidol did not improve the CGS 19755-induced deficiency in prepulse inhibition. It is suggested that CGS 19755, as well as phencyclidine, disrupts prepulse inhibition via a dopamine receptor-independent mechanism. Interestingly, the phencyclidine-induced psychosis in the human is sometimes untreatable with typical neuroleptics such as haloperidol (Allen and Young, 1978). It has been proposed that phencyclidine-induced disruption of prepulse inhibition might reflect neuroleptic-resistant psychosis in humans. Furthermore, as mentioned above, dysfunction of glutamatergic transmission might be a feature of the pathophysiology of schizophrenia, and therefore, the disruption of prepulse inhibition induced by NMDA receptor antagonists has been hypothesized to be an animal model of refractory symptoms in schizophrenic patients.

In conclusion, our findings that CGS 19755 disrupted prepulse inhibition without reduction of the startle amplitude in the P alone trial and that the effect of CPP is stereoselective, clearly demonstrate that competitive NMDA receptor antagonists as well as non-competitive NMDA receptor antagonists induce a sensorimotor gating deficit in mice. Furthermore, the failure of haloperidol to improve the CGS 19755-induced deficit in prepulse inhibition means that this NMDA receptor antagonist-induced disruption of prepulse inhibition is predominantly dopamine-independent. It is suggested that the NMDA receptor in the brain may be relevant to the neuroleptic-resistant symptoms of schizophrenia.

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