Latent inhibition of the classically conditioned rabbit nictitating membrane response is unaffected by the NMDA antagonist MK801

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Induction of long-term potentiation, a putative substrate for behavioral plasticity, appears to be dependent on NMDA receptor activation. Blockade of NMDA receptor activity has been shown to impair both classical conditioning and spatial cognition. The present study evaluated the effects of NMDA-receptor blockade by MK801 on latent inhibition of the classically conditioned nictitating membrane response in rabbits. Nonreinforced preexposure to a tone conditioned stimulus retarded acquisition rates in subjects pretreated with either saline or MK801. Thus, latent inhibition does not appear to be mediated by or dependent on NMDA activity or related NMDA-dependent long-term potentiation. Findings are discussed in relation to potential substrates for latent inhibition.

Long-term potentiation (LTP) of synaptic transmission is a prominent candidate for a neurobiological mechanism underlying behavioral plasticity. LTP occurs in brain systems known to play a significant role in learning, such as the hippocampus (Bliss & Gardner-Medwin, 1973), and LTP-like changes develop as a consequence of stimulus pairings during associative learning (Berger, Alger, & Thompson, 1976; G. B. Robinson, 1992; Weisz, Clark, & Thompson, 1984). Moreover, prior potentiation of hippocampal synapses has been shown to facilitate acquisition of conditioned responses (CRs) (Berger, 1984; G. B. Robinson, Port, & Berger, 1989). The induction of LTP at both hippocampal CA1 and dentate granule cell synapses appears to be dependent on N-methyl-D-aspartate (NMDA) receptors (Collingridge, Kehl, & McLennan, 1983; Harris, Ganong, & Cotman, 1984). Antagonists of NMDA-mediated activity have been reported to impair both LTP (G. B. Robinson & Reed, 1992) and classical conditioning (G. B. Robinson, 1993) in the rabbit, as well as performance on the Morris milk maze (Heale & Harley, 1990), spontaneous alternation (Walker & Gold, 1991), and operant conditioning with differential reinforcement of low rates of responding (Tonkiss, Morris, & Rawlins, 1988) in rats.

In the present study, we evaluated the effects of MK801, a potent and selective noncompetitive NMDA receptor/channel antagonist (Wong et al., 1986), on latent inhibition, wherein nonreinforced preexposure to a conditioned stimulus (CS) typically retards subsequent conditioning efforts (see Lublow, 1989). Previous studies have shown that damage to the hippocampal formation abolishes the retarding effect of CS preexposure (Ackil, Mellgren, Halgren, & Frommer, 1969; Solomon & Moore, 1975).

METHOD

Subjects

Twenty-three male New Zealand white rabbits (2–2.5 kg) were maintained on a 12:12-h light:dark schedule with ad-lib access to food and water. All procedures occurred during the light portion of the schedule.

Surgery

The subjects were deeply anesthetized with halothane and placed in a stereotaxic instrument. A headgear assembly (to hold the air-jet and potentiometer) was attached to the skull with anchoring screws and dental acrylic, and a small nylon loop was sutured in the nictitating membrane (NM). Each animal received penicillin (360,000 units, i.m.; two daily injections of 60,000 units for 3 days) and was permitted at least 2 weeks to recover.

Procedure

On Day 1 after recovery, each subject received an adaptation session during which it was placed in a Plexiglas box within a sound-attenuation chamber. The animals were then divided into four groups to allow the effects of MK801 versus saline to be crossed with the presence or absence of CS preexposure. CS preexposure involved 108 presentations

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of a nonreinforced tone (82 dB, 1 kHz; 400-msec duration), with an average intertrial interval of 60 sec. On Days 2–5, 6 subjects received daily subcutaneous injections of saline (Sal-108PE) and 5 received injections of 0.10 mg/kg of MK801 (MK801-108PE) approximately 75 min prior to CS preexposure. The remaining two groups served as controls for latent inhibition effects and received subcutaneous injections of saline (Sal-OPE; $n = 6$) or MK801 (MK801-OPE; $n = 6$) but no stimulus presentations during preexposure sessions. On Day 6, classical conditioning commenced, with each subject receiving daily training that consisted of 12 blocks of nine trials each; eight of the trials were reinforced with a corneal airpuff unconditioned stimulus (US; 5 psi; 100-msec duration) that coterminated with the CS and one trial served as a nonreinforced test trial. The intertrial interval averaged 60 sec. Daily training continued until a criterion of greater than 80% conditioned responses (CRs; at least 0.5 mm of NM extension during the CS-US interval) occurred over an entire session.

Once criterion performance was attained, both CS and US sensitivity tests were conducted on rabbits from the MK801-OPE group. The procedures were similar to those described elsewhere (G. B. Robinson, 1993), with the following two exceptions: the intertrial interval averaged 60 sec, and a CS sensitivity test was also performed, with the CS intensity varying randomly from trial to trial. Each animal received both the CS and the US sensitivity tests. The order of testing following saline, 0.05-mg/kg MK801, and 0.10-mg/kg MK801 administration was randomized. The 0.05-mg/kg dose, although not used in the latent inhibition tests, was also examined for comparison with the results of a subsequent study (G. B. Robinson, 1993). The data were analyzed using a within-subject repeated measures analysis of variance (ANOVA).

RESULTS

A repeated measures ANOVA was performed on the number of conditioned responses per block (for purposes of analysis, 18 trials constituted one block) of training. The results indicated a significant effect of block [$F(17,323) = 64.10, p < .001$], indicative of learning (response rates increased over time). The main effect of group was not significant [$F(3,19) = 1.91, p = .162$], but the group $\times$ block interaction was significant [$F(51,323) = 2.61, p < .001$]. Further analysis of the interaction (Tukey's; $p < .05$) revealed that the two latent inhibition groups were retarded during early acquisition stages. The Sal-108PE group performed at rates inferior to both Sal-OPE and MK801-OPE on Blocks 9–18; the MK801-108PE group performed at rates inferior to those of Sal-OPE, on Blocks 8–13 and on Block 16, and at rates inferior to those of MK801-OPE, on Blocks 8–14 and on Block 16. The two latent inhibition groups did not differ significantly from each other. The two control groups also did not differ significantly. The CR rates are shown in Figure 1.

As is evident in Figure 1, both the Sal- and the MK801-OPE groups reached criterion within three training sessions. The two latent inhibition groups, Sal- and MK801-108PE, also met criterion for acquisition of the behavioral response (Figure 2). Both latent inhibition groups, however, required longer to reach criterion and exhibited greater variability than did either of the control groups. A one-way ANOVA, however, demonstrated that the groups did not differ significantly on either the main criterion (greater than 80% CRs over an entire session) or the number of trials until eight CRs occurred within nine consecutive trials.

Figure 1. Mean conditioned response rates plotted over the initial 3 days of training for the two control groups (Sal-OPE and MK801-OPE) that received no conditioned stimulus (CS) preexposure and for the two CS preexposure groups (Sal-108PE and MK801-108PE). The SEMs for Training Blocks 10 and 18 are also illustrated.

Figure 3 illustrates the results of the US sensitivity tests. The effect of the drug dosage on the percent URs (top panel) was not quite significant [$F(2,10) = 3.48, p = .071$]. There was a significant effect of US intensity on the percent URs [$F(2,10) = 21.00, p < .001$]. The dose $\times$ intensity interaction was not significant [$F(4,20) = 1.58, p = .219$]. Both drug dose [$F(2,10) = 7.30, p < .011$] and US intensity [$F(2,10) = 40.67, p < .001$] had a significant effect on UR amplitude. The dose $\times$ intensity interaction was not significant [$F(4,20) = 0.82, p = .528$]. Follow-up tests (Tukey's) indicated that the 0.10-mg/kg dose of MK801 resulted in a significantly smaller UR amplitude than was found following saline administration. There was no significant difference between the effects of the 0.05- and 0.10-mg/kg doses of MK801 or between the effects of saline and the 0.05-mg/kg dose of MK801 on UR amplitude.

The results of the CS sensitivity tests were virtually identical, whether CS intensity varied by block (Figure 4, top panel) or by trial (Figure 4, bottom panel). Varying CS intensity by block or by trial revealed a significant effect of dose [$F(2,10) = 11.24, p < .003$ and $F(2,10) = 10.64, p < .003$, respectively] and intensity [$F(3,15) = 17.51, p < .001$ and $F(3,15) = 33.94, p < .001$, for block and trial, respectively] on the percent CRs. The dose $\times$ intensity interaction was not significant [$F(6,30) = 0.28, p = .940$ and $F(6,30) = 0.65, p < .689$, for block and trial, respectively]. Follow-up analyses (Tukey's;

Figure 2. Rate of conditioned response (CR) acquisition, according to two different criteria: the number of days until a CR rate of greater than 80% was obtained over an entire session (top panel); and the number of trials until eight CRs occurred within nine consecutive trials (bottom panel). The error bars represent the SEM.

$p < .05$) indicated that the .10-mg/kg dose of MK801 resulted in significantly fewer CRs than did either saline or the 0.05-mg/kg dose of MK801, which did not differ significantly from each other.

DISCUSSION

The results of the present experiment indicate that blockade of NMDA-mediated activity by MK801 has no significant effect on latent inhibition. As a consequence of CS preexposure, both MK801- and saline-treated subjects showed marked retardation of learning during early stages of acquisition. This result appears to indicate that latent inhibition is neither mediated by nor dependent on NMDA receptor activity or NMDA-dependent induction

of LTP. Consequently, some forms of behavioral plasticity appear to be modulated by NMDA-dependent increases in synaptic transmission, whereas other forms are apparently mediated by other, independent neurobiological processes (Malenfant, O'Hearn, & Fleming, 1991; Morris, Halliwell, & Bowery, 1989).

The present experimental design, however, may have limited the probability of observing an NMDA-mediated contribution to latent inhibition learning. As noted earlier, the induction of NMDA-dependent LTP *facilitates* acquisition (Berger, 1984), and NMDA blockade *retards* acquisition (G. B. Robinson, 1993) of the classically conditioned NM response. In those studies, learning was assessed on a trial-by-trial basis; in the present study, however, the success of latent inhibition was not determined

Figure 3. Effect of MK801 on percent unconditioned responses (URs; top panel) and UR amplitude (bottom panel), as a function of unconditioned stimulus intensity. The error bars represent the SEM. The 0.10-mg/kg dose did not affect the percent URs but did significantly reduce UR amplitude.

Figure 4. Effect of MK801 on percent conditioned responses (CRs) as a function of conditioned stimulus (CS) intensity. The CS intensity was varied by block (top panel) and by trial (bottom panel). Compared to both the saline and the 0.05-mg/kg doses, the 0.10-mg/kg dose significantly reduced the percent CRs. The error bars represent the SEM.

until after training (i.e., during subsequent acquisition of the conditioned NM response). If the number of CS pre-exposure trials was sufficient to get both the saline and the MK801 groups to a ceiling prior to the start of paired training, any possible MK801-induced antagonism of the strength of latent inhibition learning might not be noticeable. Nevertheless, the present data indicate that NMDA receptor-mediated plasticity is not essential to latent inhibition learning.

Other evidence would also appear to favor the view that latent inhibition is dependent on other neurochemical mechanisms than NMDA-mediated synaptic activity (Solomon, 1987). This belief is compatible with recent speculation that latent inhibition is mediated by nucleus accu-

bens/medial raphe input to the hippocampus (Weiner, 1990), a structure involved in latent inhibition learning (Solomon & Moore, 1975). Hippocampal plasticity appears to occur in various forms, with CS-US training producing heightened responsivity (Berger et al., 1976) and repetitive sensory input yielding reduced reactivity (Vinogradova, 1975). Conditioning-related potentiation of hippocampal activity, although not essential for learning in a standard delay paradigm (Schmaltz & Theios, 1972), may contribute to temporal aspects of simple CRs (James, Hardiman, & Yeo, 1987; Port, Mikhail, & Patterson, 1985). Determination of the effects of CS preexposure on ongoing hippocampal activity and on the subsequent development of heightened hippocampal responses during classical conditioning in raphe-lesioned animals would help to clarify the feasibility of Weiner's (1990) model.

The differential effects of NMDA antagonism on various forms of behavioral plasticity may help to clarify the neurobiological substrates underlying learning and memory. NMDA antagonism is reported to affect passive avoidance (Venable & Kelly, 1990), instrumental performance under a schedule with differential reinforcement of low rates (Welzl, Berz, & Bättig, 1991), classical conditioning (G. B. Robinson, 1993), and spatial cognition (G. S. Robinson, Crooks, Shinkman, & Gallagher, 1989; Shapiro & Caramanos, 1990; Upchurch & Wehner, 1990). However, some controversy exists about the contributions of sensory and motor impairments to learning deficits observed following NMDA antagonist administration (Hargreaves & Cain, 1992; Keith & Rudy, 1990). In the present study, 0.10-mg/kg MK801 significantly affected sensitivity to the CS (but did not block latent inhibition learning) and to the US. It should be noted that, except on the sensitivity tests, the US was never presented following MK801 administration. Behaviors spared by NMDA antagonism include maternal experience effects (Malenfant et al., 1991) and instrumental conditioning with continuous reinforcement (Seybold, Miller, & Port, 1991). In summary, although latent inhibition effects must converge on the system underlying basic associative plasticity, the present data suggest that fundamentally different mechanisms may mediate these forms of behavioral plasticity.

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