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The Delayed Effects of DTG and MK-801 on Latent Inhibition in a Conditioned Taste-Aversion Paradigm

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TURGEON, S. M., A. E. AUERBACH, M. DUNCAN-SMITH, J. R. GEORGE AND W. W. GRAVES. The delayed effects of phencyclidine (PCP) have been shown to disrupt latent inhibition (LI) in a conditioned taste-aversion paradigm. In an attempt to understand the mechanism of this disruption, the delayed effects of the selective sigma receptor agonist 1,3-Di(2-tolyl)guanidine (DTG) and the selective NMDA receptor antagonist MK-801 on latent inhibition were assessed in the same paradigm. Water-deprived male rats were allowed access to either water (non-preexposed; NPE) or 5% sucrose (preexposed; PE) for 30 min on 2 consecutive days. On the third day, animals were allowed access to sucrose and subsequently injected with lithium chloride. On the forth day, animals were allowed access to both sucrose and water. LI was assessed by comparing the percent sucrose consumed in PE and NPE groups on the fourth day. DTG (1.0, 5.0, or 10.0 mg/kg), MK-801 (0.5, 1.0, or 2.0 mg/kg), or vehicle was administered IP 20 h before preexposure (days 1 and 2) and conditioning (day 3). In vehicle-treated groups, PE animals consumed a significantly higher percent sucrose on the test day than NPE animals, indicating the presence of LI. DTG (10.0 mg/kg) and MK-801 (2.0 mg/kg) decreased the percent sucrose consumed by animals in the PE group to the level observed in the NPE group, indicating disrupted LI. However, this dose of MK-801 was found to produce a decrease in percent sucrose consumed in PE animals not treated with lithium chloride, indicating that the decrease observed in the LI paradigm could be due to MK-801-induced decrease in taste preference for sucrose rather than a disruption of LI. Lower doses of MK-801 that did not produce a decrease in taste preference for sucrose did not significantly disrupt LI. None of the doses of DTG tested altered taste preference for sucrose. These data suggest a role for sigma receptors in the previously observed PCP-induced disruption of LI. Published by Elsevier Science Inc., 2000

Requests for reprints should be addressed to Sarah M. Turgeon, Amherst College, Department of Psychology and Neuroscience Program, P.O. Box 5000, Campus Box 2256, Amherst, MA 01002-5000.
aversion paradigm (58). However, recent findings suggest that the delayed effects of a high dose of PCP may be better able than the acute effects to produce a schizophrenia-like state. This suggestion is based on the findings that the psychotomimetic effects of PCP increase with higher doses of the drug (3), and can persist for days or weeks following administration (1,28).

The delayed effects of both acute and chronic PCP treatment have been investigated in a variety of paradigms. The delayed effects of a single high dose of PCP impairs performance on a water maze task thought to model cognitive dysfunction seen in schizophrenia (44), and enhances behavioral responsiveness to amphetamine (59). Chronic PCP also enhances amphetamine responsiveness (31), impairs performance on cognitive tasks sensitive to prefrontal cortex function in rats (30) and monkeys (29), produces immobility in a forced swim test (41), and induces social withdrawal in rats (46). In addition, we recently found that the delayed effects of a high dose PCP disrupt LI in a conditioned taste-aversion paradigm (58).

Thus, the delayed effects of PCP appear to be able to produce a variety of behaviors in animals that model schizophrenia. However, the neurochemical mechanism by which PCP produces these behavioral effects is unclear. PCP is a non-competitive antagonist at the glutamate N-methyl-D-aspartate (NMDA) receptor (2,28), as well as a sigma receptor agonist (50) and an indirect dopamine agonist (10,25,27,39). Each of these neurochemical systems has been implicated in the pathophysiology of schizophrenia (9,23,24,56,57), and are thus candidate mechanisms for the production of schizophrenia-like behaviors by the delayed effects of PCP.

Thus far, PCP’s actions at NMDA and sigma receptors have been separately implicated in the delayed effects of PCP. Decreases in regional cerebral glucose metabolism seen 24 h after a high dose of PCP are argued to be mediated by PCP’s action as an NMDA receptor antagonist, as similar decreases can be produced by low, NMDA-receptor specific doses of PCP (17). Likewise, PCP-induced increases in hippocampal NMDA receptor binding seen 24 h postinjection (18) are mimicked by the selective NMDA receptor antagonist MK-801 (19). However, the sigma receptor antagonist NE-108 was found to reverse the cognitive dysfunction induced by the delayed effects of PCP in a water maze task (44). In an attempt to investigate the possible involvement of NMDA and sigma receptors in the previously observed PCP-induced disruption of LI (58), we investigated the delayed effect of 1,3-Di(2-toly)guanidine (DTG), a selective sigma receptor agonist, and MK-801, a selective NMDA receptor antagonist on latent inhibition in a conditioned taste-aversion paradigm.

METHOD

Subjects

One hundred and fifty-two male Sprague–Dawley rats (Charles River) weighing between 250 and 350 g were individually housed and maintained on a 12-h reverse light–dark cycle for the duration of the experiment. All rats were handled on at least three occasions prior to the onset of the experiment. Twenty-four hours prior to the onset of the experiment, animals were water deprived and subsequently only allowed access to water as defined by the experimental protocol. Animals were weighed and their drinking recorded daily during the experiment.

Procedure

Latent inhibition. Latent inhibition was assessed using a conditioned taste aversion paradigm adapted from Ellenbroek et al. (13). This paradigm measures the ability of preexposure to sucrose solution to prevent subsequent acquisition of conditioned taste aversion to sucrose. On days 1 and 2 of the experiment, rats were given access to either 50 ml of a 5% sucrose solution (preexposed; PE) or 50 ml of tap water (nonpreexposed; NPE) for 30 min. On day 3, all animals were given access to 50 ml of a 5% sucrose solution for 30 min immediately followed by an injection of lithium chloride (LiCl; 50 mg/kg in 2 ml/kg dH2O, IP). On day 4, all animals were given access to both 5% sucrose and water for 30 min. Latent inhibition was assessed by comparing the percent sucrose consumed on day 4 (ml sucrose consumed/ml water consumed)) in the PE vs. NPE animals.

Effects of drugs on LI. The delayed effects of DTG and MK-801 on latent inhibition were assessed by administering DTG or MK-801 20 h prior to each preexposure (days 1 and 2) and conditioning (day 3) session. Thus injections were given approximately 4 h after the initiation of water deprivation and 3.5 h after preexposure sessions on days 1 and 2. DTG was dissolved in a small volume of acetic acid then brought to volume with saline and NaOH to neutralize the solution (pH = 7.0) and administered IP in 10 ml/kg at doses of 1 mg/kg (n = 6 PE and 6 NPE), 5 mg/kg (n = 6 PE and 6 NPE), or 10 mg/kg (n = 6 PE and 6 NPE). MK-801 was dissolved in dH2O and administered IP in 2 ml/kg at doses of 0.5 mg/kg (n = 5 PE and 5 NPE), 1.0 mg/kg (n = 6 PE and 6 NPE), or 2.0 mg/kg (n = 5 PE and 5 NPE). Control animals were given either saline (10 ml/kg; n = 10 PE and 10 NPE) or dH2O (2 ml/kg; n = 8 PE and 8 NPE). No differences were noted between control groups so they were combined.

Effects of drugs on taste preference for sucrose. To control for the possibility that the drugs were altering taste preference for sucrose in the PE groups, the effects of the drugs themselves on sucrose preference were assessed. Animals were run through the above protocol modified such that the LiCl injection was replaced with a dH2O injection (10 ml/kg). The following groups were tested: 1 mg/kg DTG (n = 4 PE), 5 mg/kg DTG (n = 4 PE), 10 mg/kg DTG (n = 4 PE), 0.5 mg/kg MK-801 (n = 4 PE), 1.0 mg/kg MK-801 (n = 8 PE), and 2.0 mg/kg MK-801 (n = 8 PE). saline vehicle (n = 4 PE), dH2O vehicle (n = 8 PE). Again, no differences were noted between vehicle groups, so they were combined.

Effects of treatment group on total consumption. To ascertain that an effect of treatment group on total consumption did not lead to the appearance of disrupted LI in drug treatment groups, total consumption was analyzed in groups that displayed altered LI.

Statistics

The effects of DTG and MK-801 on latent inhibition were assessed by comparing the difference between PE and NPE groups in the drug-treated groups and the vehicle groups using a 2 × 2 ANOVA with the main factors of drug and exposure. The effects of each drug alone on sucrose consumption were compared with ANOVAs followed by a Tukey’s post hoc test. Total consumption was analyzed with a three-way mixed ANOVA with exposure and drug treatment as between-subjects variables and day as a within-subjects variable. Due to concerns about the effects of different levels of sucrose consumption during the preexposure phase on subsequent acquisition of LI, a Pearson correlation was performed between sucrose consumption on days 1 and 2 and percent sucrose consumed on the test day (day 4) in PE-vehicle animals.
RESULTS

Effects of Drugs on Taste Preference for Sucrose

Taste preference for sucrose in PE rats was not altered by any dose of DTG tested ($F(3, 20) = 0.30$ (Fig. 1a). An ANOVA revealed that MK-801 did alter sucrose preference, $F(3, 28) = 5.31, p < 0.01$, with a post hoc test demonstrating a difference between the 2.0 mg/kg MK-801 group and vehicle (Fig. 1b).

Effects of Drugs on LI

Animals in the vehicle control groups displayed clear latent inhibition that was not significantly altered by 1.0 mg/kg or 5.0 mg/kg DTG (Fig. 2a). This assertion is supported statistically as a $2 \times 2$ ANOVA revealed significant main effects of exposure, $F(1, 44) = 38.62, p < 0.001$, and $F(1, 44) = 30.37, p < 0.001$, but no effect of drug, $F(1, 44) = 3.09$, and $F(1, 44) = 1.08$, or drug $\times$ exposure, $F(1, 44) = 0.01$, and $F(1, 44) = 0.01$. However, 10.0 mg/kg DTG did suppress LI as demonstrated by significant main effects of exposure, $F(1, 44) = 13.74, p < 0.005$, drug, $F(1, 44) = 4.72, p < 0.05$, and drug $\times$ exposure, $F(1, 44) = 5.97, p < 0.05$.

Latent inhibition was not significantly affected by 0.5 mg/kg MK-801 or 1.0 mg/kg MK-801 (Fig. 3a) as supported by significant effects of exposure, $F(1, 44) = 18.57, p < 0.001$, and $F(1, 44) = 18.86, p < 0.001$, but not of drug, $F(1, 44) = 3.80$, and $F(1, 44) = 0.58$, or drug $\times$ exposure, $F(1, 44) = 2.31$, and $F(1, 44) = 3.65$. However, 2.0 mg/kg did suppress LI as supported by significant effects of exposure, $F(1, 44) = 14.23, p < 0.005$, drug, $F(1, 44) = 8.25, p < 0.01$, and drug by exposure, $F(1, 44) = 4.88, p < 0.05$.

Effects of Drugs on Total Consumption

Comparing daily consumption in the 10.0 mg/kg DTG-treated groups to vehicle (Fig. 2b), the three-way ANOVA revealed a main effect of day, $F(3, 132) = 25.33, p < 0.001$, but no main effect of drug, $F(1, 44) = 4.03$, or exposure, $F(1, 44) = 0.02$. Two-way interaction effects of day $\times$ drug, $F(3, 132) = 13.33, p < 0.001$, day $\times$ exposure, $F(3, 132) = 7.17, p < 0.001$, and drug $\times$ exposure, $F(1, 44) = 4.32, p < 0.05$, were revealed. The three-way interaction of day $\times$ drug $\times$ exposure was not significant, $F(3, 132) = 2.34$. In the 2.0-mg/kg MK-801 groups compared to vehicle (Fig. 3b), there was a main effect of day, $F(3, 132) = 39.88, p < 0.001$, but no main effect of drug, $F(1, 44) = 3.12$, or exposure, $F(1, 44) = 3.57$. There was no two-way interaction effect of drug $\times$ exposure, $F(1, 44) = 0.18$, but there were interaction effects of day $\times$ drug, $F(3, 132) = 3.65, p < 0.05$, and day $\times$ exposure, $F(3, 132) = 18.62, p < 0.001$. The three-way interaction of day $\times$ drug $\times$ exposure was not significant, $F(3, 132) = 0.28$.

There was no correlation between the amount of sucrose consumed during the preexposure phase in PE-vehicle animals and subsequent percent sucrose consumed on the test day, $r(16) = -0.083, p = 0.745$.

DISCUSSION

These results demonstrate that the delayed effects of the sigma receptor agonist DTG impair latent inhibition in a conditioned taste-aversion paradigm. The delayed effects of the NMDA receptor antagonist MK-801 also impaired latent inhibition, but only at a dose that was high enough to cause a decrease in taste preference for sucrose. Animals preexposed to sucrose and treated with 2.0 mg/kg MK-801 demonstrated a decrease in the percent sucrose consumed in the absence of LiCl. Data presented as mean $\pm$ SEM. *$p < 0.05$ Tukey’s post hoc test.

LiCl-induced taste aversion. This suggests that 2.0 mg/kg MK-801, given 3.5 h after each day of preexposure to sucrose, produced an unpleasant experience that was paired with the sucrose and created a decrease in taste preference for sucrose.
Because a decrease in percent sucrose consumed in the PE group is indicative of disrupted LI, the presence of an MK-801–induced decrease in PE groups not treated with LiCl suggests that the apparent disruption of LI by 2.0 mg/kg MK-801 may be due to drug-induced decreases in taste preference for sucrose acquired in the PE group during the preexposure phase of the experiment.

In addition to drug-induced changes in taste preference for sucrose, another potential confound that needs to be considered is the possibility that drug treatment altered drinking behavior so as to give the appearance of disrupted LI. Three-way ANOVAs revealed no main effect of drug in either the 10.0 mg/kg DTG or the 2.0 mg/kg MK-801. However, there were two-way interaction effects of day × drug for both groups. An inspection of the data reveals that MK-801- and DTG-treated groups drank less sucrose than the vehicle-treated groups on day 3, the conditioning day. However, if this variation were to affect the outcome on day 4, one would expect that animals that drank the most sucrose to have the strongest taste aversion. In fact, PE-V animals drank the most sucrose but demonstrated the least taste aversion, suggesting that this variation is unlikely to explain the outcome on day 4. In comparing 10.0 mg/kg DTG to vehicle groups, there was also a drug × exposure effect. Examination of the data reveals that PE-V animals drank more overall than NPE-V animals while NPE-10.0 mg/kg DTG animals drank more overall than PE-10.0 mg/kg DTG animals. Lower levels of drinking on days 1 and 2 in the PE-10.0 mg/kg DTG group could potentially lead to the absence of adequate preexposure to the stimulus and thus to the appearance of disrupted LI. However, this explanation seems unlikely, as the animals in this group were clearly preexposed to sucrose, drinking an average of 12 and 16 ml of sucrose on each of the preexposure days. In addition, there was no correlation between the amount of sucrose consumed during the preexposure phase (with a range of 24 to 48 ml combined consumption on days 1 and 2, thus encompassing the levels seen in the PE-10.0 mg/kg DTG group) and percent sucrose consumed on the test day in PE-vehicle animals, suggesting that lower levels of sucrose consumption during preexposure do not impair the acquisition of LI. Thus, differences in total daily consumption between groups are unlikely to account for the observed changes in LI.

The suggestion that the delayed effects of PCP may be mediated via PCP’s action at sigma receptors is consistent with the report by Okuyama et al. (44) that the sigma antagonist NE-100 decreases the deleterious delayed cognitive effects of PCP on performance in a water-maze task. However, the present data do not eliminate the possibility that the action of PCP as an NMDA receptor antagonist contributes to PCP-induced disruption of LI. While disruption of LI in the lower dose MK-801 groups was not supported statistically by a 2 × 2 ANOVA,
there is a trend toward decreased sucrose consumption in the PE groups. This observation, combined with the difficulty interpreting the effect of the highest dose of MK-801 due to MK801–induced decrease in taste preference for sucrose, leaves open the possibility that PCP’s action as an NMDA receptor antagonist contributes to PCP-induced disruption of LI.

In addition to the possibility that a direct effect of PCP on NMDA receptors contributes to PCP-induced disruption of LI, an indirect effect of PCP on NMDA receptor function may be exerted via PCP’s action as a sigma agonist. Selective sigma ligands have been found to regulate NMDA receptor-mediated function in a variety of paradigms. DTG and other sigma agonists have been found to potentiate NMDA-induced firing in CA3 neurons (40) as well as MK-801–induced head weaving (33). In addition, the sigma agonist (+)pentazocine has been found to inhibit NMDA receptor-induced $[^{3}H]$dopamine release in hippocampal and striatal slices (7,21).

Whether the acute effects of sigma receptor ligands on NMDA receptor function could lead to a sufficiently long-lasting change to explain the delayed effects of DTG is unclear. One of the few studies to examine delayed changes in NMDA receptor systems following exposure to a drug with sigma agonist properties reported that a high dose of PCP leads to increases in NMDA-sensitive $[^{3}H]$glutamate receptor binding in the hippocampus 24 h postinjection (18). While such an effect seems a plausible mechanism for the previously observed effect of PCP on LI given the involvement of the hippocampus in LI (61), this effect is most likely a compensatory response to NMDA receptor antagonism, and thus would not likely be elicited by DTG. In fact, a similar upregulation of NMDA receptors was seen following 1.0 mg/kg MK-801 (19). This finding suggests that PCP’s action as an NMDA receptor antagonist is sufficient to induce NMDA receptor upregulation, and that DTG would be unlikely to elicit such an effect. More importantly, this finding suggests that NMDA receptor upregulation in the hippocampus is not responsible for PCP-induced disruption of LI, as this dose of MK-801 did not significantly disrupt LI in the present study. Thus, a delayed effect of DTG on NMDA receptor systems remains plausible, but currently not evident.

The delayed effect of DTG on LI may involve alterations in dopamine systems, as sigma receptors have been shown to modulate dopamine system function in a variety of paradigms. Electrophysiological studies have demonstrated sigma receptor agonist-induced increases in firing of ventral tegmental dopamine neurons (16) and decreases in firing of substantia nigral dopamine neurons (8,51,52). DTG, specifically, did not increase firing of ventral tegmental neurons (16), but decreased (52) or had no effect on (70) firing of substantia nigra neurons. However, systemic (45) and intranigral (5) injections of DTG have been shown to increase striatal dopamine release and dopamine metabolites, respectively. This increase in nigrostriatal dopamine activity appears to have behavioral consequences, as unilateral intranigral injections of DTG (5) and other sigma agonists (60) have been shown to produce rotational behavior. Enhanced nigrostriatal dopamine activity could underlie DTG-induced disruption of LI, as nigrostriatal administration of the indirect dopamine agonist amphetamine has been shown to disrupt LI in a conditioned taste aversion paradigm (14). Thus, DTG may be producing an increase in nigrostriatal dopamine activity that leads to impaired LI. Such an effect of DTG could be the result of a direct action on dopamine neurons, as sigma receptor binding in the substantia nigra pars compacta and the striatum decreases following intrastriatal 6-hydroxydopamine lesions, suggesting that there are sigma receptors located directly on dopaminergic neurons (23). However, altered NMDA receptor function may be involved in sigma receptor-mediated increases in striatal dopamine, as 6-OHDA lesions only reduced sigma binding by about 15–30% (23), and the NMDA receptor antagonist CPP was found to block increases in striatal dopamine metabolites induced by the sigma agonists (+)-SKF 10,047 and (+)-pentazocine (26).

While the connection between sigma ligands and the nigrostriatal dopamine system suggests a plausible mechanism by which DTG could disrupt LI, the timing of the drug effect needs to be considered because the reported effects of DTG on striatal dopamine were acute rather than delayed. DTG increased levels of striatal dopamine and dopamine metabolites between 40 and 120 min after injection (5,45). Although time points beyond 120 min were not examined, levels in most groups had begun to decline by this time point, making it unlikely that levels remained elevated 20 h postinjection. However, the activation of DA systems by DTG could be producing a sensitization effect that leads to enhanced responsiveness within the system at the 20-h time point. This hypothesis is supported by the observation that a single dose of PCP leads to enhanced behavioral responsiveness to amphetamine as well as enhanced AMPH-induced striatal c-Fos 24 h postinjection (59). Whether or not the delayed effects of DTG would produce the same enhanced responsiveness remains to be determined.

There are at least two subtypes of sigma receptors, designated $\sigma_1$ and $\sigma_2$ (6). Because DTG acts at both subtypes, the results of this experiment do not permit determination of their selective involvement in the impairment of LI. Okuyama et al. (44) demonstrated that the delayed effects of PCP on cognitive dysfunction were decreased by the selective $\sigma_1$ antagonist NE-100. If the effect of DTG observed here is due to activation of the same sigma receptors implicated in the delayed effects of PCP on cognitive dysfunction, we might predict that $\sigma_1$ receptors are involved in the delayed effects of DTG on LI. However, if nigrostriatal dopamine systems are integral to the observed effects of DTG, sigma, receptors may be involved as rotational behavior induced by intranigral injections of a variety of sigma agonists is correlated with the affinity of the ligands for the $\sigma_1$ receptor (60). Further investigation with more selective sigma ligands will be necessary to determine which subtype of sigma receptor mediates DTG-induced disruption of LI. In addition, assessing the degree to which selective sigma receptor antagonists are able to reverse PCP-induced disruption of LI would help to determine whether or not PCP’s action at NMDA receptors plays an appreciable role in PCP-induced disruption of LI.

A role for sigma receptors in schizophrenia is suggested by a number of lines of evidence. The sigma agonist SKF 10,047 induces delusions, hallucinations, depersonalization, and dysphoria (24). A number of effective antipsychotic drugs have been found to be potent sigma receptor ligands, including haloperidol and chlorpromazine (55). In addition, a number of newer drugs with antipsychotic efficacy have been found to possess high affinity for the sigma binding site (34). However, not all attempts to demonstrate antipsychotic efficacy of sigma ligands have proven successful (20). Finally, there is some evidence for alterations in sigma receptors in schizophrenic patients; however, these changes were noted in medicated populations (47,48,69). These data have lead some to suggest that sigma receptors may be a potential target for antipsychotic medications (34). However, others predict involvement of sigma receptors in psychosis but are more cau-
tious about the potential value of targeting sigma receptors therapeutically due to possible involvement of extrapyramidal side effects and the limited demonstrated clinical antipsychotic efficacy of selective sigma ligands (9).

Although the present results support a role for sigma receptors in the etiology of PCP-induced behavioral states, support for a role of sigma receptors in schizophrenia remains speculative. As discussed previously (58), the validity of PCP-induced disruption of LI in a conditioned taste aversion paradigm as a model for schizophrenia requires further consideration. Disruption of LI has been observed in a subpopulation of schizophrenic patients (4); however, not all studies have replicated this finding (54). In addition, while antipsychotic drugs reverse amphetamine-induced disruption of LI in conditioned emotional response (CER) paradigms (49,65), the effects of antipsychotic drugs on PCP or amphetamine-induced disruption of LI in a CTA paradigm have not yet been tested. Finally, LI as assessed in different paradigms may be regulated by different substrates. The nucleus accumbens plays a role in LI as assessed by a CER paradigm (61,63), while the striatum has been implicated in LI as assessed by a CTA paradigm (14). Thus results obtained in one LI paradigm may not generalize to other paradigms.

In summary, while not ruling out a contribution from PCP’s action as an NMDA receptor antagonist, the present findings implicate a role for sigma receptors in the delayed effects of PCP. The relevance of these findings to the study of schizophrenia remains speculative; however, the data are consistent with the suggestion that sigma receptors may play a role in the etiology of schizophrenia, and/or may be a potential target for therapy.

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