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Subchronic MK-801 behavioural deficits in rats: Partial reversal by the novel nitrate GT 1061

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ABSTRACT

Cognitive deficits are a core feature of schizophrenia that may be linked to abnormalities in GABA and nitric oxide (NO). Subchronic treatment with glutamate receptor antagonists produces similar deficits, providing a useful model to examine potential therapeutics. The present study investigated the effects of subchronic MK-801 (intraperitoneally; 0.5 mg/kg twice daily for 7 days) on amphetamine-induced locomotor activity and reversal learning in the water maze in rats, and the ability of the novel compound GT 1061 (4-methyl-5-(2-nitroxyethyl) thiazole HCl), containing dual pharmacophores producing NO- and GABA-mimetic activity, to ameliorate these effects. MK-801 enhanced locomotor responses to amphetamine. GT 1061 (0.1; not 0.0001, 0.001, 0.01, 1.0 mg/kg) further enhanced locomotion; the pro-GABA drug chlormethiazole (0.1, 1.0 mg/kg) had no significant effect. In saline-pretreated rats GT 1061 (0.1; not 0.0001, 0.001 mg/kg) increased amphetamine-induced locomotion; chlormethiazole (0.1, 1.0 mg/kg) had no effect. In the water maze, MK-801 impaired reversal learning after platform relocation. GT 1061 (0.001, 0.01, 0.1; not 0.0001 or 1.0 mg/kg) attenuated this impairment; chlormethiazole had no significant effect. These ameliorative effects of GT 1061 may be linked to the activation of NO- and GABA-dependent signaling and suggests a new direction for treating cognitive dysfunction in schizophrenia.

Schizophrenia affects approximately 1% of the population. Although psychosis is often the most prominent feature (American Psychiatric Association, 2000), cognitive impairments are a core component (Green and Nuechterlein, 1999); they may be the most persistent (Heaton et al., 2001; Rund 1998) and debilitating feature of the illness and the best predictor of long-term functional outcome (Green, 1996; Green et al., 2004). Traditional anti-psychotics primarily target positive symptoms and remain largely ineffective at treating cognitive impairments. Many researchers advocate for the development of novel therapeutics that target the negative and cognitive symptoms of schizophrenia and are based on identifying and targeting the molecular bases of these symptoms (Insel and Scolnick, 2006; Lewis and Gonzalez-Burgos, 2006).

Abnormalities in multiple neurotransmitter systems have been reported in schizophrenia suggesting a general dysfunction in neurocircuitry (review: Lisman et al., 2008). Changes in γ-aminobutyric acid (GABA; Lewis et al., 2004) and nitric oxide (NO; Bernstein et al., 2005) may be particularly relevant for developing novel therapeutic strategies for the cognitive impairments of schizophrenia. Thus, in post-mortem brain studies cell counts of GABAergic neurons (Reynolds and Beasley, 2001; Reynolds et al., 2004) and interneurons (Benes et al., 1991; Benes et al., 1998) were reduced in the prefrontal cortex (PFC) and hippocampus, and GABA concentration was reduced in the PFC (Ohnuma et al., 1999) of schizophrenic patients compared to controls. The parvalbumin (PV)-containing subtype of GABA neurons have been implicated in schizophrenia in particular (Lewis and Gonzalez-Burgos, 2006). Thus, the density of PV-immunoreactive neurons (Beasley and Reynolds, 1997), PV mRNA expression per neuron and the density of neurons containing the 67 kDa isoform of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD67) were decreased in the PFC of schizophrenia brains (Hashimoto et al., 2003). Abnormalities in PFC GABA function are thought to contribute to the cognitive deficits of schizophrenia (Lewis and Gonzalez-Burgos, 2006; Volk and Lewis, 2002).

NO is a membrane-permeable gas formed from L-arginine by the enzyme nitric oxide synthase (NOS). A highly diffusible gas, it has diverse functions including regulating vascular tone, modulating signal transduction pathways and modulating the release of neurotransmitters such as glutamate, GABA and dopamine (DA) (review: Prast and Philippu, 2001). Although the precise alterations are unclear, NO has been repeatedly implicated in schizophrenia (Bernstein et al., 2005). Higher total plasma nitrate levels (Zoroglu et al., 2002), higher platelet NOS activity (Das et al., 1995) and higher caudate NO levels

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have been reported in schizophrenia patients (Yao et al., 2004). Lower calcium-dependent constitutive NOS (Xing et al., 2002) and reduced cell density of NOS-containing neurons (Bernstein et al., 1998) have also been found in the PFC and paraventricular nucleus, respectively, of schizophrenic patients. Thus, although the direction of abnormality is under debate, NO is altered in schizophrenia.

NO has been implicated in cognitive processes such as learning and memory (Monfort et al., 2004; Muller, 1996; Schwighofer and Ferriol, 2000). In the brain, NO binds to soluble guanylate cyclase (sGC) leading to the production of cyclic guanosine monophosphate (cGMP) that activates cGMP-kinases that can potentiate synaptic transmission (Bon and Garthwaite, 2001; Zhuo et al., 1994a). Through downstream activation of cGMP-dependent protein kinase (PKG), NO also activates extracellular signal-regulated kinase (ERK1/2; Andoh et al., 2003), implicated in synaptic plasticity and memory (review: Adams and Sweatt, 2002). At high concentrations NO has the potential to exert toxic effects (Brorson et al., 1999; Connop et al., 1996) through reactivity with superoxide anion to produce peroxynitrite ($OONO^-$), a highly reactive molecule involved in neurotoxicity (Lipton et al., 1993). NO may contribute to the cognitive deficits of schizophrenia. This is supported by recent evidence of altered genes for the neuronal isoform of NOS that were associated with altered PFC activity and impaired cognitive function in schizophrenic patients (Reif et al., 2006).

A new class of NO-mimetic drugs, S-nitrates, may be potential therapeutics for the cognitive dysfunction of schizophrenia. S-nitrates appear to produce the beneficial effects of NO at therapeutic doses without producing harmful concentrations (Thatcher et al., 2004b). These hybrid compounds incorporate additional functional groups onto an organic nitrate and thereby produce additional biological activity over parent pharmacophores (Thatcher et al., 2004b). 4-Methyl-5-(2-nitroxyethyl) thiazole HCl (GT 1061) is one drug from this class. It is derived from chlormethiazole, a neuroprotective agent that potentiates $GABA_A$ receptor activity (Wilby and Hutchinson, 2004) and GABA neurotransmission (Thatcher et al., 2005). GT 1061 therefore produces both NO- and GABA-mimetic activity.

GT 1061 and 2,3-dinitrooxy-(2,3-bis-nitrooxy-propyldisulfanyl)-propane (GT 715), a related S-nitrate, have demonstrated neuroprotective and memory enhancing effects. In a model of excitotoxic neuronal injury using malonate, GT 715 prevented losses of striatal GABA content (Thatcher et al., 2004a). In an animal model of dementia using the anticholinergic scopolamine, both GT 715 (Smith et al., 2000) and GT 1061 (Thatcher et al., 2004a) improved water maze task acquisition and, after cortical and hippocampal cholinergic depletion, GT 1061 improved performance on water maze tasks (Bennett et al., 2007). These results suggest that GT 1061 may represent a novel therapeutic approach for the cognitive deficits of schizophrenia.

Animal models that mimic neurobiological and behavioural features of schizophrenia provide insight into the pathophysiology and potential treatments (review: Marcotte et al., 2001). One model is based on subchronic (e.g., 7 days) treatment with non-competitive *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonists such as phencyclidine (PCP), ketamine and dizocilpine (MK-801). Like amphetamine (Connell, 1958; Young and Scoville, 1938) these substances reliably induce psychosis in humans; however, they also produce cognitive deficits (Luby et al., 1959). In the rat, these substances create abnormalities similar to those seen in schizophrenia including enhanced sensitivity to amphetamine (Balla et al., 2001), increased locomotor activity (Jentsch et al., 1998b), and impaired social interaction (Snigdha and Neill, 2008), as well as cognitive deficits including impaired working memory (Jentsch et al., 1997), behavioral inhibition (Jentsch and Taylor, 2001) and reversal learning (Abdul-Monim et al., 2007). Subchronic, compared to acute, administration creates more persistent and qualitatively closer deficits to those seen in schizophrenia and is considered an animal model with greater validity (review: Jentsch et al., 1999; Morris et al., 2005). The subchronic model creates a variety of abnormalities in neurotransmission similar to those in schizophrenia including decreased PV mRNA expression in the PFC (Cochran et al., 2003), decreased density of PV immunoreactive interneurons and increased density of NADPH-diaphorase-positive neurons, a marker for NOS activity, in the hippocampus (Keilhoff et al., 2004), and in a cerebrocortical neuronal cell culture, decreased PV and GAD_{67} immunoreactivity in PV-containing GABA neurons (Kinney et al., 2006). Similar neurochemical changes have been linked to cognitive deficits. Thus, subchronic PCP impaired reversal learning of a lever-pressing task and was linked to large decreases in PV cell density in the dentate gyrus and CA2 and CA3 regions of the hippocampus and more subtle changes in PV cell density in the frontal cortex (Abdul-Monim et al., 2007). The subchronic NMDA receptor antagonist model was used in the present study.

We investigated the effects of the novel S-nitrate GT 1061 and its parent compound chlormethiazole HCl on cognitive deficits induced by subchronic treatment with MK-801. The effects of GT 1061 and chlormethiazole on amphetamine sensitivity, an additional behavioural measure related to schizophrenia (Geyer and Moghaddam, 2002), were also investigated. We hypothesized that GT 1061 would mitigate the effects of MK-801 on amphetamine-induced locomotor activity and on performance in a reversal learning task in the water maze with greater efficacy than chlormethiazole.

1. Methods

1.1. Subjects

Experimentally naïve male albino Sprague–Dawley rats ($N=228$; Charles River, St. Constant, Quebec) weighing from 225-275 g on arrival were housed in pairs in clear Plexiglas cages (45×25×22 cm deep). Rats were kept in a climate-controlled colony room (21±1 °C; humidity 40–70%) on a reversed 12-h light/dark schedule, with lights off at 0700 h. Rats were handled for approximately 2 min/day for 6 consecutive days prior to the initiation of testing. Unlimited access to food (labDiet rodent feed # 5001) and water was available in the home cages.

Treatment of the animals was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and pertinent University Policy and was approved by the Queen's University Animal Care Committee.

1.2. Drugs

MK-801 (dizocilpine; [5R-10 S]-[+]5-methyl-10,11-dihydro-5H-dibenzo[a,d]cylcohepten-5,10-imine maleate salt), purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario), dextroamphetamine sulfate (USP, Rockville, MD), chlormethiazole hydrochloride (Tocris Cookson Inc., Ellisville MO) and GT 1061 (a gift from Dr. G.R. Thatcher, University of Illinois at Chicago) were dissolved in saline.

1.3. Apparatus

1.3.1. Activity monitors

Activity was measured as the number of breaks across seven pairs of photocells positioned at a height of 5.0 cm above the metal-rod floor in each of 6 experimental chambers (50×40×40 cm high) constructed from plexiglass and housed in wooden, Styrofoam-insulated outer boxes. Each chamber was illuminated with a 2.5 W incandescent bulb and ventilated by a small fan that also provided background noise. Beam breaks were recorded on an experimenter-controlled circuit board connected to a personal computer. For details of the apparatus see Beninger et al. (1985).

1.3.2. Water maze

The water maze was a circular pool (1700 cm diam×60 cm high) filled with water (21 °C) to a depth of 40 cm and non-toxic tempera

white paint (2 L) was added to make the water opaque. Four release points, equally spaced around the pool, were designated by the four cardinal compass positions that also specified 4 quadrants. A moveable platform (20 cm diam) was hidden approximately 3 cm below the surface of the water in the center of one of the quadrants and approximately 30 cm from the edge of the pool. Visual stimuli were placed on the walls around the pool to provide the rats with spatial cues. A video camera was situated directly above the center of the pool and all trials were monitored and recorded using the HVS Water 2020 (HVS Image; Hampton, UK) tracking system.

1.4. Procedure

Rats were subchronically treated with either saline (1.0 ml/kg twice daily for 7 days) or MK-801 (0.5 mg/kg twice daily for 7 days). Injections were given i.p. (1.0 ml/kg) at approximately 0830 and 1800 h. Testing commenced 7 days after the last injection. Drug dose and administration schedule were based on the subchronic NMDA receptor antagonist model developed by Jentsch et al. (1998a; Jentsch and Roth, 1999).

1.4.1. Activity

Activity was measured over 3.5 h using a protocol with three distinct phases. Activity counts were recorded every 10 min. The habituation phase had a duration of 1.0 h. Rats were then removed from the chamber, injected with saline (1.0 ml/kg i.p.) and returned to the chamber for another 1.0-h session. Rats were again removed from the chamber, and independent groups of MK-801- and saline-pretreated rats were injected with GT 1061 (0, 0.0001, 0.001, 0.01, 0.1, or 1.0 mg/kg i.p.) and returned to their home cage for 20 min, or chlormethiazole (0.1, 1.0 mg/kg i.p.) and returned to their home cage for 25 min. All rats were then injected with amphetamine (1.5 mg/kg i.p.) immediately before being returned to the chamber and tested for an additional 90 min.

1.4.2. Water maze

Two days after the completion of activity testing, rats were tested in the water maze. Twenty minutes prior to the first trial, independent groups of MK-801 and saline-pretreated rats were injected with GT-1061 (0, 0.0001, 0.001, 0.01, 0.1, or 1.0 mg/kg i.p.) or chlormethiazole (0.1, 1.0 mg/kg i.p.). Dosing groups remained the same as in activity testing. Testing consisted of two sessions of four trials. For each trial, rats were released from one of the two farthest points from the platform. Release points were counter-balanced and the farthest release points were chosen in an effort to reduce the variability in time to find the platform that results from very short swim times that can occur when the rat is released near the platform. A trial began with release into the water facing the wall. The rat swam until it located the hidden platform or, if it failed to find the platform in 60 s, the rat was manually guided to the platform by the experimenter and the maximum of 60 s was recorded as the time-to-platform for that trial. Rats then remained on the platform for 15 s before the next trial began. After 4 trials (session 1), rats spent 15 min under a heat lamp and then 5 min in their home cage before receiving 4 more similar trials but with the platform in a new position (session 2). The dependent variable for the trials was time to the platform.

1.5. Data analyses

Habituation, saline and amphetamine activity scores (counts/10 min) were analysed separately with 2-way mixed design analyses of variance (ANOVA) followed by Dunnett's *T*-test for post-hoc analyses where appropriate. Variables analysed were time (10-min blocks) and group. The first ANOVA compared the MK-801- vs. saline-pretreated control groups in each phase. Subsequent ANOVA of beam breaks during each phase included the MK-801-pretreated control group and compared the MK-801-pretreated GT 1061 groups and the chlormethiazole groups. Similar ANOVA compared the saline-pretreated groups.

For the water maze, time-to-platform for the MK-801- and saline-pretreated control groups were analysed using two 2-way (group×trial) mixed-design ANOVA, one for each session. A 1-way ANOVA was conducted for the first trial of the second session to test the hypothesis that the MK-801-pretreated group would take longer to find the platform in the new position, showing reduced behavioral flexibility. Subsequent ANOVA comparing performance in trial 1 of session 2 included the MK-801-pretreated control group and the GT 1061 or chlormethiazole groups. Similar ANOVA compared the saline-pretreated groups.

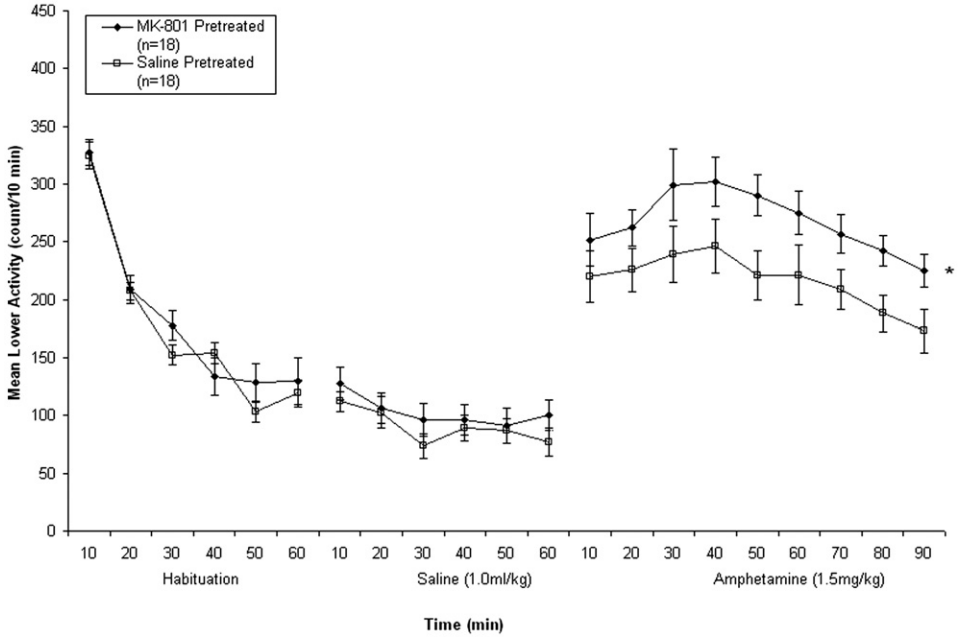

Fig. 1. Mean (±SEM) beam breaks per 10 min for habituation, saline, and amphetamine (1.5 mg/kg) phases for groups that received MK-801 (0.5 mg/kg twice daily for 7 days) or vehicle (1.0 ml/kg twice daily for 7 days). * Significant group effect.

2. Results

2.1. Activity

Locomotor activity for the saline- and MK-801-pretreated groups under the three different experimental conditions is shown in Fig. 1. During the habituation phase, both groups showed a large decrease in beam breaks over the 60-min test period. Injection of saline had no effect on locomotor activity. In contrast, acute challenge with amphetamine increased beam breaks in both groups, but the MK-801-pretreated group showed a larger increase. The group×time (10-min blocks) ANOVA revealed significant main effects of time, but not group, for the habituation ($F(5.170)=148.30$, $p<.001$) and saline ($F(5.170)=2.72$, $p=.02$) phases. In contrast, following amphetamine, there was a significant main effect of time ($F(8.272)=6.87$, $p<.001$) and group, with MK-801-pretreated rats showing significantly more beam breaks ($F(1.34)=4.93$, $p=.03$). Thus, the locomotor activity level of MK-801- and saline-pretreated rats differed only during the amphetamine phase.

As there was no time×group interaction in the comparison of MK-801- vs. saline-pretreated groups, beam breaks were averaged over time blocks for subsequent analysis of possible drug effects. MK-801 pretreated groups that were to receive GT 1061 or chlormethiazole did not differ in locomotor activity during the habituation and saline phases (Fig. 2A). In the acute amphetamine challenge phase, groups treated with GT 1061 showed a dose-dependent increase in locomotor activity; groups treated with chlormethiazole showed little change. The ANOVA

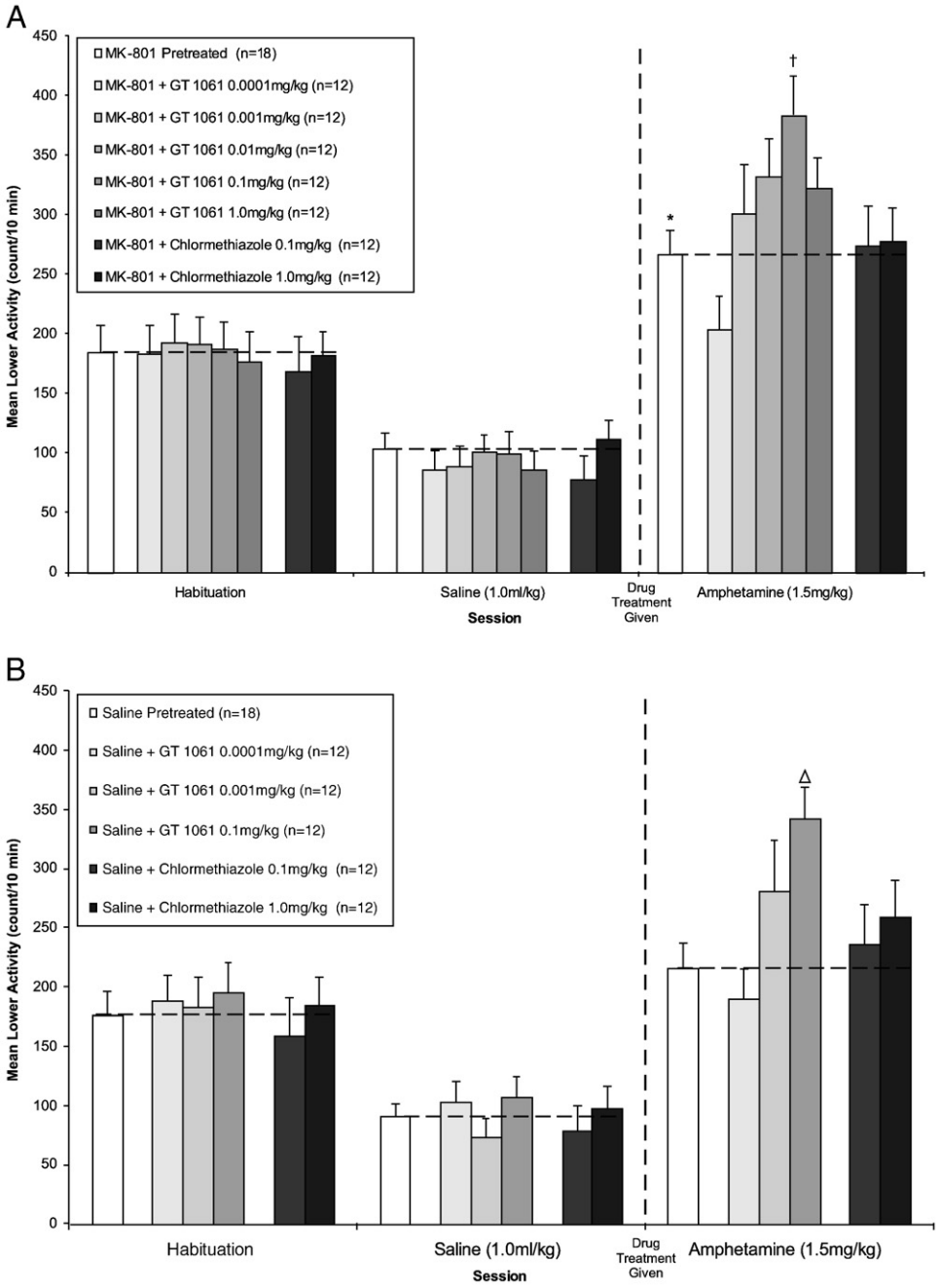

Fig. 2. Mean (±SEM) beam breaks per 10 min for habituation, saline, and amphetamine (1.5 mg/kg) phases for groups that received (A) MK-801 or MK-801 with GT 1061 (0, 0.0001, 0.001, 0.01, 0.1, 1.0 mg/kg) or chlormethiazole (0.1, 1.0 mg/kg); (B), saline or saline with GT 1061 (0, 0.0001, 0.001, 0.1 mg/kg) or chlormethiazole (0.1, 1.0 mg/kg). * Significant difference between MK-801- and saline-pretreated groups; † significant difference between MK-801-pretreated and GT 1061 groups; Δ significant difference between saline-pretreated and GT 1061 groups.

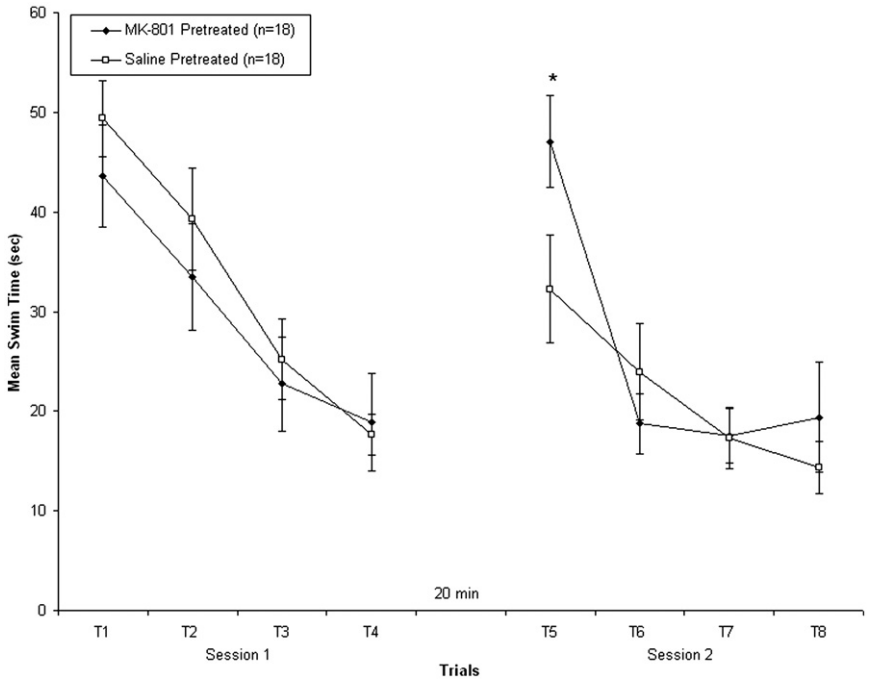

Fig. 3. Mean (±SEM) swim time (s) per trial in the water maze for groups that received MK-801 (0.5 mg/kg twice daily for 7 days) or vehicle (1.0 ml/kg twice daily for 7 days). * Significant difference between MK-801- and saline-pretreated groups.

comparing the MK-801 alone with the five GT 1061 treated groups revealed a significant effect ($F(5.71)=7.22$, $p<.001$), and Dunnett's post-hoc test comparing each group to the MK-801 alone group showed that the group receiving 0.1 mg/kg GT 1061 had significantly more beam breaks ($p=.001$).

There were few differences among saline-pretreated groups during the habituation and saline phases (Fig. 2B). Although ANOVA showed a significant effect for GT 1061 during the saline phase ($F(3.50)=2.80$, $p<0.05$), Dunnett's post-hoc test revealed no significant differences between particular GT 1061 groups and the saline-pretreated group. During the amphetamine phase, groups treated with GT 1061 showed a dose-dependent increase in beam breaks; chlormethiazole produced little change. ANOVA comparing the saline alone group and the GT 1061 groups revealed a significant effect of drug treatment ($F(3.50)=8.18$,

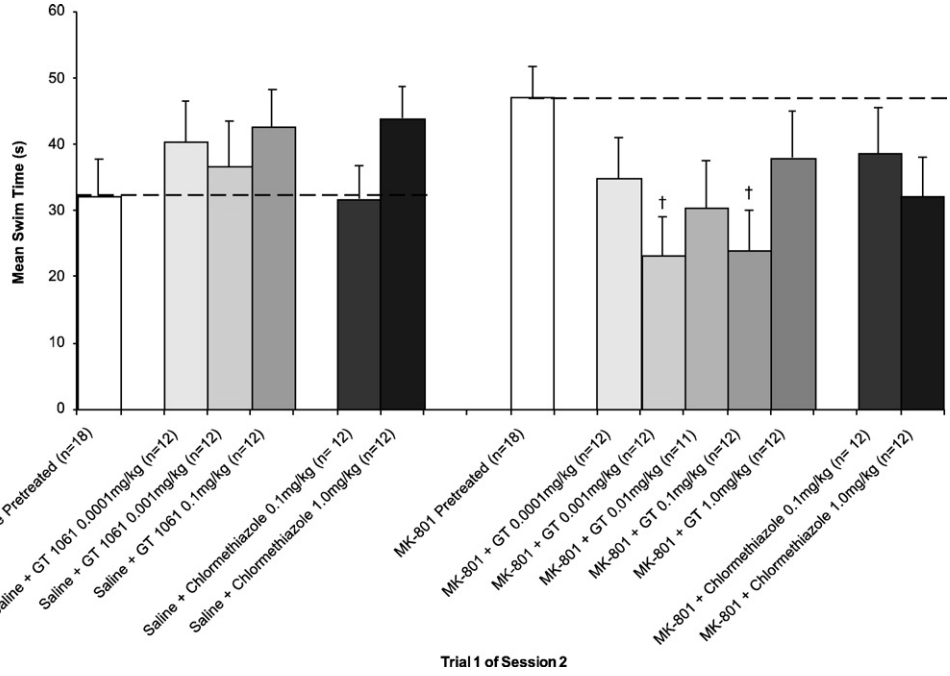

Fig. 4. Mean (±SEM) swim time (s) for trial 1 of session 2 (reversal) in the Morris water maze for groups that received MK-801, or MK-801 and GT 1061 (0, 0.0001, 0.001, 0.01, 0.1, 1.0 mg/kg) or chlormethiazole (0.1, 1.0 mg/kg) (left panel), and saline, or saline and GT 1061 (0, 0.0001, 0.001, 0.1 mg/kg) or chlormethiazole (0.1, 1.0 mg/kg) (right panel). * Significant difference between MK-801 and saline-pretreated groups; † significant difference between MK-801-pretreated and GT 1061 groups.

$p<.001$). Dunnett's post-hoc tests comparing the saline alone group to each GT 1061 group showed that the group receiving 0.1 mg/kg GT 1061 ($p<0.001$) made significantly more beam breaks.

2.2. Water maze

During session 1 and session 2 the saline- and MK-801-pretreated groups showed decreased swim times over trials (Fig. 3) and ANOVA revealed main effects of trial ($F(3.78)=16.72$, $p<.001$), and ($F(3.75)=11.77$, $p<.001$), respectively. As hypothesized, on trial 1 of session 2, the MK-801-pretreated group exhibited a longer swim time than the saline-pretreated group ($F(1.28)=4.32$, $p<.05$). Subsequent analyses of drug treatment effects focused on this trial.

During trial 1 of session 2, drug treatments had little systematic effect on the swim time of saline-pretreated rats (Fig. 4, left panel); in MK-801-pretreated rats GT 1061 and chlormethiazole tended to reduce swim time at some doses with GT 1061 producing the largest effect (Fig. 4; right panel). ANOVA comparing the saline alone to the GT 1061- or chlormethiazole-treated groups yielded no significant effects. ANOVA comparing the MK-801 alone and chlormethiazole groups also yielded no significant effects; however, ANOVA comparing the MK-801 alone and the GT 1061-treated groups revealed a group effect ($F(5.68)=2.38$, $p<.05$), and Dunnett's post-hoc tests comparing each group to the MK-801 alone group showed the 0.1 and 0.001 mg/kg GT 1061 groups to have significantly reduced swim times. Thus GT 1061 reduced the swim-time of MK-801-, but not saline-pretreated rats at mid-range but not low or high doses.

3. Discussion

The results can be summarized as follows: rats subchronically pretreated with MK-801 demonstrated increased locomotor activity following acute amphetamine injection but not during either habituation or following acute saline injection. GT 1061 dose-dependently increased the stimulant effect of amphetamine in both MK-801- and saline-pretreated rats. MK-801-pretreated rats did not show a deficit in initial task acquisition to find a hidden platform in the water maze, but exhibited a deficit in reversal learning as demonstrated by increased swim time on the first trial following the repositioning of the hidden platform. GT 1061, but not chlormethiazole, dose-dependently reversed this deficit in MK-801-pretreated rats but did not significantly affect the performance of saline-pretreated rats.

The observations that MK-801- and saline-pretreated groups did not differ in locomotor activity during the habituation or saline phases, nor did their swim times differ during the initial 4 acquisition trials in the water maze suggest that MK-801 pretreatment did not significantly affect general activity or ability to acquire the water maze task. Significant differences were only seen after amphetamine administration and on the first trial in the water maze following relocation of the platform. Differences in general well being, body weight or swim speed therefore do not seem to account for the observed effects.

The finding that MK-801-pretreated rats showed an enhanced locomotor response to amphetamine is in agreement with previous studies using subchronic doses of NMDA receptor antagonists (Balla et al., 2001; Jentsch et al., 1998a); similar effects also occur following acute administration of NMDA receptor antagonists (Hoffman, 1994; Wolf et al., 1994). In other studies, subchronic PCP induced hyperlocomotion in otherwise untreated rats (Jentsch et al., 1998a), but subchronic MK-801 did not (Dall'Olio et al., 1992; Mandillo et al., 2003). Our finding of enhanced amphetamine-induced hyperlocomotion following subchronic MK-801 in the absence of increased locomotion during the habituation and saline phases is consistent with these observations.

In previous studies, subchronic PCP administration impaired reversal learning in an operant lever-pressing task (Abdul-Monim et al., 2007) and in a T-maze (Jentsch and Taylor, 2001), without affecting initial acquisition. Perinatal PCP led to impaired reversal learning in the water maze (Anderson and Pouzet, 2004). Acute doses of MK-801 impaired reversal learning in developing (Chadman et al., 2006) and adult rats (van der Meulen et al., 2003) without affecting initial acquisition, as have acute doses of the NMDA receptor antagonists D-2-amino-5-phosphonopentanoic acid (AP-5; Palencia and Ragozzino, 2004), and PCP (Abdul-Monim et al., 2003; Idris et al., 2005). Our finding that subchronic MK-801 administration impaired reversal learning confirms and extends previous findings that subchronic doses of PCP, as well as acute doses of other NMDA receptor antagonists, impair reversal learning.

GT 1061 at 0.1 mg/kg significantly increased the stimulant effects of amphetamine in both MK-801- and saline-pretreated rats. GT 1061 at 0.001 and 0.1 mg/kg significantly decreased the swim time of MK-801- but not saline-pretreated rats on the first reversal learning trial in the water maze. Although not significant, the mean swim time of the MK-801 pretreated group treated with 0.01 mg/kg of GT 1061 was among the shortest. Results suggest that GT 1061 doses ranging from 0.001 to 0.1 mg/kg are effective at ameliorating cognitive deficits produced by subchronic MK-801. One possible explanation for these beneficial effects is that decreased swim time reflects increased activity. However, this is not likely for two reasons: i) even though GT 1061 had significant effects on amphetamine-stimulated activity in both MK-801- and saline-pretreated rats, it decreased swim time in MK-801-pretreated rats only; and ii) of the three doses that produced the largest decreases in swim time in MK-801-pretreated rats, only one had a significant effect on activity.

The current finding that GT 1061 dose-dependently increased amphetamine-induced hyperlocomotion in both saline- and MK-801-pretreated rats may be related to its dual potentiation of GABA and NO. Increases in locomotor activity following amphetamine administration are regulated by the mesoaccumbens DA system (Vezina and Kim, 1999). Both NO and GABA modulate striatal DA release; however, whereas NO can potentiate striatal DA (review: West et al., 2002), GABA exerts an inhibitory tone on striatal DA release both indirectly through inhibition of excitatory PFC glutamate afferents to the striatum (Karreman & Moghaddam, 1996), and directly in interneurons that exert an inhibitory tone on DA neurons in the striatum (Smolders et al., 1995). The dual activation of GABA and NO pathways by GT 1061 may therefore have resulted in competing potentiation and inhibition of striatal DA by NO and GABA, respectively. Thus, the 0.1 mg/kg dose enhanced amphetamine-induced hyperlocomotion in saline- and MK-801-pretreated rats whereas the other doses did not. Additional studies are needed to further define those doses of GT 1061 that increase striatal DA release.

The finding that GT 1061 dose-dependently reversed the MK-801-induced reversal learning deficit is in line with previous studies showing that NO donors and agonists can mitigate the cognitive and behavioral deficits induced by acute NMDA receptor antagonism. Thus, sodium nitroprusside (SNP), an NO donor, blocked PCP-induced hyperactivity, stereotyped behaviour and ataxia (Bujas-Bobanovic et al., 2000), L-arginine, a substrate of NOS, and S-Nitroso-N-acetylpenicillamine, a generator of NO, reversed MK-801-induced impairment of spontaneous alternation behaviour (Yamada et al., 1996), and molsidomine, an NO donor, attenuated MK-801-induced impairment in an object recognition task (Pitsikas et al., 2006). To our knowledge, this is the first study to examine the effects of a pro-NO drug on cognitive deficits produced by subchronic NMDA receptor blockade. As chlormethiazole and GT 1061 both augment GABA neurotransmission, why was GT 1061 effective at ameliorating the reversal learning deficit produced by subchronic MK-801 whereas chlormethiazole was not? In previous studies from our lab (in preparation) we found that chlormethiazole had ameliorative effects on cognitive deficits induced by acute NMDA receptor blockade but was less effective when cognitive deficits resulted from more chronic insult such as that following neonatal ventral hippocampal lesions. The present observation that chlormethiazole was without effect on cognitive deficits produced by subchronic MK-801 agrees with these

findings. Although speculative, these results may suggest that pro-GABA compounds have some efficacy at ameliorating cognitive deficits resulting from milder or acute insults (e.g., acute MK-801) but that cognitive deficits resulting from more severe or chronic insults (e.g., subchronic MK-801) are resistant to these compounds. Clinical schizophrenia is characterised by diverse abnormalities in neurotransmission that likely reflect a dysfunction in broader neural circuitry (Carlsson et al., 2001) and stem from complex epistatic and multifactorial bases (Riley and Kendler, 2006). Potential therapeutics that target only one of these systems may be insufficient to address the broader dysfunction in neurotransmission present in schizophrenia. Compounds such as GT 1061 that combine the potentiation of GABAA receptor function with potentiation of NO function, and thereby address multiple factors implicated in the pathophysiology of schizophrenia may therefore have greater efficacy following systemic insults that more closely model those in schizophrenia.

In conclusion, the present study demonstrated that subchronic MK-801, while not significantly affecting locomotor activity in otherwise untreated rats, increased amphetamine-stimulated locomotor activity; it also impaired reversal learning in the water maze without affecting initial acquisition. The novel nitrate GT-1061 dose-dependently attenuated MK-801-induced reversal learning deficits. Although more research needs to be conducted to confirm the exact therapeutic mechanisms of NO mimetics and to further clarify those doses of S-nitrates that enhance cognition without adversely affecting striatal dopamine levels, these findings demonstrate the potential therapeutic effects of combined GABA modulation plus NO mimetic activity for the cognitive deficits of schizophrenia.

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