Pharmacology, Biochemistry and Behavior 91 (2008) 71–76

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Ketamine induces hyperactivity in rats and hypersensitivity to nicotine in rat striatal slices

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ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 5 June 2008
Accepted 24 June 2008
Available online 29 June 2008

Keywords:
Dopamine
Glutamate
Ketamine
Nicotine
Nicotinic acetylcholine receptors
NMDA receptors
Rats
Schizophrenia
Striatum

ABSTRACT

The symptoms of schizophrenia can be modeled in rats through blockade of ionotropic glutamate receptors, which induces changes in central dopamine circuits. These circuits also contain nicotinic acetylcholine receptors that are activated by nicotine. A role for nicotine in the etiology of schizophrenia is supported by clinical observations of high tobacco use rates in individuals experiencing the psychopathology. The present study investigated the effect of the ionotropic glutamate receptor antagonist ketamine on the function of striatal nicotinic acetylcholine receptors to understand better the potential role of these receptors in schizophrenia. Ketamine (0.1–300 μM) was ineffective to evoke [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]dopamine. Application of psychotomimetic ketamine concentrations (1–10 μM) to striatal slices augmented nicotine-evoked [^{3}H] overflow. Finally, rats received ketamine (30–50 mg/kg) injections for 30 days, to model the development of the disorder, and hyperactivity was observed, although repeated ketamine treatment did not significantly alter nicotine-evoked [^{3}H]dopamine overflow. These data indicate that the function of nicotinic acetylcholine receptors that mediate dopamine release are altered by ketamine, and support a role for nicotinic acetylcholine receptors in schizophrenia pathology.

1. Introduction

Schizophrenia is a complex, debilitating disorder, of which we do not fully understand the pathophysiology underlying the development, progression, and treatment of the disorder. Subanesthetic doses of *N*-methyl-D-aspartate (NMDA) receptor antagonists, like ketamine, induce behavioral and neurochemical changes associated with symptoms of schizophrenia in humans and animals (Jentsch and Roth, 1999; Krystal et al., 1994; Lahti et al., 2001). Ketamine was developed as a dissociative anesthetic (Collins et al., 1960); however, subanesthetic doses (30–50 mg/kg) induce psychotomimetic effects (Rao et al., 1989; Verma and Moghaddam, 1996). For example, acute and subchronic ketamine administration induces hyperactivity, which models positive symptoms of schizophrenia (Imre et al., 2006; Leccese et al., 1986). The effects of subchronic drug exposure are generally more persistent and resemble the psychopathology of schizophrenia more closely than the effects of acute exposure (Jentsch and Roth, 1999).

The dopamine hypothesis of schizophrenia posited that hyperfunction of dopamine neurons is the underlying cause of the symptoms (Snyder, 1976). Subsequently, the glutamate hypothesis posited that glutamatergic hypofunction is the underlying cause of symptoms (Kornhuber and Kornhuber, 1986). More recently, the dopamine and glutamate hypotheses have been integrated to suggest that cortical glutamatergic projections modulate the firing of subcortical dopamine neurons (Carlsson et al., 1999). The striatal complexes mediate both motor and cognitive functions as they receive direct innervation from substantia nigra and neocortex, and hyperactivity of dopamine neurons in this region is postulated to contribute to the development of the positive symptoms of schizophrenia (Carlsson, 1995).

Regarding the effect of ketamine on dopamine release, germane to the development of schizophrenia, in vivo ketamine administration increases (Rao et al., 1989; Verma and Moghaddam, 1996), decreases (Rao et al., 1989), and does not change (Lannes et al., 1991; Micheletti et al., 1992) dopamine release from striatum. In vitro ketamine superfusion either increases (Tso et al., 2004) or induces no change (Mantz et al., 1994) in dopamine release. This lack of consensus on ketamine's effect on dopamine release warrants further investigation for the development of ketamine as a rodent model of schizophrenia and glutamate receptor regulation of dopamine neurons.

Tobacco cigarette smoking is more prevalent among people diagnosed with schizophrenia than in the general population (Kalman et al., 2005). Clinical studies suggest that individuals diagnosed with schizophrenia who are chronic smokers have more severe positive symptoms and less severe negative symptoms of schizophrenia

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doi:10.1016/j.pbb.2008.06.012

(Aguilar et al., 2005; Ziedonis et al., 1994). The high prevalence of smoking among people with schizophrenia suggests that nicotinic acetylcholine receptor function may be altered in the disorder. Nicotine evokes dopamine release in a concentration-dependent manner (Rapier et al., 1988) by binding to and activating nicotinic acetylcholine receptors (Sargent, 1993). The effect of ketamine on nicotine's properties and the effects of long-term (subchronic or chronic) glutamate receptor blockade have not been addressed thoroughly. Considering the prevalence of tobacco smoking in individuals with schizophrenia and the correlation of smoking with the exacerbation of positive symptoms of schizophrenia, it is important to determine the effect of NMDA receptor antagonists on the function of nicotinic acetylcholine receptors that mediate dopamine release in striatum.

The purpose of this study was to examine the effect of ketamine on striatal dopamine neurons and nicotinic acetylcholine receptors. Experiment 1 determined the efficacy of ketamine to evoke [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]dopamine. This experiment was designed to clarify previous controversial findings and evaluate the intrinsic activity of ketamine across a range of subanesthetic, anesthetic, and supra-anesthetic concentrations. Experiments 2 and 3 investigated the effect of ketamine on nicotine-evoked [^{3}H] dopamine release. In Experiment 2, ketamine was applied directly to striatal slices followed by nicotine, modeling acute in vitro drug challenge. In Experiment 3, rats were administered ketamine for 30 days and then nicotine-evoked [^{3}H]dopamine release was measured, investigating the effect of subchronic NMDA receptor blockade.

2. Methods

2.1. Subjects

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri. Male Sprague–Dawley rats (Harlan, Indianapolis, IN; 175–200 g upon arrival to the laboratory) were housed two per cage with ad libitum access to tap water and standard rat chow. The colony was maintained under a 12-h/12-h light/dark cycle and the experiments were conducted during the light phase of the cycle.

2.2. Drugs and chemicals

Pargyline hydrochloride, (±)-ketamine hydrochloride, (−)-nicotine ditartrate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company (St. Louis MO). Alpha-D(+)-glucose, sodium phosphate, and L(+)-ascorbic acid were purchased from Acros Organics (Geel, Belgium). Radiolabeled dopamine (dihydroxyphenylethylamine 3,4-[7-^{3}H], specific activity=34.8–60.0 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston MA). (±)-Ketamine hydrochloride (Ketasthesia) solution was purchased from Butler Animal Health Supply (Chicago IL). Scintigest tissue solubilizer and all of the other chemicals were purchased from Fisher Scientific (Fairlawn NJ).

2.3. Apparatus

Locomotor activity was monitored automatically using Med Associates' (Georgia VT) Open Field Test Environments (ENV-515), comprised of a 16×16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Sensors surrounded an acrylic cage (43.2×43.2×30.5 cm), and each sensor bank and cage was housed in a large sound-resistant cubicle (ENV-017M). Locomotor activity data were collected in 5-min intervals using Med Associates' Open Field Activity Software (SOF-811) that records the number of sensor breaks along the spatial dimensions of the monitor and computes these data as measures of distance traveled (in cm).

2.4. Experiment 1: effect of ketamine to evoke [^{3}H] overflow

Rats (n=6) were euthanized via rapid decapitation. Striata were dissected and sliced (750 μm). Slices were incubated in oxygenated buffer (in mM, 108 NaCl, 25 $NaHCO_3$, 11.1 glucose, 4.7 KCl, 1.3 $CaCl_2$, 1.2 $MgSO_4$, 1.0 Na_2HPO_4, 0.11 ascorbic acid, 0.001 EDTA) at 37 °C for 30 min. Slices were transferred to fresh buffer, [^{3}H]dopamine (0.1 μM) was added, and slices were incubated for an additional 30 min. Six slices were transferred to individual reaction chambers in an automated superfusion system (Suprafusion 2500, Brandel, Gaithersburg MD). Each reaction chamber (0.2 ml) was bound by glass microfiber filters (GF/B, Whatman, Madistone England). Pargyline (10 μM) was included in buffer to inhibit monoamine oxidase (Westerink and Kikkert, 1986). Slices were superfused with buffer at a rate of 0.75 ml/min. After 60 min, sample collection commenced at a rate of 1 sample/3 min. After the collection of three baseline samples, ketamine (0.1–300 μM) was added to buffer, and superfusion continued for 6 min. One chamber was superfused with only buffer and served as a control condition. Subsequently, all slices were superfused with buffer that did not contain ketamine for 15 min. When collection was complete, slices and filters were removed from the reaction chambers and incubated with tissue solubilizer (0.25 ml/sample). Radioactivity in superfusate samples and slices/filters was measured by liquid scintillation spectroscopy (LS 6500 Scintillation counter, Beckman–Coulter, Fullerton CA; counting efficiency≈60%).

2.5. Experiment 2: effect of acute NMDA receptor blockade on nicotine-evoked [^{3}H] overflow

Slices and buffer were prepared as described in Experiment 1. Three separate conditions were investigated in this experiment, as slices were continuously superfused with buffer containing 0 μM (n=8), 1 μM (n=8), or 10 μM (n=7) ketamine. The concentration–response profile for nicotine (1–100 μM) was then determined in the presence (1 or 10 μM) and absence (0 μM) of ketamine. The nicotine concentrations were selected from previous experiments in our laboratory (Dopheide et al., 2007). The ketamine concentrations were selected based on results from Experiment 1 as concentrations that did not evoke [^{3}H] overflow. Furthermore, these ketamine concentrations are also below those (38–105 μM) observed in plasma during anesthesia (Rogers et al., 2004), which elicit little psychotomimetic effects (White et al., 1980). After the collection of three baseline samples, nicotine was added to buffer, and superfusion continued for 6 min. Subsequently, nicotine was removed and slices were superfused for 15 min.

2.6. Experiment 3: effect of subchronic NMDA receptor blockade on nicotine-evoked [^{3}H] overflow

Rats were weighed and assigned randomly to one of three ketamine injection groups (0 mg/kg (n=9), 30 mg/kg (n=10), or 50 mg/kg (n=8) ketamine). On Day 1, the exposure regimen began. Rats were injected (IP) with either 30 or 50 mg/kg of ketamine or saline (0 mg/kg ketamine) once daily for 30 days (Days 1–30). On Day 30 behavioral changes (hyperactivity) associated with positive symptoms of schizophrenia were assessed using a locomotor activity assay. Rats were administered their respective ketamine or saline injection and then placed in the locomotor activity monitor for 60 min. Rats were not administered any injections on Days 31–36. On Day 37, one week after the final ketamine or saline injection, rat brain slices and buffer were prepared as described in Experiment 1. After the collection of three baseline samples, nicotine (1–100 μM) was added to buffer, and superfusion continued for 6 min. Ketamine was not added to buffer for any of these slices. Subsequently, all slices were superfused with buffer that did not contain nicotine for 15 min.

2.7. Data analysis

For all experiments, a significance level of $p<0.05$ was established a priori and paired *t*-tests were performed as post hoc comparisons using Bonferroni adjustment when appropriate. All data are expressed as the mean (±S.E.M).

For all [^{3}H] overflow experiments, fractional release (i.e., the time course) was calculated by dividing the [^{3}H] collected in each 3-min sample by the total [^{3}H] present in the tissue at the time of sample collection. A second dependent measure, total [^{3}H] overflow, was calculated by subtracting the cumulative overflow in the presence of drug from average basal overflow.

For Experiment 1 (effect of ketamine to evoke [^{3}H] overflow), total [^{3}H] overflow data were analyzed via one-way repeated measures analysis of variance (RM-ANOVA) with Ketamine Concentration as a within-subject factor. Fractional [^{3}H] release was analyzed via two-way RM-ANOVA with Ketamine Concentration and Time as within-subject factors.

For Experiment 2 (effect of acute NMDA receptor blockade on nicotine-evoked [^{3}H] overflow), total [^{3}H] overflow data were analyzed via two-way RM-ANOVA with Nicotine Concentration as a within-subject factor and Ketamine Concentration as a between-groups factor. Subsequent analyses evaluated the concentration–response for nicotine in each of the three ketamine conditions. Fractional [^{3}H] release was analyzed via three-way RM-ANOVA with Nicotine Concentration and Time as within-subject factors and Ketamine Concentration as a between-groups factor.

For the locomotor activity probe for Experiment 3 (effect of subchronic NMDA receptor blockade on nicotine-evoked [^{3}H] overflow), distance traveled data were analyzed via two-way RM-ANOVA with Ketamine Dose as a between-groups factor and Time as a within-subjects factor. For the [^{3}H] overflow assay for Experiment 3, total [^{3}H] overflow data were analyzed via two-way RM-ANOVA with Nicotine Concentration as a within-subject factor and Ketamine Dose as a between-groups factor. Additional analyses evaluated the concentration–response for nicotine within each of the three ketamine groups. Fractional [^{3}H] release was analyzed via three-way RM-ANOVA with Nicotine Concentration and Time as within-subject factors and Ketamine Dose as a between-groups factor.

3. Results

3.1. Experiment 1: effect of ketamine to evoke [^{3}H] overflow

The concentration–response profile for ketamine (0.1–300 μM) was determined in Experiment 1. The main effect of Ketamine Concentration was not significant for the measure of total [^{3}H] overflow ($p=0.09$). There was comparable total [^{3}H] overflow among the ketamine concentrations. Thus, ketamine, within a range of subanesthetic, anesthetic, and supra-anesthetic concentrations, did not significantly evoke [^{3}H] overflow from striatal slices.

3.2. Experiment 2: effect of acute NMDA receptor blockade on nicotine-evoked [^{3}H] overflow

Nicotine evoked [^{3}H] overflow in a time- and concentration-dependent manner from striatal slices. For the measure of total [^{3}H] overflow, a significant Ketamine Concentration × Nicotine Concentration interaction ($F(8,80)=3.15$, $p<0.05$) was found. Subsequent analyses evaluated the nicotine concentration–response curves in the presence and absence of ketamine. The nicotine concentration–response curves are presented in Fig. 1A.

Nicotine-evoked [^{3}H] overflow in a concentration-dependent manner from slices superfused in the absence (0 μM) of ketamine and the presence of 1 and 10 μM ketamine ($F(4,28)=12.37$, $p<0.05$; $F(5,35)=44.79$, $p<0.05$; $F(5,30)=15.28$, $p<0.05$; respectively). For each of the three conditions, post hoc analyses determined that there was greater

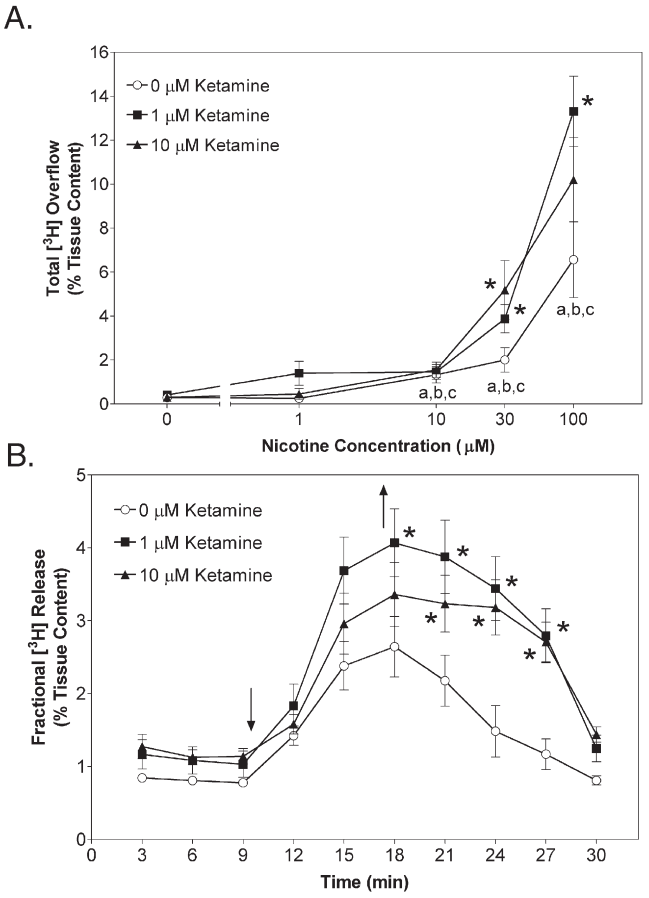

Fig. 1. Panel A depicts the nicotine concentration–response profiles in the presence and absence of ketamine. Ketamine (1–10 μM) augmented the effect of nicotine to evoke [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]dopamine. Superfusion buffer contained ketamine (0, 1, or 10 μM) through the entirety of the experiment and then nicotine was added. Data are expressed as mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine. Asterisks designate a significant ($p<0.05$) between-group difference from 0 μM ketamine (superfusion in the absence of ketamine) at the respective nicotine concentration. The letters "a", "b" and "c" designate a significant ($p<0.05$) within-subjects difference from the 0 μM nicotine concentration for the 0 μM, 1 μM and 10 μM ketamine groups, respectively. Panel B depicts the time course of nicotine (100 μM)-evoked [^{3}H]dopamine release. Arrows signify the addition and removal of nicotine, respectively, and asterisks designate a significant ($p<0.05$) difference from 0 μM ketamine at each respective time point. ($n=7$–8 rats/group).

[^{3}H] overflow in the presence of 10–100 μM nicotine than in the absence of nicotine. Furthermore, for the 0, 1, and 10 μM ketamine conditions, the threshold nicotine concentration to evoke [^{3}H] overflow was 10 μM.

Comparison of the three concentration–response curves for nicotine to evoke [^{3}H] overflow revealed significant differences among the three ketamine conditions. At the 30 and 100 μM nicotine concentrations, there was greater [^{3}H] overflow for the 1 μM ketamine group than for the 0 μM ketamine group. At the 30 μM nicotine concentration, there was greater [^{3}H] overflow for the 10 μM ketamine group than for the 0 μM ketamine group. There were no differences among the groups at any other nicotine concentrations.

Fractional [^{3}H] release (i.e., the time course) also was evaluated to assess differences among the ketamine groups. The Ketamine Concentration × Nicotine Concentration × Time interaction ($F(72,720)=3.22$, $p<0.05$) was significant. Post hoc analyses did not determine any significant differences in fractional [^{3}H] release among the three ketamine groups for the 0 or 1 μM nicotine concentrations. However, there were significant differences among the three ketamine groups for the 10 μM nicotine concentration. Fractional [^{3}H] release was greater for the 1 μM ketamine group than for the 0 μM ketamine group at the 21 and 24 min time points (data not shown). Fractional [^{3}H] release was greater

for the 10 μM ketamine group than for the 0 μM ketamine group at the 24 min time point (data not shown). There were no other significant differences among ketamine groups during superfusion with 10 μM nicotine. Regarding the 30 μM nicotine concentration, fractional [^{3}H] release was greater for the 1 μM ketamine group than the 0 μM ketamine group at the 15, 18, 21, 24, and 27 min time points (data not shown). Fractional [^{3}H] release was greater for the 10 μM ketamine group than for the 0 μM ketamine group at the 24 and 27 min time points (data not shown). Finally, for the 100 μM nicotine concentration, there were significant differences among the three ketamine groups and these data are presented in Fig. 1B. Post hoc analyses revealed that fractional [^{3}H] release was greater for the 1 μM ketamine group than for the 0 μM ketamine group at the 15, 18, 21, 24, and 27 min time points. Also, fractional [^{3}H] release was greater for the 10 μM ketamine group than for the 0 μM ketamine group at the 21, 24, and 27 min time points. Thus, these results demonstrate that application of 1 and 10 μM ketamine to striatal slices enhanced the effect of nicotine to evoke [^{3}H] release.

3.3. Experiment 3: effect of subchronic NMDA receptor blockade on nicotine-evoked [^{3}H] overflow

The presence of ketamine-induced behavioral changes associated with the positive symptoms of schizophrenia was evaluated using a locomotor activity assay on Day 30 of repeated drug treatment. The main effect of Ketamine Dose ($F(2,24)=51.61$, $p<0.05$) and the Ketamine Dose×Time interaction ($F(22,264)=6.37$, $p<0.05$) were significant. As depicted in Fig. 2, there was greater distance traveled in the ketamine-treated rats (30–50 mg/kg) than in the saline-treated rats at all time points except at the 10 min time point.

Nicotine-evoked [^{3}H] overflow was evaluated 1 week after the final injection. Fig. 3 depicts the concentration–response profiles for nicotine to evoke [^{3}H] overflow from striatal slices from rats repeatedly administered ketamine (30 or 50 mg/kg) or saline.

For rats repeatedly administered saline or 30 mg/kg ketamine, nicotine-evoked [^{3}H] overflow in a concentration-dependent manner ($F(5,35)=13.79$, $p<0.05$; $F(5,45)=17.56$, $p<0.05$; respectively). For both groups, post hoc analyses determined that total [^{3}H] overflow was greater for 10, 30, and 100 μM nicotine than for 0 μM nicotine. Thus, the threshold nicotine concentration to evoke [^{3}H] overflow was 10 μM for the saline-treated and 30 mg/kg ketamine-treated rats. However, for the rats administered 50 mg/kg ketamine, the threshold nicotine concentration to evoke [^{3}H] overflow was 1 μM. Nicotine-evoked [^{3}H] overflow in a concentration-dependent manner ($F(5,35)=79.69$, $p<0.05$) for rats in the 50 mg/kg ketamine group and post hoc analyses revealed greater total [^{3}H] overflow at the 1, 3, 10, 30, and 100 μM nicotine concentrations that at the 0 μM nicotine concentration.

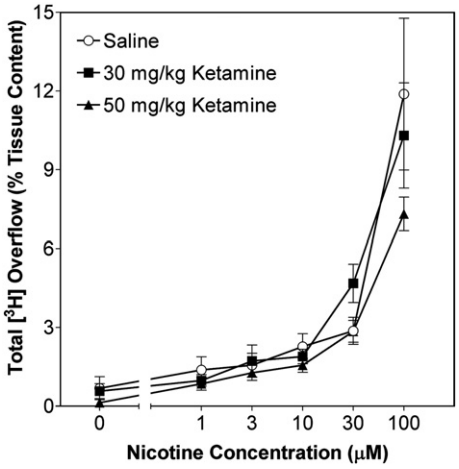

Fig. 3. Ketamine treatment does not alter the effect of nicotine to evoke [^{3}H] overflow from striatal slices preloaded with [^{3}H]dopamine. Data are expressed as the mean (±S.E.M.) total [^{3}H] overflow after the addition of nicotine to buffer. ($n=8$–10 rats/group).

For the measure of total [^{3}H] overflow, the Ketamine Dose×Nicotine Concentration interaction was not significant ($p=0.32$). Thus, there were no differences in the shape of the concentration–response curves for nicotine among the three ketamine groups. Regarding fractional [^{3}H] release, the Ketamine Dose×Nicotine Concentration×Time interaction was not significant ($p=0.07$). Thus, there were no differences in the efficacy of nicotine with repeated ketamine treatment as exhibited in Experiment 2 (Fig. 1A).

4. Discussion

In the present study, NMDA receptor blockade by ketamine induced hypersensitivity to nicotine in striatal slices. In line with previous studies, superfusion with ketamine (0.1–300 μM) did not evoke dopamine release (Mantz et al., 1994), and nicotine evoked

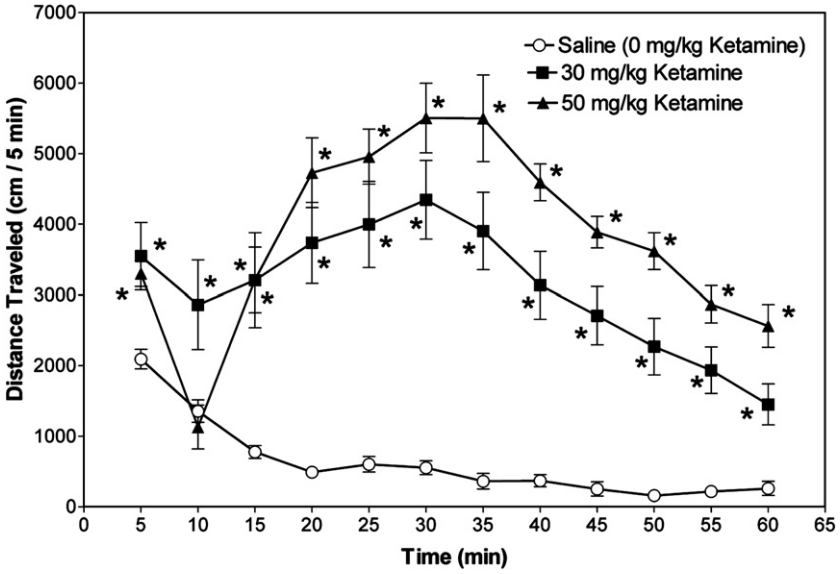

Fig. 2. Ketamine induces hyperactivity. Rats were administered ketamine (30 or 50 mg/kg) or saline on Days 1–30 and locomotor activity was measured after the last injection on Day 30. The data are presented as mean (±S.E.M.) distance traveled on Day 30. Rats were injected with ketamine or saline and placed in locomotor activity monitors for 60 min. Asterisks designate a significant ($p<0.05$) between-group difference from saline-treated group at each respective time point. ($n=8$–10 rats/group).

dopamine release in a concentration-dependent manner from slices (Dopheide et al., 2007; Rapier et al., 1988). More importantly, acute NMDA receptor blockade by ketamine augmented the efficacy of nicotine to evoke dopamine release.

The effect of ketamine to evoke striatal dopamine release is controversial. In vivo ketamine (30–50 mg/kg) administration increased or decreased dopamine release in striatum (Rao et al., 1989; Verma and Moghaddam, 1996). Furthermore, repeated ketamine treatment (15 mg/kg/day for 50 or 150 days) did not induce changes in striatal dopamine content (Lannes et al., 1991; Micheletti et al., 1992). In these in vivo studies ketamine was administered systemically to rats in which the cortical-subcortical neurocircuitry was intact. Presently, the cortical-subcortical neurocircuitry was not intact, as slices were used. Previous in vitro studies using striatal synaptosomes have shown that pharmacologically-relevant ketamine concentrations (10–100 μM) had no effect on dopamine release (Mantz et al., 1994). The present results are consistent with Mantz and colleagues as ketamine did not evoke dopamine release from striatal slices within a similar, relevant concentration range.

Electrophysiological studies have been conducted by many other groups to investigate the neural circuitry that regulates dopamine in striatum. Electrophysiological studies have shown that activation of local NMDA receptors present on GABA interneurons (Miyoshi et al., 1991; Wheeler et al., 1995) facilitates dopamine neurotransmission via a disinhibition mechanism (Christoffersen and Meltzer, 1995). This disinhibition results in a burst activity firing pattern in dopamine neurons, which is efficacious to trigger neurotransmitter release (Chergui et al., 1994). NMDA receptor blockade abolishes this disinhibitory mechanism, which results in a reduction in the frequency and amplitude of burst activity in dopamine neurons (Connelly and Shepard, 1997). As a result, there is less dopamine released from neurons with NMDA receptor blockade. The present study used a superfusion technique that is not sufficient to measure neuron firing patterns and used slices that primarily contain neurons' terminal regions. However, based on the electrophysiological studies, it is possible that ketamine superfusion decreased membrane polarization, but was not sufficient to engender detectable [^{3}H]dopamine release from preloaded slices.

In Experiment 2, the NMDA receptor antagonist was present when the effect of nicotine was determined. Subanesthetic ketamine (1–10 μM) concentrations that are sufficient for producing behavioral changes associated with schizophrenia (Collins et al., 1960; Verma and Moghaddam, 1996) augmented nicotine-evoked dopamine release. This is a novel finding and is consistent with previous research investigating the interaction of another NMDA receptor antagonist and nicotine. Ro 25-6981, a selective NMDA receptor antagonist, augmented nicotine-evoked dopamine release in nucleus accumbens (Kosowski and Liljequist, 2004), suggesting that NMDA receptor blockade induces hypersensitivity to nicotine. In the present study, the effect of ketamine on nicotine-evoked dopamine release could also possibly be mediated by NMDA receptor blockade. The ketamine concentrations (1–10 μM) that augmented nicotine-evoked dopamine release are within the range of ketamine's affinity (K_i value = 3.1 μM) for NMDA receptors in rat brain preparations (Seeman et al., 2005). As stated above, electrophysiological studies have shown that blockade of dopamine neuron disinhibition results in a decrease in the amplitude and frequency of bursts (Connelly and Shepard, 1997). The addition of the dopamine-releaser nicotine to the slices superfused with ketamine may have been sufficient to result in augmented dopamine release. Together, these mechanisms may increase sensitivity to stimulants and subsequently, enhance drug-evoked dopamine release. Thus, present and previous results indicate that NMDA receptor antagonist application induces a state of hypersensitivity to nicotine.

In Experiment 3, ketamine was administered subchronically to investigate the long-lasting neural adaptations that might be observed following the onset of schizophrenia's symptoms. The effect of ketamine administration on locomotor activity was determined to probe behavioral changes. On Day 30 of repeated ketamine treatments, ketamine (30–50 mg/kg) induced marked hyperactivity, consistent with a previous study (Leccese et al., 1986). Regarding the effect of repeated ketamine treatment on nicotine-evoked dopamine release, the threshold concentration for nicotine to evoke [^{3}H]overflow was 1 μM for rats repeatedly administered 50 mg/kg of ketamine and the threshold concentration for nicotine to evoke [^{3}H]overflow was 10 μM for rats repeatedly administered 30 mg/kg of ketamine or saline. However, statistical comparisons did not reveal a significant difference in nicotine-evoked dopamine release between groups of rats that received repeated ketamine injections and rats that received repeated saline injections.

In summary, the present study showed that ketamine, at psychotomimetic concentrations, enhanced the effect of nicotine to evoke dopamine release from striatal slices. The results of the present studies are consistent with findings indicating a role for nicotinic receptors in the pathophysiology of schizophrenia (Kosowski and Liljequist, 2004). The involvement of cholinergic systems in the pathophysiology of schizophrenia has significant implications for treatment as tobacco cigarette smoking is more prevalent in patients with schizophrenia. However, nicotine may be exacerbating the positive symptoms of schizophrenia (Aguilar et al., 2005; Ziedonis et al., 1994) that are mediated by subcortical dopamine systems. A better understanding of the neurobiological interactions of multiple neurotransmitter systems underlying schizophrenia will be beneficial for the development of novel treatments.

Acknowledgements

Portions of this research were presented at the annual meeting (2007) of the American Society of Pharmacology and Experimental Therapeutics (Washington DC). This research was partially supported by grants from the University of Missouri Alumni Association and Department of Psychological Sciences.

References

Aguilar MC, Gurpegui M, Diaz FJ, de Leon J. Nicotine dependence and symptoms in schizophrenia: naturalistic study of complex interactions. Br J Psychiatry 2005;186:215–21.

Carlsson A. Neurocircuitries and neurotransmitter interactions in schizophrenia. Int Clin Psychopharmacol 1995;10(Suppl 3):21–8.

Carlsson A, Waters N, Carlsson ML. Neurotransmitter interactions in schizophrenia—therapeutic implications. Biol. Psychiatry 1999;46(10):1388–95.

Chergui K, Suaud-Chagny MF, Gonon F. Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. Neuroscience 1994;62(3):641–5.

Christoffersen CL, Meltzer LT. Evidence for *N*-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: possible preferential role for *N*-methyl-D-aspartate receptors. Neuroscience 1995;67(2):373–81.

Collins VJ, Gorospe CA, Rovenstine EA. Intravenous nonbarbiturate, nonnarcotic analgesics: preliminary studies. 1. Cyclohexylamines. Anesth Analg 1960;39:302–6.

Connelly ST, Shepard PD. Competitive NMDA receptor antagonists differentially affect dopamine cell firing pattern. Synapse 1997;25(3):234–42.

Dopheide MM, Morgan RE, Rodvelt KR, Schachtman TR, Miller DK. Modafinil evokes striatal [3H]dopamine release and alters the subjective properties of stimulants. Eur J Pharmacol 2007;568(1–3):112–23.

Imre G, Fokkema DS, Den Boer JA, Ter Horst GJ. Dose–response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity. Brain Res Bull 2006;69(3):338–45.

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20(3):201–25.

Kalman D, Morissette SB, George TP. Co-morbidity of smoking in patients with psychiatric and substance use disorders. Am J Addict 2005;14(2):106–23.

Kornhuber J, Kornhuber ME. Presynaptic dopaminergic modulation of cortical input to the striatum. Life Sci 1986;39(8):699-174.

Kosowski AR, Liljequist S. The NR2B-selective *N*-methyl-D-aspartate receptor antagonist Ro 25-6981 [(+/−)-(R*,S*)-alpha-(4-hydroxyphenyl)-beta-methyl-4-(phenylmethyl)-1-pipe ridine propanol] potentiates the effect of nicotine on locomotor activity and dopamine release in the nucleus accumbens. J Pharmacol Exp Ther 2004;311(2):560–7.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994;51(3):199–214.

Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 2001;25(4):455–67.

Lannes B, Micheletti G, Warter JM, Kempf E, Di Scala G. Behavioural, pharmacological and biochemical effects of acute and chronic administration of ketamine in the rat. Neurosci Lett 1991;128(2):177–81.

Leccese AP, Marquis KL, Mattia A, Moreton JE. The anticonvulsant and behavioral effects of phencyclidine and ketamine following chronic treatment in rats. Behav Brain Res 1986;22(3):257–64.

Mantz J, Varlet C, Lecharny JB, Henzel D, Lenot P, Desmonts JM. Effects of volatile anesthetics, thiopental, and ketamine on spontaneous and depolarization-evoked dopamine release from striatal synaptosomes in the rat. Anesthesiology 1994;80(2):352–63.

Micheletti G, Lannes B, Haby C, Borrelli E, Kempf E, Warter JM, et al. Chronic administration of NMDA antagonists induces D2 receptor synthesis in rat striatum. Brain Res Mol Brain Res 1992;14(4):363–8.

Miyoshi R, Kito S, Doudou N, Nomoto T. Influence of age on *N*-methyl-D-aspartate antagonist binding sites in the rat brain studied by in vitro autoradiography. Synapse 1991;8(3):212–7.

Rao TS, Kim HS, Lehmann J, Martin LL, Wood PL. Differential effects of phencyclidine (PCP) and ketamine on mesocortical and mesostriatal dopamine release in vivo. Life Sci 1989;45(12):1065–72.

Rapier C, Lunt GG, Wonnacott S. Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. J Neurochem 1988;50(4):1123–30.

Rogers R, Wise RG, Painter DJ, Longe SE, Tracey I. An investigation to dissociate the analgesic and anesthetic properties of ketamine using functional magnetic resonance imaging. Anesthesiology 2004;100(2):292–301.

Sargent PB. The diversity of neuronal nicotinic acetylcholine receptors. Annu Rev Neurosci 1993;16:403–43.

Seeman P, Ko F, Tallerico T. Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol Psychiatry 2005;10(9):877–83.

Snyder SH. The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. Am J Psychiatry 1976;133(2):197–202.

Tso MM, Blatchford KL, Callado LF, McLaughlin DP, Stamford JA. Stereoselective effects of ketamine on dopamine, serotonin and noradrenaline release and uptake in rat brain slices. Neurochem Int 2004;44(1):1–7.

Verma A, Moghaddam B. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. J Neurosci 1996;16(1):373–9.

Westerink BH, Kikkert RJ. Effect of various centrally acting drugs on the efflux of dopamine metabolites from the rat brain. J Neurochem 1986;46(4):1145–52.

Wheeler D, Boutelle MG, Fillenz M. The role of *N*-methyl-D-aspartate receptors in the regulation of physiologically released dopamine. Neuroscience 1995;65(3):767–74.

White PF, Ham J, Way WL, Trevor AJ. Pharmacology of ketamine isomers in surgical patients. Anesthesiology 1980;52(3):231–9.

Ziedonis DM, Kosten TR, Glazer WM, Frances RJ. Nicotine dependence and schizophrenia. Hosp Community Psychiatry 1994;45(3):204–6.