

EFFECTS OF KETAMINE AND *N*-METHYL-D-ASPARTATE ON GLUTAMATE AND DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX: MODULATION BY A GROUP II SELECTIVE METABOTROPIC GLUTAMATE RECEPTOR AGONIST LY379268

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Abstract—Previous studies have shown that the metabotropic glutamate receptor (mGluR)2/3 agonist LY354740 attenuated glutamate release in medial prefrontal cortex (mPFC) induced by the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine. In the present study we examined the effects of the more potent mGluR2/3 selective agonist LY379268 on ketamine-evoked glutamate and dopamine (DA) release in mPFC of male rats. Subjects were implanted with a unilateral microdialysis probe in the mPFC and were tested 12–24 h after implantation. Ketamine (18 mg/kg, s.c.) evoked a significant release of glutamate and DA, although the glutamate response was slower in onset compared with DA. Pretreatment with either systemic (3 mg/kg s.c.) or local (1 μ M, in the probe) LY379268 blocked ketamine-evoked glutamate, but not DA, release. When applied directly to the mPFC via the dialysis probe, ketamine (1 mM in the probe) had no effect on glutamate release but did significantly enhance the release of DA. Application of NMDA (500 μ M in the probe), on the other hand, decreased DA while increasing glutamate release. The effect of NMDA on evoking glutamate release was blocked by systemic but not local administration of LY379268. These findings indicate that systemic ketamine increases both glutamate and DA release in mPFC and that the effect on glutamate can be blocked by stimulating mPFC group II mGluR receptors. Local ketamine, on the other hand, does not increase glutamate but does increase DA release. This suggests that ketamine acts outside of the mPFC to enhance glutamate, but within the mPFC to enhance DA release. The origin of the ketamine effect on mPFC glutamate is currently not known. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: microdialysis, metabotropic glutamate receptors, schizophrenia, antipsychotic, neurotransmitter release.

Recent preclinical discoveries have brought into focus the potential utility of group II metabotropic glutamate receptor (mGluR2/3) agonists in the treatment of schizophrenia.

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, dopamine; EDTA, ethylenediaminetetraacetic acid; HVA, homovanillic acid; mGluR, metabotropic glutamate receptor; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclo-hepten-5,10-iminemaleate; mPFC, medial prefrontal cortex; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; TTX, tetrodotoxin.

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Specifically, phencyclidine's (PCP) neurochemical and behavioral effects in rats were reversed by pretreatment with the mGluR2/3 selective agonist LY354740 (Moghaddam and Adams, 1998; Cartmell et al., 1999). PCP, a non-competitive *N*-methyl-D-aspartate (NMDA) channel blocker with known psychotomimetic properties in humans (Javitt and Zukin, 1991), increased glutamate release in medial prefrontal cortex (mPFC), activated locomotion, and impaired working memory in rats. Pretreating animals with LY354740 reversed each of these effects suggesting that mGluR2/3 agonists may have potential in treating the symptoms of PCP psychosis in humans, and possibly schizophrenia via a novel mechanism of action, i.e. by attenuating glutamatergic neurotransmission in the mPFC. Known antipsychotics such as clozapine and haloperidol, however, were without effect in blocking PCP-evoked glutamate release in the mPFC (Adams and Moghaddam, 2001). We should also note that not all evidence supports overactivity of glutamate systems in schizophrenia. In fact, the opposite case has also been suggested. For example, biochemical analysis of brains and cerebrospinal fluid from schizophrenics have revealed, in some cases, decreased concentrations of glutamate and increased cortical glutamate receptors (for review see Carfagno et al., 2000).

Although several studies have shown that systemically administered NMDA receptor antagonists increase glutamate release in the mPFC (Moghaddam et al., 1997; Moghaddam and Adams, 1998; Adams and Moghaddam, 2001), other studies provide conflicting results (Yonezawa et al., 1993; Steciuk et al., 2000). For example, activating NMDA receptors in mPFC via local application of NMDA has been shown to increase glutamate release (Del Arco and Mora, 2002). In the striatum, NMDA but not PCP increased glutamate release and the effect of NMDA on glutamate was blocked by local application of PCP (Yamamoto et al., 1999). Likewise, immobilization or foot-pinch stress increased mPFC glutamate release (Bagley and Moghaddam, 1997; Steciuk et al., 2000) and this effect was blocked by MK-801 (Steciuk et al., 2000). In this experiment systemically administered MK-801 had no effects of its own on glutamate release. Taken together, these findings show that both NMDA receptor blockade as well as NMDA receptor activation may enhance glutamate release, while NMDA antagonists diminish stress-evoked release. Clearly more experimentation is necessary to fully appreciate the role played by the NMDA receptor in regulating glutamate release. In addition, it would be important

to know if mGluR2/3 agonists block NMDA-evoked glutamate release or whether the inhibition is selective to glutamate release induced by NMDA receptor blockade.

Although LY354740 was reported to reduce PCP-evoked glutamate release, it had no effect on basal or PCP-evoked dopamine (DA) release in the mPFC (Moghaddam and Adams, 1998). This finding is in stark contrast to reports using a different group II agonist LY379268 (Cartmell et al., 2000a, 2001). In these two studies, LY379268, a more potent mGluR2/3 agonist than LY354740 (Monn et al., 1999; Schoepp et al., 1999), was shown to increase DA release in the mPFC. In addition to increases in extracellular DA, LY379268 also elicited increases in the DA metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid (HVA) in a manner which paralleled the changes in these metabolites produced by atypical antipsychotics clozapine and risperidone (Cartmell et al., 2000b). It is not clear why LY379268 increased DA release while LY354740 was ineffective; however, such discrepancies are important to resolve.

The present experiments focus on ketamine, which, like PCP, induces schizophrenia-like symptoms in humans, to confirm and extend the role of group II mGluR activation on mPFC glutamate and DA release. We first confirmed that this non-competitive NMDA receptor antagonist increases mPFC glutamate and DA release and then tested the ability of LY379268 to block these effects. Secondly, because of the conflicting reports described above, we also tested whether LY379268 alone could evoke DA release. Thirdly, because mGluR2/3 receptors have been localized in prefrontal cortex on presynaptic processes (Petralia et al., 1996; Marek et al., 2000, 2001), we examined whether selective activation of these receptors by local LY379268 delivery to the mPFC could mimic the effects of systemic LY379268. Lastly, since NMDA was shown recently to increase mPFC glutamate release (Del Arco and Mora, 2002) we examined the effect of NMDA delivered to the mPFC on glutamate and DA release and whether these effects could be prevented by systemic and locally applied LY379268. In the same experiment we also tested whether local administration of ketamine to the mPFC via the microdialysis probe could increase glutamate release.

EXPERIMENTAL PROCEDURES

Animals and surgery

Male Sprague–Dawley rats (300–350 g) were purchased from Harlan (San Diego, CA, USA) and were maintained under a 12-h light/dark cycle. All microdialysis experiments occurred during the light phase of the light/dark cycle. All procedures were performed in accordance with *The Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee. The total number of animals as well as their suffering was minimized whenever possible. Before and after surgeries, rats were allowed free access to standard rat chow and water. Temperature and relative humidity were maintained at 22–24 °C and 50–55%, respectively.

Under 3% isoflurane (in 1% O₂) anesthesia, rats were implanted with a unilateral microdialysis probe (CMA/7 with a 2-mm dialysis tip for glutamate-release experiments and CMA/11 with a

3-mm dialysis tip for experiments in which DA was measured) in the mPFC (anteroposterior, +3.2; mediolateral, –0.8; and dorsoventral, –5.0) (Paxinos and Watson, 1998). The microdialysis probe was slowly lowered into position and was then fixed to the skull by means of three anchoring screws and application of dental acrylic. Immediately following surgery rats were placed in their testing arena and allowed to recover for a minimum of 14 h.

In vivo microdialysis

For all microdialysis experiments, rats were tethered to a CMA awake animal system by means of a plastic collar (CMA Microdialysis, Acton, MA, USA). All fluid connections were made using FEP tubing (internal volume of 1.2 µl/100 mm, CMA). Following the appropriate post-surgery recovery period (approximately 14 h=approximately 0700 h) flow through the dialysis probe with artificial cerebrospinal fluid (aCSF; 145-mM NaCl; 2.7-mM KCl; 1.0-mM MgCl₂ and 1.2-mM CaCl₂; pH 7.4) was increased and maintained at 3.0 µl/min. A 2-h stabilization period was then allowed prior to sample collection. Fractions were then collected at 20-min intervals (60 µl) into 250-µl glass vials via a BAS honeycomb fraction collector maintained at 4 °C (BAS HoneyComb; BAS, West Lafayette, IA, USA). Samples were subsequently analyzed for either DA or glutamate by high-performance liquid chromatography (HPLC) (see details below) within a 24-h period.

Administration of drugs

Ketamine (in the form of Ketaset, 100 mg/ml) was obtained from Fort Dodge Animal Health (Fort Dodge, IA, USA). NMDA and tetrodotoxin (TTX) were purchased from Sigma (St. Louis, MO, USA). LY379268 was provided by the Chemistry Department (Merck Research Laboratories, San Diego, CA, USA). For systemic administration drugs were prepared in saline (pH adjusted to 7.0) and delivered s.c. in a volume of 1 ml/kg. For central administration, drugs were prepared in aCSF (pH adjusted to 7.4) and delivered to the mPFC via the microdialysis probe by manually switching the inlet tubing from a syringe containing aCSF to one containing the appropriate concentration of drug. The actual concentration of drug reaching the surrounding tissue is estimated to be approximately 10% of the concentration perfused.

Verification of probe placement

At the completion of each experiment, rats were killed by CO₂ inhalation and their probes perfused with approximately 200 µl of Cresyl Violet dye. Brains were then removed and a single coronal cut was made at the level where the probe penetrated the cortex. The resulting coronal section was placed onto a glass slide and scanned into a personal computer. Only those animals demonstrating dye within the mPFC were included in the results.

Glutamate analysis

Glutamate content was determined by HPLC coupled to fluorescence detection. Individual samples were placed in a Shimadzu auto-sampler (model SIL-10ADVP) maintained at 10 °C. Each sample was subject to pre-column derivitization. Briefly, a 60-µl aliquot (20-min dialysis sample) was reacted with 30 µl of an OPA/mercaptoethanol solution (27 mg OPA, 1 ml methanol, 10 µl β-mercaptoethanol and 9 ml 0.1-M sodium tetraborate, pH 9.4). The reaction period was 2 min. Separation occurred on a C18 reverse-phase column (Hypersil Elite, 150×4.6 mm, 5-µm particles) and detection was performed using a Shimadzu SCL-10AVP controller unit with a fluorescence flow cell. A gradient program of two mobile phases was used. Mobile phase A consisted of a 0.1-M sodium phosphate buffer (pH 6.4) with 50 mg/l EDTA and 10% methanol (v/v); mobile phase B was a 90:10 (v/v) methanol/water mixture. The rate of mobile phase flow through the system was 1

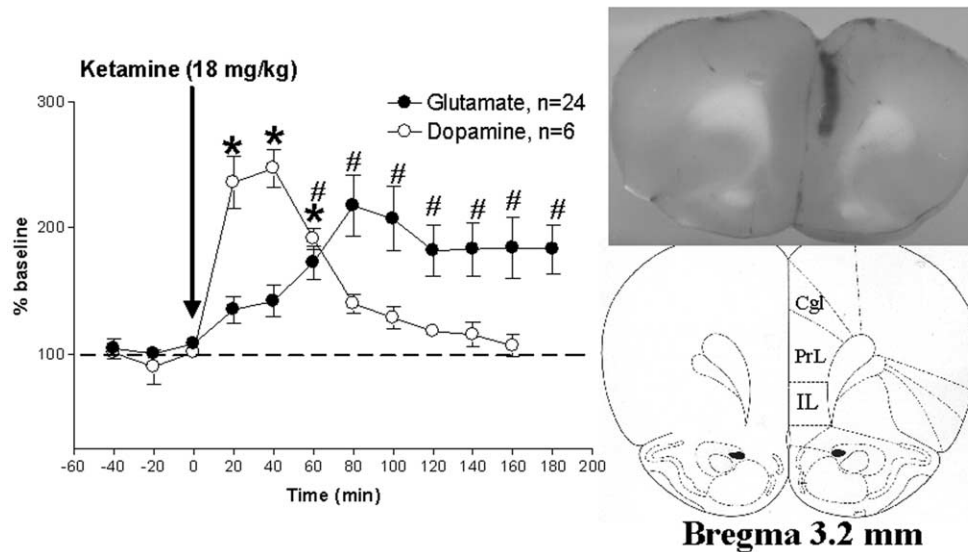


Fig. 1. Left: Effects of ketamine on glutamate and DA release in the mPFC of the awake rat. Data (mean±S.E.M.) are reported as percentage of baseline. Under the current testing conditions basal glutamate was calculated to be 50 ± 24 pg/ μ l dialysate and DA was calculated to be 0.02 ± 0.01 pg/ μ l dialysate. */# $P < 0.05$: Significantly greater dialysate levels compared with time “0” for glutamate (#) and for DA (*), as revealed by ANOVA followed by Dunnett’s post hoc comparisons. Right: Representative coronal section showing the spread of dye from the active portion of the dialysis membrane to the surrounding tissue in an individual subject with an appropriately placed microdialysis probe (top). Drawing of the rat brain (taken from Paxinos and Watson) (Paxinos and Watson, 1998) depicting the regions of the mPFC assayed by the microdialysis probe (bottom). Cgl, cingulate, PrL, prelimbic, IL, infralimbic cortex.

ml/min. Glutamate eluted with a retention time of approximately 3.5 min under these conditions. Homoserine (1 ng/ μ l) was added to each sample and served as an internal standard.

DA analysis

DA content was determined by HPLC coupled to electrochemical detection. Samples were loaded into an ESA autosampler (model 465). Separation occurred on a C18 reverse-phase column (Phenomenex Luna, 150×2.0 mm, 3- μ m particles) and was detected by an ESA Coulochem controller unit using a model 5041 microdialysis analytical cell fitted with a glassy carbon electrode set to oxidize at +250 mV. The mobile phase used was a phosphate buffer purchased from ESA (MD-TM). The rate of mobile-phase flow through the system was 0.2 ml/min. DA eluted with a retention time of approximately 4.5 min under these isocratic conditions.

Data analysis

Data are reported as percentages of baseline, not corrected for *in vitro* recovery. All time-course data obtained on glutamate and DA release were analyzed with repeated measure analysis of variance (ANOVA) using either a one-factorial design for detection of change in release across time within a single treatment or a two-factorial design for comparison of two treatment groups (with treatment as the between factor and time as the within factor). Post hoc analyses were made using either Tukey’s multiple comparisons test or Dunnett’s method.

RESULTS

Effects of ketamine on glutamate and DA release

For glutamate-release studies, 24 control animals received an 18 mg/kg s.c. ketamine challenge injection (Fig. 1). These 24 animals are a compilation of three separate groups, each serving as a control group for one of three

treatment groups; 1) systemic LY379268 (3 mg/kg), 2) local LY379268 (1 μ M) and 3) TTX (1 μ M). Here we report on the pooled data (24 subjects). In the section to follow individual control groups are analyzed with respect to their treatment group. The ANOVA conducted on the pooled data revealed a significant increase in glutamate release following ketamine challenge [$F(9,207)=8.3$, $P < 0.001$]. Dunnett’s post hoc comparisons revealed a delayed yet long-lasting increase in glutamate release reaching statistical significance by 60 min and remaining elevated throughout the 180-min sampling period ($P < 0.05$).

Ketamine (18 mg/kg) also produced a significant increase in dialysate DA concentrations [Fig. 1, $F(9,45)=25.0$, $P < 0.001$]. Dunnett’s post hoc comparisons revealed an immediate increase in DA reaching statistical significance by 20 min and returning to baseline values by 80 min ($P < 0.05$).

Effects of LY379268 and TTX on ketamine-evoked glutamate release

For each individual control ketamine-challenged subgroup, ketamine led to a significant increase in dialysate glutamate (Fig. 2). Separate one-factor repeated measures ANOVA revealed significant increases in glutamate across time [$F(9,54)=2.49$, $P < 0.05$ (Fig. 2A); $F(9,63)=2.13$, $P < 0.05$ (Fig. 2B); $F(9,72)=3.68$, $P < 0.001$ (Fig. 2C)]. Dunnett’s post hoc comparisons revealed a long-lasting increase in glutamate release reaching significance between 80 and 180 min ($P < 0.05$) in each group.

Systemic administration of LY379268 (3 mg/kg, s.c.) 30 min prior to ketamine blocked the evoked glutamate release (Fig. 2A). A two-factor ANOVA revealed significant

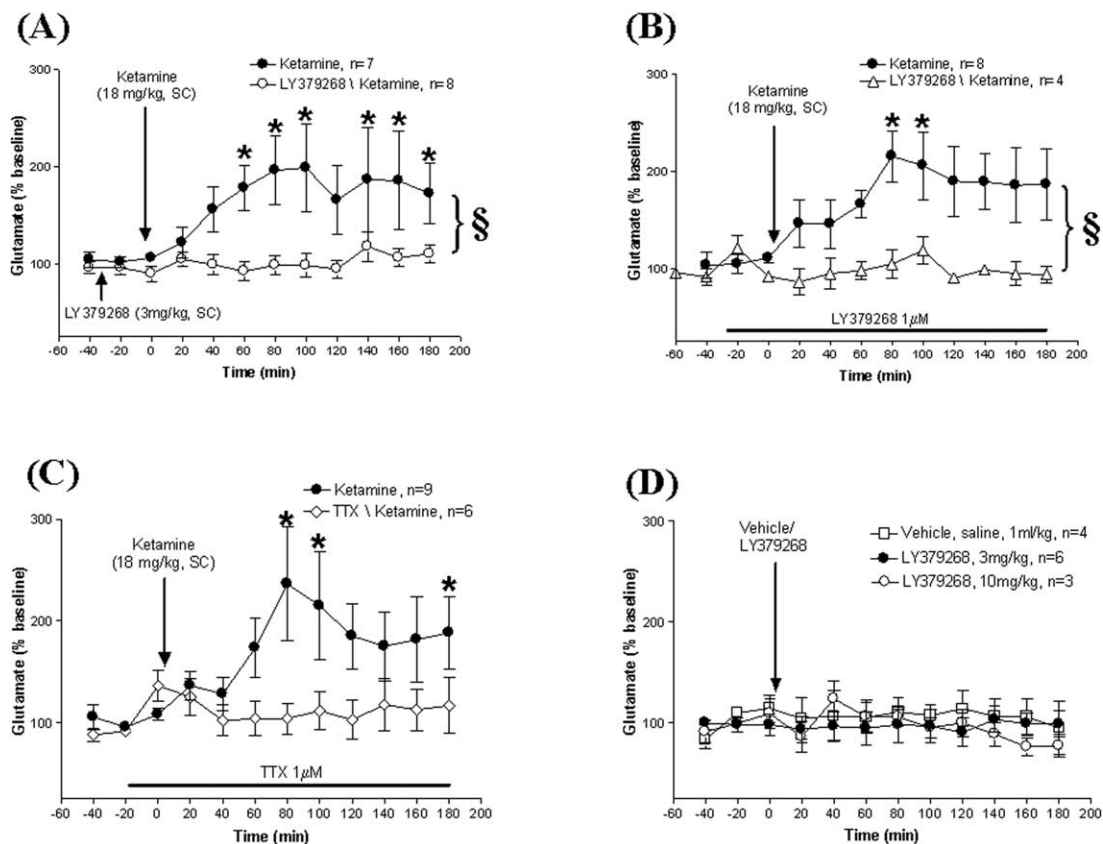


Fig. 2. Effects of the group II agonist LY379268 and TTX on ketamine-evoked glutamate release in the mPFC of the awake rat. Systemic (A) and local (B) administration of LY379268 prevented glutamate release elicited by ketamine. The glutamate release following ketamine appears to be of neuronal origin because no increase was observed in animals receiving TTX+ketamine (C). Neither a vehicle injection nor LY379268 had effects of its own on glutamate release (D). Data (mean±S.E.M.) are reported as percentage of baseline. * $P < 0.05$: Significantly greater glutamate levels in the saline–ketamine group compared with baseline (time “0”), as revealed by one-factor ANOVA followed by Dunnett’s post hoc comparisons. $^{\$}P < 0.05$: Significant effect of pretreatment as revealed by two-factor ANOVA followed by Tukey’s post hoc comparisons.

effects of treatment [$F(1,13)=4.9$, $P < 0.05$] and of time [$F(9,117)=3.2$, $P < 0.01$] and a significant treatment \times time interaction [$F(9,117)=2.2$, $P < 0.05$, Fig. 2A]. Post hoc comparisons revealed significant increases in the ketamine-only group from 60 min to 180 min ($P < 0.05$). No changes in glutamate release were observed at any of the time points in the ketamine plus LY379268-treated group.

Ketamine-evoked glutamate release was also blocked when LY379268 (1 μM) was administered directly to the mPFC via the dialysis probe (Fig. 2B). The ANOVA revealed significant effects of treatment [$F(1,10)=6.7$, $P < 0.05$] but not of time nor was there a significant treatment \times time interaction. A one-way ANOVA on the LY379268-treated group revealed no increase in glutamate release following ketamine injection.

The sodium channel blocker TTX (1 μM) perfused through the probe prevented ketamine-evoked glutamate release (Fig. 2C). Although no overall effect of treatment on glutamate release was revealed by the ANOVA [$F(1,13)=2.4$, $P = 0.147$] a significant treatment \times time interaction was observed [$F(9,117)=2.8$, $P < 0.01$]. Similar to that reported above using a one-factorial ANOVA, post hoc

comparisons on the two-factorial ANOVA revealed significant increases in glutamate in the ketamine control group from 80 min to 180 min. No increases were seen in the TTX-treated group.

LY379268 (3 and 10 mg/kg, s.c.) had no effect of its own on glutamate release (Fig. 2D).

Effects of LY379268 on ketamine-evoked DA release

Ketamine-evoked DA release in the mPFC was not blocked by pretreatment with LY379268 (3 mg/kg) (Fig. 3A). A two-factor ANOVA revealed no significant effects of treatment [$F(1,9)=0.55$, $P = 0.48$] but did show significant effects of time [$F(7,63)=37.3$, $P < 0.001$], although there was no significant treatment \times time interaction [$F(7,63)=1.46$, $P = 0.20$]. Post hoc comparisons showed DA increased significantly in both saline- and LY379268-treated groups from 20 min to 100 min and returned to baseline levels by 120 min after injection ($P < 0.05$). LY379268 (3 and 10 mg/kg) had no effect of its own on DA levels (Fig. 3B). The DA metabolite HVA did, however, slowly increase in the high-dose group (10 mg/kg) reaching significance at time=100 min after injection ($P < 0.05$, data not shown).

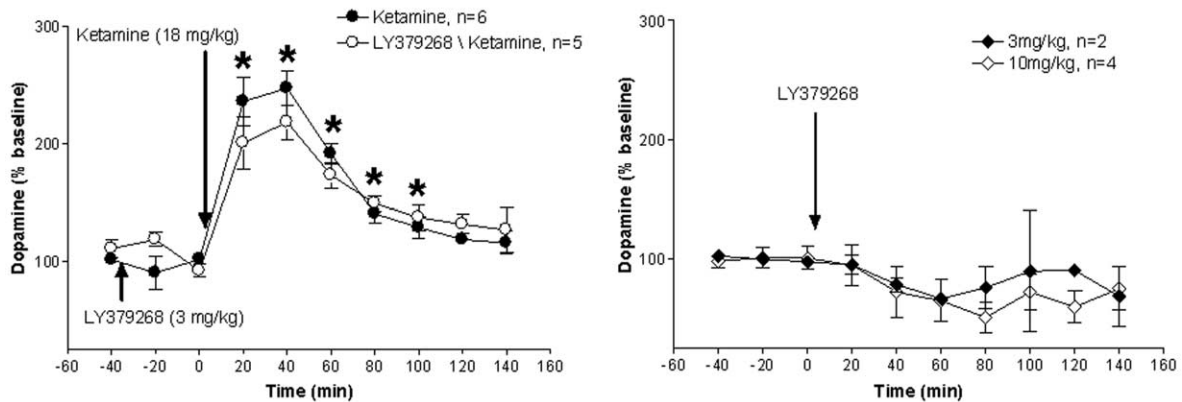


Fig. 3. Ketamine-evoked DA release in the mPFC was not blocked by pretreatment with LY379268 (3 mg/kg). LY379268 had no effect of its own on dopamine release. Data (mean \pm S.E.M.) are reported as percentage of baseline (time "0"). * $P < 0.05$: Significantly greater DA levels above baseline in both ketamine and LY379268+ketamine groups as revealed by two-factor ANOVA post hoc Tukey comparisons for factor of time.

Effects of local ketamine and local NMDA on glutamate and DA release

Unlike our systemic results, direct application of ketamine (1 mM) to the mPFC via the microdialysis probe failed to increase glutamate levels (Fig. 4 A). Direct application of NMDA (500 μ M), on the other hand, increased glutamate efflux [$F(8,40)=3.8$, $P < 0.01$]. This effect was immediate and short lasting, reaching significance only during the first 20-min sample of the 40-min perfusion period (Dunnett's post hoc, $P < 0.05$).

In contrast to the effect on glutamate, local perfusion of ketamine increased DA release while NMDA reduced DA levels (Fig. 4B). Ketamine perfusion (1 mM in the probe) led to an immediate increase in DA that lasted throughout the 40-min perfusion period [$F(7,21)=24.7$, $P < 0.001$]. Dunnett's post hoc comparisons revealed DA was elevated above baseline from 20 min to 60 min ($P < 0.05$). NMDA perfusion (500 μ M in the probe) suppressed DA release [$F(7,14)=6.3$, $P < 0.01$]. Dunnett's post hoc comparisons revealed DA was significantly lower than baseline during the 40-min sample-collection time ($P < 0.05$).

Effects of LY379268 on NMDA-evoked glutamate release

Systemic (3 mg/kg), but not local (1 μ M), administration of LY379268 prevented NMDA-evoked glutamate release (Fig. 5). A two-factor ANOVA on these data revealed significant effects of treatment [$F(2,11)=12.1$, $P < 0.01$] and of time [$F(7,77)=5.6$, $P < 0.001$] as well as a significant treatment \times time interaction [$F(14,77)=3.4$, $P < 0.001$]. Tukey's post hoc analysis on treatment revealed an effect of systemic LY379268 but not of local LY379268 against NMDA-evoked glutamate release ($P < 0.05$). Tukey's post hoc analysis showed that NMDA alone and local LY379268 plus NMDA evoked significant increases in dialysate glutamate during sample time 20–40 min and during sample time 20 min, respectively.

DISCUSSION

We demonstrate that the mGluR2/3-selective agonist LY379268 blocks ketamine-evoked as well as NMDA-evoked glutamate release in the mPFC of the awake rat.

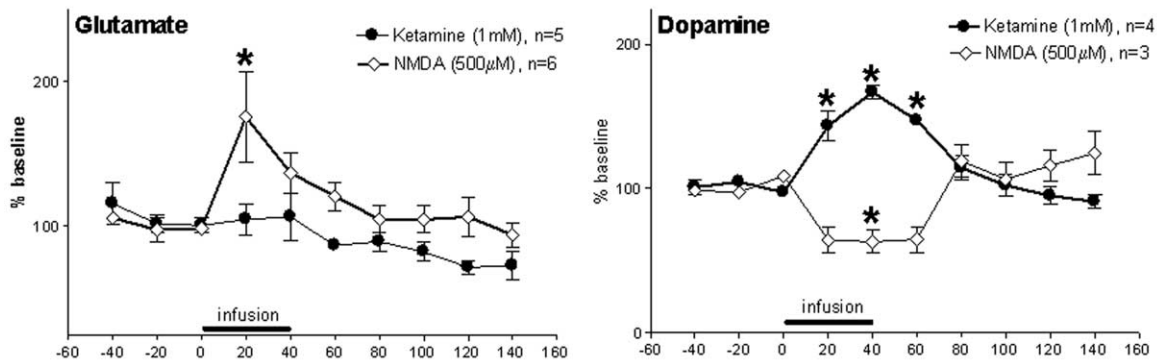


Fig. 4. Effects of local ketamine and local NMDA on glutamate and DA release in the mPFC. Local ketamine had no effect on glutamate release while local NMDA led to an immediate increase in release (left graph). As with systemic administration, local ketamine increased DA release while local NMDA decreased DA release (right graph). Bar at bottom of graph indicates time of drug delivery via the microdialysis probe. Data (mean \pm S.E.M.) are reported as percentage of baseline. * $P < 0.05$: Significantly different from time "0" as revealed by post hoc Dunnett's method.

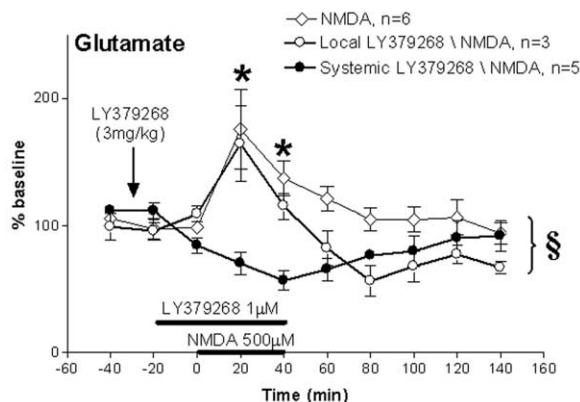


Fig. 5. Effects of LY379268 on the increase in dialysate glutamate by NMDA. Systemic (3 mg/kg), but not local (1 μ M), administration of LY379268 prevented NMDA-evoked glutamate release. Data (mean \pm S.E.M.) are reported as percentage of baseline. $\$P < 0.05$: Significant effect of treatment (NMDA versus systemic LY379268 + NMDA) as revealed by two-factor ANOVA followed by Tukey's post hoc comparisons. $*P < 0.05$: Significant increase above time "0" in the NMDA and local LY379268 + NMDA groups as revealed by Tukey's post hoc analysis on time. A two-factor ANOVA on these data revealed significant effects of treatment [$F(2,11) = 12.1, P < 0.01$] and of time [$F(7,77) = 5.6, P < 0.001$] and a significant treatment \times time interaction [$F(14,77) = 3.4, P < 0.001$]. Tukey's post hoc analysis on treatment revealed an effect of systemic LY379268 but not of local LY379268 against NMDA-evoked glutamate release ($P < 0.05$). NMDA and NMDA plus local LY379268 evoked significant increases in dialysate glutamate during sample time 20–40 min and during sample time 20 min, respectively.

Systemic administration of ketamine and local infusion of NMDA increased glutamate release; pretreating animals with a systemic injection of LY379268 blocked both effects. Locally applied LY379268 into the mPFC also prevented the increase in glutamate release evoked by ketamine (but not by NMDA). These results suggest that stimulation of mGluR2/3 receptors located within the mPFC is sufficient to prevent ketamine-evoked glutamate release. Ketamine also increased DA release but this effect was not altered by LY379268. These findings are in line with one report showing that LY354740 blocked PCP-evoked glutamate, but not DA, release in the mPFC (Moghaddam and Adams, 1998). We also show that when delivered directly to the mPFC via the microdialysis probe ketamine had no effect on glutamate release (but did enhance DA release). This suggests that systemically administered ketamine increases glutamate release by acting outside the mPFC. Direct application of NMDA, on the other hand, increased glutamate while decreasing DA release. The effect on NMDA-evoked glutamate release was prevented by systemic but not local administration of LY379268. These findings indicate that at the level of the mPFC NMDA receptor activation, but not NMDA receptor blockade, increases glutamate release.

In the current study LY379268 had no effects of its own on dialysate levels of either glutamate or DA. We did, however, see an increase in the DA metabolite HVA using the 10 mg/kg dose of LY379268 (data not shown). The lack of an effect of an mGluR2/3 agonist on DA per se is consistent with one report (Moghaddam and Adams, 1998)

but not with others (Cartmell et al., 2000a,b, 2001). The reason for this discrepancy is not clear. It is conceivable that the use of nomifensine (Cartmell et al., 2001) could have influenced LY379268's effect on DA release; however they also report an increase without the use of nomifensine (albeit a much-delayed increase) (Cartmell et al., 2000b). DA release in the nucleus accumbens was also measured following treatment with another group II selective agonist DCG-IV (Hu et al., 1999). Here local administration through the dialysis probe of 1 mM DCG-IV significantly reduced DA release.

Effects of LY379268 on ketamine-evoked glutamate and DA release

Studies using LY354740, a selective mGluR2/3 agonist (Schoepp et al., 1997; Monn et al., 1999) revealed a high density of specific binding throughout the cerebral cortex (Schaffhauser et al., 1998). More recently, autoradiography using [3 H]LY354740 has demonstrated substantial binding within the mPFC (Marek et al., 2000, 2001). We used this information along with results from studies showing an increase in 2-deoxyglucose uptake in the mPFC of the rat following a systemic ketamine challenge (Duncan et al., 1999) to guide our decision on microdialysis-probe placement. The expression of [3 H]LY354740 binding overlapped closely with the area that showed an increase in 2-deoxyglucose uptake. Within the same region we observed an increase in glutamate release following systemic ketamine that is blocked by both systemic as well as locally applied LY379268. These findings, together with our results showing TTX sensitivity, are the first direct *in vivo* demonstration that mGluR2/3 receptors located within the mPFC modulate glutamate release, presumably by a pre-synaptic effect (see Fig. 6).

In addition to blocking ketamine-evoked glutamate release, mGluR2/3 agonists have been shown to block 5-HT-evoked glutamate release. Ex vivo slice recordings from the prefrontal cortex revealed an increase in excitatory currents following 5-HT administration that was enhanced by the mGluR2/3 antagonist LY341495 and inhibited by LY354740 (Marek et al., 2000, 2001). LY354740 was also shown to inhibit the excitatory effects induced by the partial 5-HT_{2A} agonist DOI. Because ketamine has been shown to increase serotonergic activity in the prefrontal cortex (Lindfors et al., 1997; Martin et al., 1998), it is conceivable that the glutamate response observed in this study is mediated in part through 5-HT_{2A} receptor activation. This may be unlikely since systemic but not local ketamine increased prefrontal glutamate release, even though both conditions increased prefrontal 5-HT levels (unpublished observations). Although it remains unclear whether ketamine and 5-HT share a common mechanism with respect to evoking glutamate release, both ketamine (Krystal et al., 1994; Malhotra et al., 1996) and 5-HT receptor ligands (Aghajanian and Marek, 2000) share psychotomimetic properties in humans. It is presently unknown whether, within a clinical setting, mGluR2/3 agonists prevent the psychotomimetic effects of ketamine and/or 5-HT agonists.

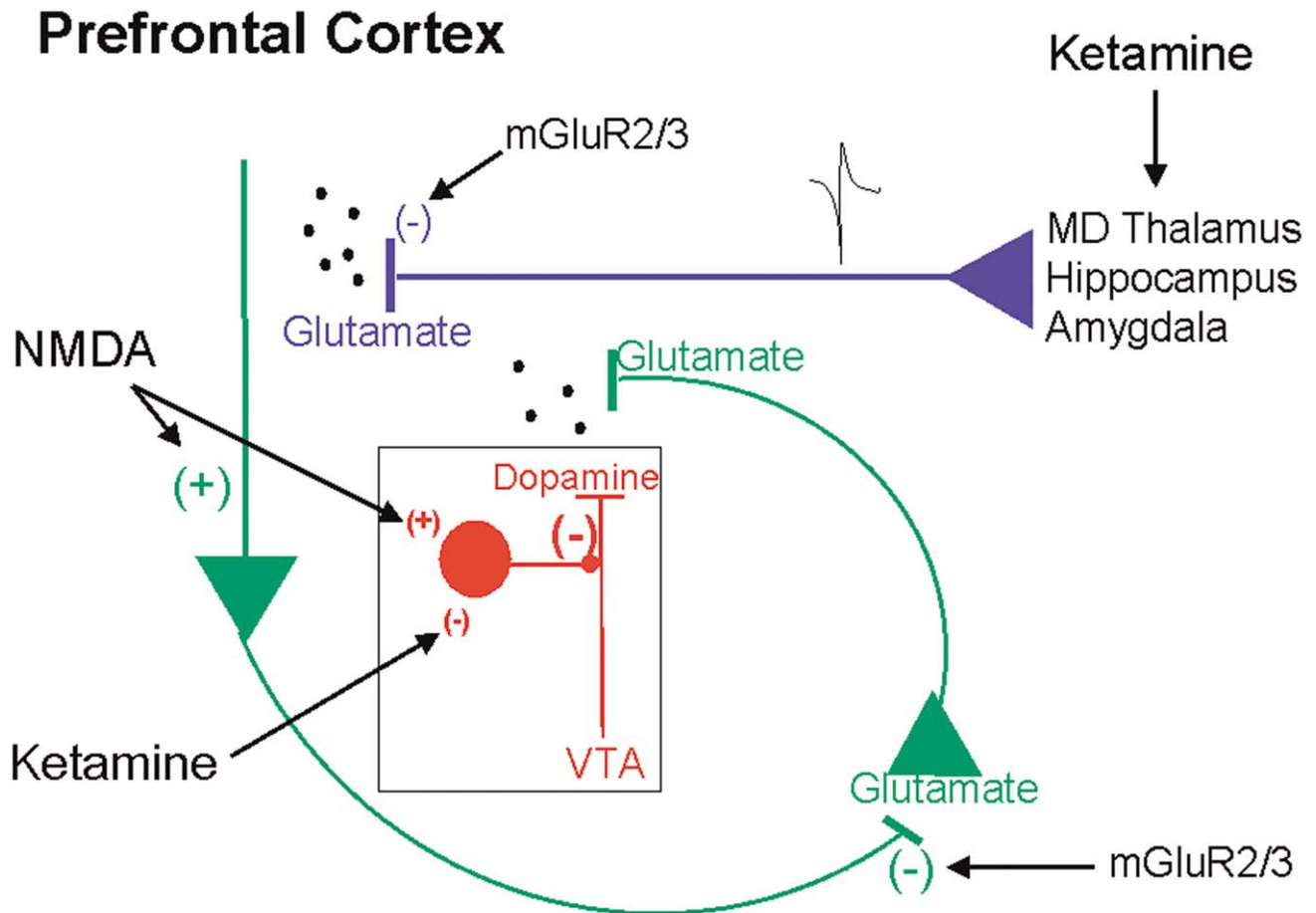


Fig. 6. Schematic representation of possible neural circuits involved in mGluR2/3 regulation of ketamine and NMDA evoked glutamate and DA release in the mPFC. Blue: Ketamine may evoke glutamate release by activating afferent input. This effect can be blocked by local mGluR2/3 receptors located on afferent terminals. Green: NMDA may evoke glutamate by activating a positive feedback loop outside of the mPFC. This effect can be blocked by mGluR2/3 receptors located somewhere along that loop. Red: Ketamine may increase while NMDA decrease DA release through inhibition and activation (respectively) of local NMDA receptors on intrinsic inhibitory neurons (boxed area). The effects of ketamine on DA release are not blocked by mGluR2/3 activation.

From a preclinical perspective, LY354740 failed to alter the discriminative stimulus properties of PCP and failed to prevent PCP's disruption of prepulse inhibition of the acoustic startle response in rat (Schreiber et al., 2000). Similar studies with more potent agonists such as LY379268 have not yet been reported.

The fact that local ketamine failed to increase glutamate release suggests that systemically administered ketamine acts in brain regions outside the prefrontal cortex to drive afferent input (see Fig. 6). In contrast, both systemic as well as local administration of ketamine increases DA release in the mPFC. A prefrontal site of action for ketamine-evoked DA release suggested by these data is also supported by previous observations. First, locally applied PCP and AP-5 increased DA release in prefrontal cortex (Yonezawa et al., 1993; Feenstra et al., 2002). Second, application of TTX to DA cell bodies within the ventral tegmental area failed to prevent systemic MK-801-evoked DA release in the prefrontal cortex. Together, these data strongly suggest

that NMDA antagonists exert their effects on DA release by blocking NMDA receptors located within the prefrontal cortex, perhaps on intrinsic inhibitory GABAergic neurons (Fig. 6). This is supported by neuroanatomical data demonstrating the expression of NMDA receptors on cortical interneurons (Conti et al., 1997). If correct, NMDA antagonists may inhibit GABAergic drive onto DA terminals thus disinhibiting DA release. Indeed local application of PCP decreased prefrontal GABA release (Yonezawa et al., 1998) while local application of NMDA increased GABA release (Del Arco and Mora, 2002). In the former experiment DA release was increased during PCP perfusion and this effect was attenuated by co-perfusing the GABA_A agonist muscimol along with PCP (Yonezawa et al., 1998). It does not appear that prefrontal glutamate neurons are under similar regulation. In fact, *in vivo* unit recordings of the mPFC have shown that local application of PCP depresses neuronal activity in the majority of deep-layer cells (Gratton et al., 1987).

Effects of LY379268 on NMDA-evoked glutamate release

When applied directly to the prefrontal cortex via the dialysis probe, NMDA increased glutamate release. These results are consistent with previous work showing increased extracellular levels of glutamate in striatal regions following administration of glutamate agonists (Morari et al., 1993; Liu and Moghaddam, 1995; Patel and Croucher, 1996; Segovia et al., 1997). Our results are also in direct agreement with a recent publication showing that at the level of the mPFC, NMDA increased glutamate release (Del Arco and Mora, 2002), although in this latter study, a prolonged increase in glutamate was shown, while we observed only a very brief increase.

It is not entirely clear which pathways are involved in NMDA-evoked glutamate release but we speculate that the response may involve NMDA receptors located on efferent projections that eventually feed back to the prefrontal cortex (see Fig. 6). For example, stimulation of NMDA receptors could drive pyramidal output neurons that directly synapse onto medial dorsal thalamus pyramidal cells that project back to the mPFC (Kuroda et al., 1998). In this case, mGluR2/3 receptor stimulation at a distant synapse (perhaps at the level of the thalamus) may block glutamate release thus interrupting the loop. This hypothesis is supported by the fact that systemic, but not local, administration of LY379268 blocked NMDA-evoked glutamate release. It is likely that the concentration of LY379268 perfused locally in these experiments (1 μ M) was sufficient to fully stimulate mGluR2/3 receptors since this concentration was able to block ketamine-evoked glutamate release. Interestingly, direct application of glutamate to the dendrites of pyramidal neurons in the prefrontal cortex has been shown to induce burst firing and this effect was mediated, at least in part, through NMDA receptors (Zhang and Shi, 1999). Del Arco and Mora (2002), on the other hand, showed that NMDA-evoked glutamate release in mPFC was insensitive to TTX and thus suggested that stimulation of NMDA receptors located presynaptically or on astrocytes led to the increase in glutamate release. Further experiments are needed to fully resolve the mechanism behind NMDA-activated glutamate release and the role of mGluR2/3 receptors in modulating this response. In particular, it would be important to know which afferents possess mGlu2/3 receptors within the prefrontal cortex and whether local connections between prefrontal neurons express these receptors.

Temporal pattern of glutamate versus DA release evoked by ketamine

While conducting the current study one aspect became clear: although ketamine increases glutamate release it does so out of phase with DA release and out of phase with overt behavioral responses associated with this drug (see Fig. 1). In the majority of subjects tested under the ketamine alone condition, glutamate was observed to increase and reach a maximal response by 80 min, remaining elevated throughout the remainder of the 180-min sam-

pling period even though DA had returned to baseline and animals were back to rest following a brief period of locomotor activation. The delayed and sustained increase in glutamate is consistent with one report using PCP (Moghaddam and Adams, 1998). We speculate that our data showing an increase in glutamate release by local activation of NMDA receptors may help to explain the delayed increase following ketamine administration. It is likely that, when given systemically, ketamine reaching the prefrontal cortex blocks NMDA receptors that may otherwise facilitate a glutamate response. Alternatively, ketamine reaching the prefrontal cortex may actually decrease glutamate release while ketamine at some distant site increases glutamate with a net increase in this area. A decrease in glutamate release, however, is not supported by our current experiments. In either case, focal injections of ketamine to regions that project to the prefrontal cortex may show a more rapid increase in mPFC glutamate. Microinfusion of PCP and MK-801 into the ventral hippocampus, for example, produced a transient increase in prefrontal cortical activity (Suzuki et al., 2001). A dual-probe microdialysis experiment could help resolve this issue.

Summary

In summary, we show that systemic administration of the non-competitive NMDA receptor antagonist ketamine increased both glutamate and DA concentrations in dialysate collected from the mPFC of male rats. Pretreatment with either systemic (3 mg/kg s.c.) or local (1 μ M) LY379268 blocked the effect of ketamine on glutamate, but not DA, release. Direct application of ketamine to the mPFC via the dialysis probe (1 mM in the probe) failed to increase glutamate release but did significantly enhance the release of DA. Direct application of NMDA (500 μ M in the probe), on the other hand, increased glutamate and decreased DA release. The effect of NMDA on glutamate release was blocked by systemic LY379268 (local administration was without effect). Together these findings suggest that ketamine acts outside of the mPFC to enhance glutamate release, but within the mPFC to enhance DA release. At the local level, NMDA receptor stimulation increased glutamate release. Further investigation is needed to determine the specific brain region that mediates the effect of ketamine on mPFC glutamate.

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