Naunyn-Schmiedeberg's Arch Pharmacol (2006) 374:177–193
DOI 10.1007/s00210-006-0115-9

ORIGINAL ARTICLE

Role of the simultaneous enhancement of NMDA and dopamine D_1 receptor-mediated neurotransmission in the effects of clozapine on phencyclidine-induced acute increases in glutamate levels in the rat medial prefrontal cortex

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Received: 26 June 2006 / Accepted: 22 October 2006 / Published online: 14 November 2006

Abstract Clozapine (CLZ) can improve both the positive and negative symptoms of treatment-resistant schizophrenia (TRS), which does not respond to typical antipsychotics. This suggests that elucidation of the pharmacological mechanism for CLZ could lead to further clarification of the pathophysiology of TRS. This study examined the effects of CLZ on phencyclidine (PCP)-induced hyperlocomotion and on the acute increases in glutamate levels that occur in the medial prefrontal cortex (mPFC) in order to test the hypothesis that CLZ effect is associated with the simultaneous enhancement of N-methyl-D-aspartate (NMDA) and dopamine D_1 receptor-mediated neurotransmission. CLZ effect on PCP-induced hyperlocomotion and increases in glutamate levels were examined by using behavioral rating scores and in vivo microdialysis, respectively. CLZ and haloperidol (HAL) dose-relatedly attenuated PCP-induced hyperlocomotion, and concentration-relatedly blocked PCP-induced acute increases in glutamate levels in the mPFC, with the decrease in saline-induced locomotor activity induced by CLZ being much weaker than that induced by HAL. CLZ also blocked, in a dose-related manner, acute increases in glutamate levels in the mPFC that were induced by local perfusion with a competitive NMDA receptor antagonist, CPP, in this region. Although an enhanced blocking effect of the sub-threshold concentration of NMDA perfusion on PCP-induced acute increases in glutamate levels in the mPFC was noted after co-perfusion with a dopamine D_1 receptor agonist, SKF-38393, perfusion with SKF-38393 did not reverse the CLZ blocking of PCP-induced increases in glutamate levels. Therefore, CLZ may block PCP-induced acute increases in glutamate levels in the mPFC by an enhancement of the NMDA receptor-mediated neurotransmission that is not accelerated by an enhanced dopaminergic transmission via dopamine D_1 receptors. This blocking effect may partially explain the CLZ-induced attenuation of PCP-induced hyperlocomotion.

Keywords Clozapine · Phencyclidine · NMDA · Dopamine D_1 · Treatment-resistant schizophrenia

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Introduction

In cases of treatment-resistant schizophrenia (TRS) that have been diagnosed by the strict criteria developed by Kane et al. (1988), clozapine (CLZ) can improve both positive and negative symptoms along with cognitive dysfunction. In TRS, symptoms and dysfunctions are resistant to typical antipsychotics such as chlorpromazine (Kane et al. 1988) and haloperidol (HAL) (Volavka et al. 2002; Bitter et al. 2004). Therefore, elucidation of the pharmacological mechanisms of the action of CLZ might lead to clarification of the pathophysiology of TRS and help in discovering pharmacological mechanisms for novel antipsychotics that would be effective in TRS treatment.

CLZ enhances N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission in vitro (Arvanov et al. 1997; Ninan et al. 2003). This enhancing effect is noteworthy

because hypofunction of NMDA receptors is considered part of the pathophysiology of dopamine D_2 receptor antagonist-resistant schizophrenia (Krystal et al. 2003; Abekawa et al. 2003). Both phencyclidine (PCP) and ketamine block NMDA receptors non-competitively. In subjects who abuse PCP (Luisada 1978; Javitt and Zukin 1991) and who are also administered ketamine (Krystal et al. 1994; Malhotra et al. 1996), these NMDA antagonists induce not only schizophrenia-like positive symptoms such as hallucinations and delusions, but also cause negative symptoms such as a flattened affect and social withdrawal. Additionally, each of these NMDA receptor antagonists can individually produce cognitive dysfunctions. Administration of ketamine to schizophrenic patients worsens positive symptoms that can be effectively treated by CLZ (Malhotra et al. 1997). Even when administered in conjunction with HAL, ketamine worsens the positive and negative symptoms of schizophrenic patients (Lahti et al. 1995). Taken together, these findings suggest that the hypofunction of NMDA receptors is part of the pathophysiology of dopamine D_2 receptor antagonist-resistant schizophrenia, and that CLZ may possibly be able to improve this NMDA receptor hypofunction.

The stimulating effect of CLZ on NMDA receptor-mediated neurotransmission is enhanced by its activating effect on dopamine D_1 receptor-mediated dopaminergic transmission in vitro (Chen and Yang 2001; Ninan and Wang 2003; Wittmann et al. 2005). Therefore, we hypothesized that CLZ can block the PCP-induced acute increases in glutamate levels in the medial prefrontal cortex (mPFC) through the activation of NMDA-mediated transmission that is modulated by the stimulating effect on dopamine D_1 receptor-mediated transmission. Blocking these glutamate increases could possibly attenuate the PCP-induced abnormal behavior.

To examine the above hypothesis, the present study examined the effects of CLZ on PCP-induced hyperlocomotion and PCP-induced acute increases in glutamate levels in the mPFC. Furthermore, this study also examined the essential role of the simultaneous activation of NMDA receptor- and dopamine D_1 receptor-mediated neurotransmission in the effect of CLZ.

Methods

Animals

Male Sprague-Dawley rats (SLC, Hamamatsu, Japan), weighing 250–290 g at the start of the experiment, were housed individually in a plastic cage 30×25×18 cm with a wire mesh top and sawdust bedding. The animal house was under controlled conditions of light (from 6:30 a.m. to 6:30 p.m.), temperature (24°C), and humidity (50%). They were allowed free access to standard laboratory diet and tap water. Animals were handled daily for at least 4 days before the start of the experiment. This study was conducted in accordance with a guide for the care and use of laboratory animals regulated by Hokkaido University School of Medicine and NIH guidelines on animal care.

Drugs

Phencyclidine hydrochloride (PCP) (synthesized in the laboratory of Hokkaido University) was dissolved in saline. Clozapine (gift from Sandoz Pharmaceutical, East Hanover, NJ) and haloperidol (HAL; gift from Dainippon Pharmaceutical, Osaka, Japan) were dissolved in 0.15% tartaric acid. This experiment employed 0.3, 1, and 10 mg/kg CLZ and 0.01, 0.1, and 1 mg/kg HAL according to a recent report by our group (Abekawa et al. 2003). The dose of PCP refers to salt. All injections were given intraperitoneally as a volume of 1 ml/kg. NMDA, CPP [(±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid], SKF-38393, and SCH-23390 (all purchased from Sigma, St. Louis, MO), excluding vehicle [artificial cerebrospinal fluid (aCSF)], were each dissolved in aCSF, and perfused in the mPFC using the reverse dialysis method. Concentrations of compounds were rationalized according to previous studies (Abekawa et al. 2000, 2003; Yonezawa et al. 1998; Ceglia et al. 2004).

Rating for locomotor activity

Visual observation of locomotor activity was conducted during microdialysis, using the rating scale devised by Sturgeon et al. (1979). Each animal was assigned a rating score of 1–5 every 10 min for 160 min, and was observed for 30 s before the assignment of a score. Ratings were made by two observers, one of whom was unaware of the treatment conditions. In most cases, the two observers gave the same score. The inter-reliability between two observers calculated using data from present experiment was very high (>0.9). In cases of inconsistency, consensus was reached by a quick review of the behavior. If two behavioral scores were observed in an observational period, both behavioral scores were recorded and the mean score was used for statistical analysis. Definition of each score for locomotor activity was as follows. 0: Stationary, with little or no movement. 1: Movement within localized area of cage; intermittent activity emitted at a low rate. 2: Movement over a small area of cage; intermittent activity emitted at a low–moderate rate. 3: Movement over small area of cage; activity emitted continuously and at a moderate–rapid rate. 4: Movement over large area of cage; activity intermittent and emitted at a low–moderate rate. 5: Movement over large area of cage; activity emitted

continuously and at a moderate–rapid rate. In our preliminary study, the rating scores for PCP (7.5 mg/kg)-induced locomotion using the rating score by Sturgeon et al. (1979) significantly correlated with counts for PCP (7.5 mg/kg)-induced hyperlocomotion using an infrared sensor ($r=0.73$, $P=0.003$).

Microdialysis

Rats were implanted stereotaxically under pentobarbital anesthesia (30 mg/kg, i.p.) with G-4 guide cannulae (Eicom, Kyoto, Japan) leading to the surface of the mPFC (A: +2.7 mm, L: 0.8 mm, DV: −1.8 mm). These coordinates were with respect to the bregma, and according to the atlas of Paxinos and Watson (1997). A dialysis probe made of regenerated cellulose with an outer diameter of 220 μm (BDP-IV-03, Eicom, Kyoto, Japan) was inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the mPFC. On the following day, in freely moving rats, perfusion was started using artificial CSF (147 mM NaCl, 2.4 mM KCl, 1.2 mM $CaCl_2$ and 1.0 mM $MgCl_2$, pH 7.4) at a flow rate of 2 μl/min. Following initial perfusion for 1.5 h, baseline samples were obtained every 20 min for 80 min. Dialysis samples were collected every 20 min for 220 min following the last baseline collection. Dialysate samples were collected in microtubes containing 40 μl 50 mM acetic acid with 20 mg/l L-cysteine. A 20 μl sample of the dialysate was used to quantify glutamate.

At the end of the microdialysis study, rats were killed and then had their brains removed. After the termination of each experiment, animals were anesthetized with pentobarbital and perfused intracardially with PBS, followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde overnight, then stored in 30% sucrose solution. Serial sections of brains were cut at 30 μm intervals and stained with cresyl violet. Probe placement was verified microscopically. Success rate in this study was more than 95%.

Biochemical measurement

The HPLC system consisted of a liquid chromatograph pump (EP-300; Eicom), a degasser (DG-300; Eicom), a fluorometric detector (FLD-370; Eicom), and a column oven (ATC-300; Eicom). Eicompak SC-5 ODS 2.1× 150 mm (Eicom) was used for measuring the concentration of glutamate. Analysis of glutamate was performed according to the pre-column derivatization method described by Lindroth and Mopper (1979) with minor modification. The minimum level of detection for glutamate is around 10 fmol/μl (Eicom). The derivatization reagent was prepared by dissolving 54 mg *o*-phthalaldehyde (OPA) in 1 ml 99.9% methanol and 9 ml 0.1 M Na_2CO_3 (pH 9.5). This solution (2.5 ml) was diluted 1:1 with 0.1 M Na_2CO_3, and 10 μl of β-mercaptoethanol was added. A 10 μl aliquot of OPA derivatization reagent was added to 20 μl dialysate, and, after a 2.5 min reaction period, 15 μl of the reactant was injected into the HPLC system coupled with a fluorometric detector with excitation and emission wavelengths of 340 nm and 445 nm, respectively. The mobile phase consisted of 0.06 M NaH_2PO_4, 0.01 M Na_2HPO_4, 5 mg/l Na_2-EDTA (pH 6.0) and 30% (v/v) methanol. Flow rate was 0.3 ml/min. Separation was conducted isocratically at 30°C.

Experimental protocol

1. Effects of systemically administered CLZ or HAL on cumulated rating scores for PCP-induced hyperlocomotion (0–160 min) and acute increases in glutamate levels in the mPFC, and on cumulated scores for locomotor activity and glutamate levels in this region emerging after saline injection (see Figs. 1, 5, and Table 1). For the behavioral study, CLZ (0.3, 1, and 10 mg/kg, i.p.), HAL (0.01, 0.1, and 1 mg/kg, i.p.), and vehicle, were each injected at 0 min. PCP (7.5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) were each injected at 30 min. For the microdialysis study, CLZ (0.3, 1, and 10 mg/kg, i.p.), HAL (0.1 and 1 mg/kg, i.p.) and vehicle were each injected at 60 min. PCP (7.5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) were each was injected at 90 min. Visual observation of locomotor activity was conducted during the microdialysis study.
2. Effects of perfusion with NMDA, SKF-38393, or co-perfusion with NMDA and SKF-38393 in the mPFC on systemically administered PCP-induced acute increases in glutamate levels in this region (see Fig. 2). NMDA (10, 100 μM, and 1 M), SKF-38393 (20 and 200 μM), NMDA (10 μM) +SKF-38393 (20 μM), NMDA (1 mM) +SKF-38393 (200 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. PCP (7.5 mg/kg, i.p.) was injected at 90 min.
3. Effects of perfusion with SCH-23390 in the mPFC on systemically administered CLZ-induced blockade of PCP-induced acute increases in glutamate levels (see Fig. 3a) and on PCP-induced acute increases in glutamate levels (Fig. 3b) in this region
   - A SCH-23390 (40 μM) was perfused in the mPFC from 40 min to 180 min. Clozapine (10 mg/kg, i.p.) and vehicle was injected at 60 min. PCP (7.5 mg/kg, i.p.) was injected at 90 min.
   - B SCH-23390 (40 μM) and vehicle (aCSF), were each perfused in the mPFC from 60 min to 180 min. PCP was injected at 90 min.
4. Effects of systemically administered CLZ or perfusion with NMDA in the mPFC on locally perfused CPP-

Table 1 Effects of clozapine (CLZ) and haloperidol (HAL) on cumulated rating scores(0–160 min) for phencyclidine (PCP)- or saline-induced locomotor activity

Groups of experiments	Cumulated scores	Groups of experiments	Cumulated scores
Vehicle/saline(n=6)	25.83±2.09	Vehicle/saline(n=7)	37.67±1.02
Vehicle/PCP(n=8)	61.13±2.08 *	–	
CLZ(0.3 mg/kg)/PCP(n=8)	59.50±2.30 *	CLZ(0.3 mg/kg)/saline(n=6)	37.75±0.73
CLZ(1 mg/kg)/PCP(n=8)	51.25±1.75 *,**	CLZ(1 mg/kg)/saline(n=6)	35.75±0.62
CLZ(10 mg/kg)/PCP(n=10)	44.00±1.92 *,**	CLZ(10 mg/kg)/saline(n=6)	26.17±1.01 *
HAL(0.01 mg/kg)/PCP(n=8)	56.00±1.89 *	HAL(0.01 mg/kg)/saline(n=6)	37.00±0.27
HAL(0.1 mg/kg)/PCP(n=8)	52.25±2.68 *	HAL(0.1 mg/kg)/saline(n=6)	26.75±0.86 *
HAL(1 mg/kg)/PCP(n=8)	28.50±1.45 **	HAL(1 mg/kg)/saline(n=7)	20.00±0.52 *

Values represents mean±SEM
*P<0.01 vs vehicle/saline group, ** P<0.01, vs vehicle/PCP group

induced acute increases in glutamate levels in this region (see Fig. 4)

A Clozapine (1 and 10 mg/kg, i.p.) and vehicle were each injected at 60 min. CPP (200 μM) was perfused in the mPFC from 60 min to 180 min.

B NMDA (10, 100 μM, and 1 mM) and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. CPP (200 μM) was co-perfused with NMDA in the mPFC from 60 min to 180 min.

5. Effects of perfusion with CPP or SCH-23390 in the mPFC on systemically administered CLZ-induced delayed increases in glutamate levels in this region (see Fig. 5b). CPP (200 μM) SCH-23390 (40 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. Clozapine (10 mg/kg, i.p.) and vehicle were each injected at 60 min. Saline (1 ml/kg) was injected at 90 min.
6. Effects of perfusion with NMDA, SKF-38393, or co-perfusion with NMDA and SKF-38393 in the mPFC on basal glutamate levels in this region emerging after saline injection (see Fig. 6). NMDA (10,100 μM, and 1 mM), NMDA (1 mM)+CPP (200 μM), SKF-38393 (20 and 200 μM), SKF-38393 (200 μM)+SCH-23390 (40 μM), NMDA (10 μM)+SKF-38393 (20 μM), NMDA (1 mM)+SKF-38393 (200 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. Saline (1 ml/kg, i.p.) was injected at 90 min.

Statistics

Data from extracellular concentrations of glutamate were analyzed by a repeated two-way ANOVA using treatment group as the between-subject variable and time as the repeated measures variable (defined as P<0.05). A post-hoc Duncan new multiple range test was then used to determine which group significantly differed from the others. Cumulated scores for locomotor activity (0–160 min) were analyzed by a one-way ANOVA followed by post-hoc Duncan tests (P<0.05).

Results

Effects of CLZ or HAL on cumulated rating scores for PCP-induced hyperlocomotion and on locomotor activity emerging after saline injection

Systemic administration of CLZ at 10 and 1 mg/kg, but not 0.3 mg/kg reduced cumulated rating scores for PCP (7.5 mg/kg)-induced hyperlocomotion (Table 1). Systemic administration of HAL at 1 mg/kg, but not 0.1 mg/kg and 0.01 mg/kg, reduced cumulated rating scores for PCP (7.5 mg/kg)-induced hyperlocomotion. Clozapine at 10 mg/kg, but not 1 mg/kg and 0.3 mg/kg, reduced cumulated rating scores for locomotor activity emerging after saline injection. HAL at 0.1 and 1 mg/kg, but not 0.01 mg/kg, reduced cumulated rating scores for locomotor activity emerging after saline injection.

Effects of systemically administered CLZ or HAL on PCP-induced acute increases in glutamate levels in the mPFC

Systemic administration of CLZ at 10 and 1 mg/kg, but not 0.3 mg/kg inhibited PCP (7.5 mg/kg)-induced acute increases in glutamate levels in the mPFC (Fig. 1a). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle/PCP (n=8), CLZ (0.3) /PCP (n=7), CLZ (1) /PCP (n=6), and CLZ (10)/PCP (n=10) groups were 0.31±0.057, 0.40±0.056, 0.39±0.079, 0.35±0.074, and 0.46±0.045, respectively. Systemic administration of HAL at 1 mg/kg, but not 0.1 mg/kg inhibited PCP (7.5 mg/kg)-induced acute increases in glutamate levels in the mPFC (Fig. 1b). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle/PCP (n=8), HAL (0.1)/PCP (n=6), HAL (1)/PCP (n=8) groups are 0.31±0.057, 0.40±0.056, 0.33±0.086, and 0.38±0.054, respectively.

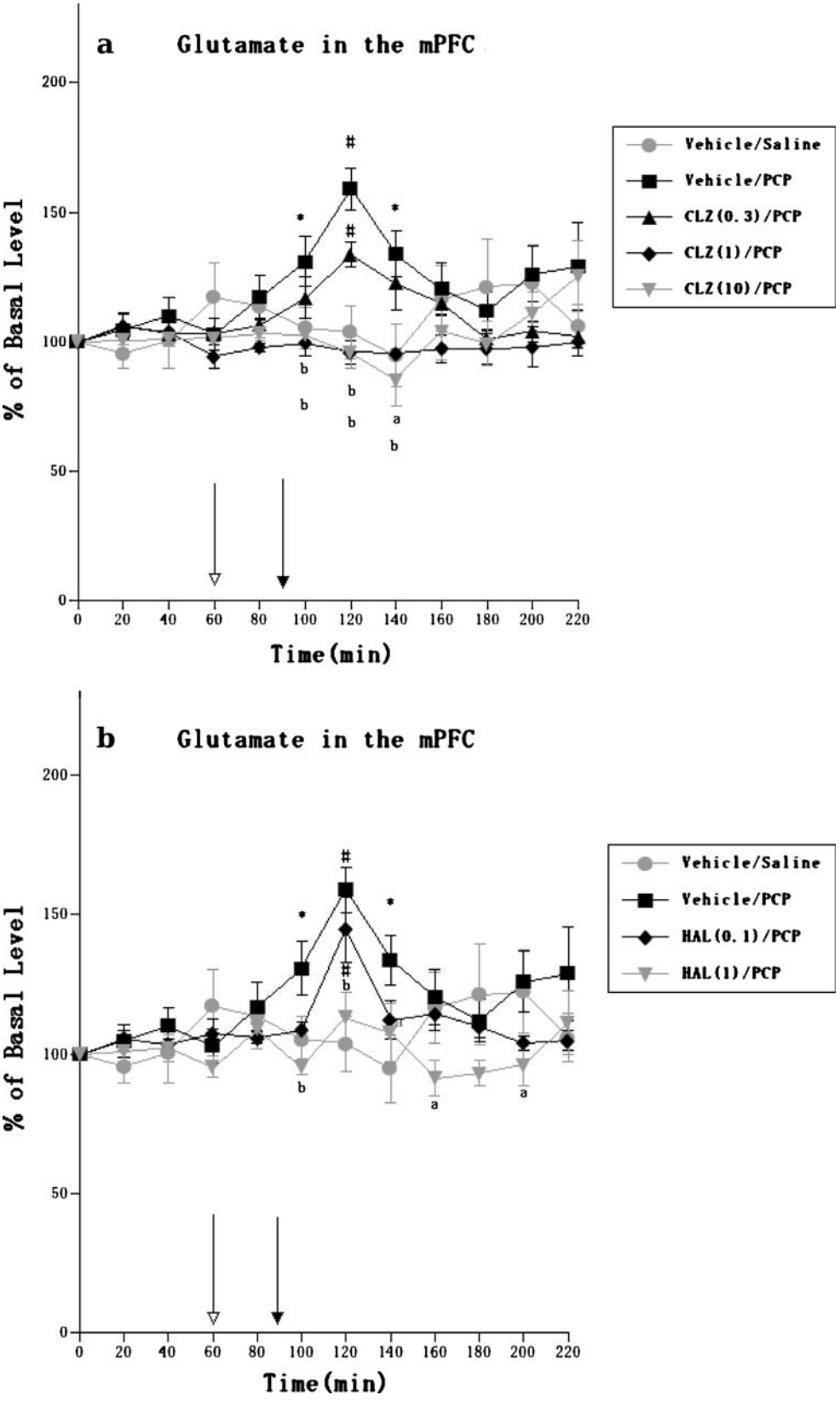


Fig. 1a, b Effect of systemically administered clozapine (CLZ; 0.3, 1, and 10 mg/kg) or haloperidol (HAL; 0.1 and 1 mg/kg) on phencyclidine (PCP; 7.5 mg/kg)-induced acute increases in glutamate levels in the medial prefrontal cortex (mPFC) and glutamate levels in this region emerging after saline injection. **a** For CLZ data, repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(40, 320)=2.31$, $P<0.01$], an effect of group [$F(4, 32)=3.97$, $P < 0.01$], and an effect of time [$F(10, 320)=2.04$, $P<0.05$]. * $P<0.05$ vs vehicle/saline; # $P<0.01$ vs vehicle/saline; $aP<0.05$, CLZ (10)/PCP vs vehicle/PCP, CLZ (1)/PCP vs vehicle/PCP; $bP<0.01$, CLZ (10)/PCP vs vehicle/PCP, CLZ (1)/PCP vs vehicle/PCP (post-hoc test). **b** For HAL data, repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30, 240)=1.90$, $P < 0.01$], an effect of group [$F(3, 24)=3.44$, $P<0.05$], and an effect of time [$F(10, 240)=3.63$, $P<0.01$]. * $P<0.05$ vs vehicle/saline; # $P<0.01$ vs vehicle/saline; $aP<0.05$, HAL (1)/PCP vs vehicle/PCP; $bP<0.01$, HAL (1)/PCP vs vehicle/PCP (post-hoc test). For the microdialysis study, CLZ (0.3, 1, and 10 mg/kg, i.p.), HAL (0.1 and 1 mg/kg, i.p.) and vehicle were each injected at 60 min (*white arrows*). PCP (7.5 mg/kg, i.p.) and saline (1 ml/kg, i.p.) were each was injected at 90 min (*black arrows*)

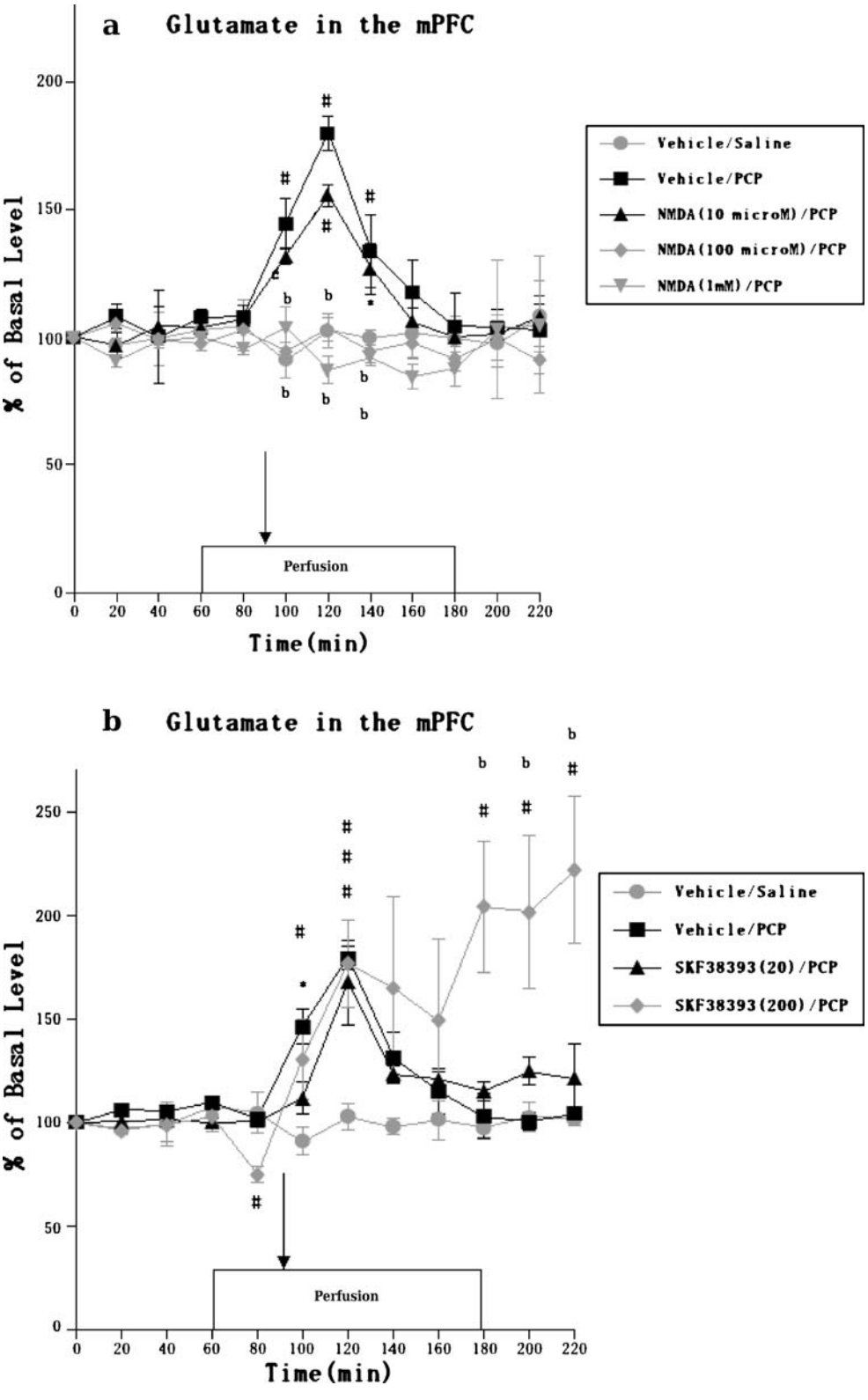
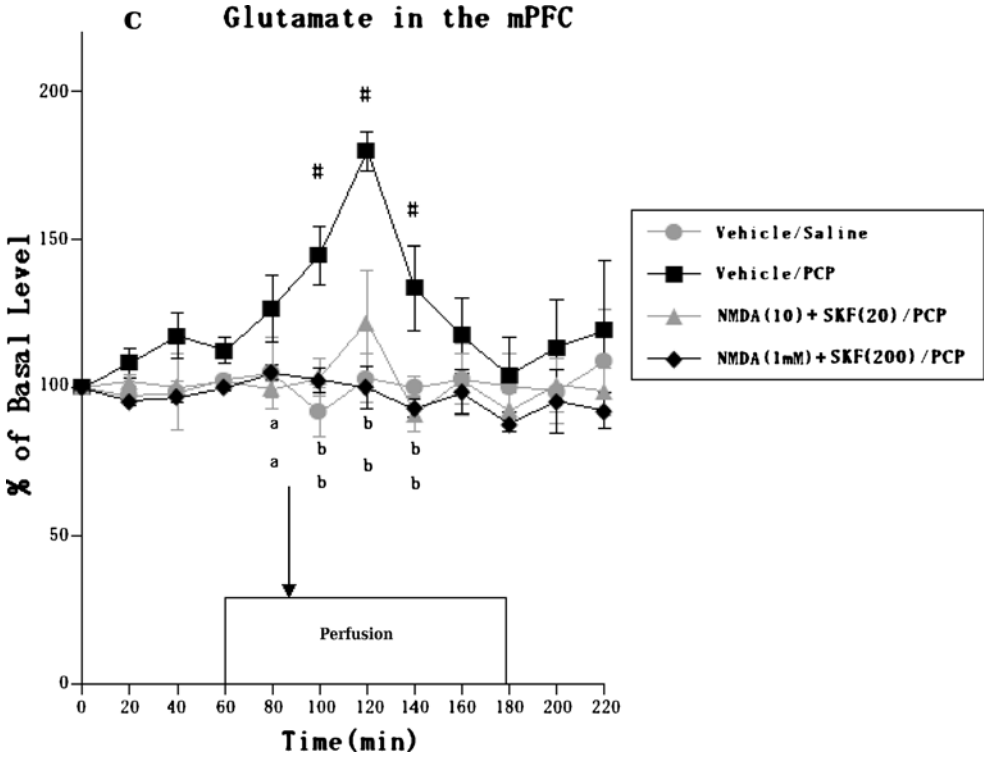


Fig. 2a–c Effect of locally administered N-methyl-D-aspartate (NMDA; 10, 100 μM, and 1 mM), SKF-38393 (20 and 200 μM), or co-perfusion with NMDA (10 μM, 1 mM) and SKF-38393 (20 and 200 μM) on PCP (7.5 mg/kg)-induced acute increases in glutamate levels and basal glutamate levels in the mPFC. **a** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(40, 280)=2.48$, $P<0.01$], an effect of group [$F(4, 28)=6.39$, $P<0.01$], and an effect of time [$F(10, 280)=5.05$, $P<0.01$]. * $P<0.05$ vs vehicle/saline; # $P<0.01$ vs vehicle/saline; *b*$P<0.01$, NMDA (1 mM)/PCP vs vehicle/PCP, NMDA (100 μM)/PCP vs vehicle/PCP (post-hoc test). **b** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30, 230)=2.60$, $P<0.01$], an effect of group [$F(3, 23)=5.02$, $P<0.01$], and an effect of time [$F(10, 230)=4.53$, $P<0.01$]. *$P<0.05$ vs vehicle/saline; # $P<0.01$ vs vehicle/saline (post-hoc test). **c** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30, 220)=1.77$, $P<0.05$], an effect of group [$F(3, 22)=8.19$, $P<0.01$], and an effect of time [$F(10, 220)=3.95$, $P<0.01$]. * $P<0.05$ vehicle/PCP vs vehicle/saline; # $P<0.01$ vehicle/PCP vs vehicle/saline; *a*$P<0.05$, NMDA (10)+SKF38393 (10)/PCP vs vehicle/PCP; NMDA (1 mM)+SKF38393 (200)/PCP vs vehicle/PCP; *b*$P<0.01$, NMDA (10)+SKF38393 (10)/ PCP vs vehicle/PCP; NMDA (1 mM)+SKF38393 (200)/PCP vs vehicle/PCP (post-hoc test). NMDA (10, 100 μM, and 1 M), SKF-38393 (20 and 200 μM), NMDA (10 μM) +SKF-38393 (20 μM), NMDA (1 mM) +SKF-38393 (200 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. PCP (7.5 mg/kg, i.p.) was injected at 90 min (*black arrow*)

Fig. 2 (continued)



Effects of perfusion with NMDA, SKF 38393, or co-perfusion with NMDA and SKF-38393 in the mPFC on PCP-induced acute increases in glutamate levels in this region

Perfusion with NMDA in the mPFC at a concentration of 100 μM or 1 mM inhibited PCP (7.5 mg/kg)-induced acute increases in glutamate levels in the mPFC (Fig. 2a). Perfusion with NMDA at any concentration did not induce delayed increases in glutamate levels after inhibiting the PCP-induced acute increases in glutamate levels. Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle/PCP (n=6), NMDA (10 μM)/PCP (n=6), NMDA (100 μM)/PCP (n=8), and NMDA (1 mM)/PCP (n=7) groups were 0.55±0.084, 0.43±0.046, 0.31±0.046, 0.32±0.047, and 0.32±0.051, respectively.

Perfusion with SKF-38393 in the mPFC at a concentration of 20 μM or 200 μM did not inhibit PCP (7.5 mg/kg)-induced acute increases in glutamate levels, and 200 μM of SKF-38393 induced delayed increases in glutamate levels in the mPFC following the PCP-induced acute effect on glutamate levels (Fig. 2b). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle/PCP (n=7), SKF38393 (20 μM)/PCP (n=6), SKF38393 (200 μM)/PCP (n=7) groups were 0.55±0.082, 0.52±0.066, 0.44±0.087, and 0.63±0.087, respectively.

Co-perfusion with NMDA (10 μM) and SKF-38393 (20 μM), each of which alone had no effect on PCP-induced acute increases in glutamate levels, completely blocked the PCP-induced acute increase in glutamate levels in the mPFC. Furthermore, co-perfusion with NMDA (1 mM) and SKF-38393 (200 μM) also inhibited PCP-induced acute increases in glutamate levels in the mPFC (Fig. 2c). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle/PCP (n=6), NMDA (10)+SKF38393 (20)/PCP (n=7), and NMDA (1 mM)+SKF38393 (200)/PCP (n=7) groups were 0.55±0.100, 0.43±0.118, and 0.39±0.046, respectively.

Effects of perfusion with SCH-23390 in the mPFC on systemically administered CLZ-induced blockade of PCP-induced acute increases in glutamate levels and on PCP-induced acute increases in glutamate levels in this region

Perfusion with SCH-23390 (40 μM) did not reverse the CLZ (10 mg/kg)-induced blocking of PCP-induced acute increases in glutamate levels in the mPFC (Fig. 3a). Basal levels of glutamate (pmol/μl) for vehicle/PCP (n=7), CLZ/PCP (n=8), and SCH23390+CLZ/PCP (n=6) groups were 0.43±0.13, 0.46±0.045, and 0.36±0.093, respectively.

Perfusion with SCH-23390 (40 μM) had no effect on PCP-induced acute increases in glutamate levels in the mPFC, and had no effect on basal glutamate levels emerging after saline injection (Fig. 3b). Basal levels of glutamate for vehicle/saline (n=6), vehicle/PCP (n=7), SCH23390 (40)/PCP (n=7), and SCH23390 (40)/saline (n=7) groups were 0.55±0.082, 0.43±0.133, 0.43±0.098, and 0.42±0.028, respectively.

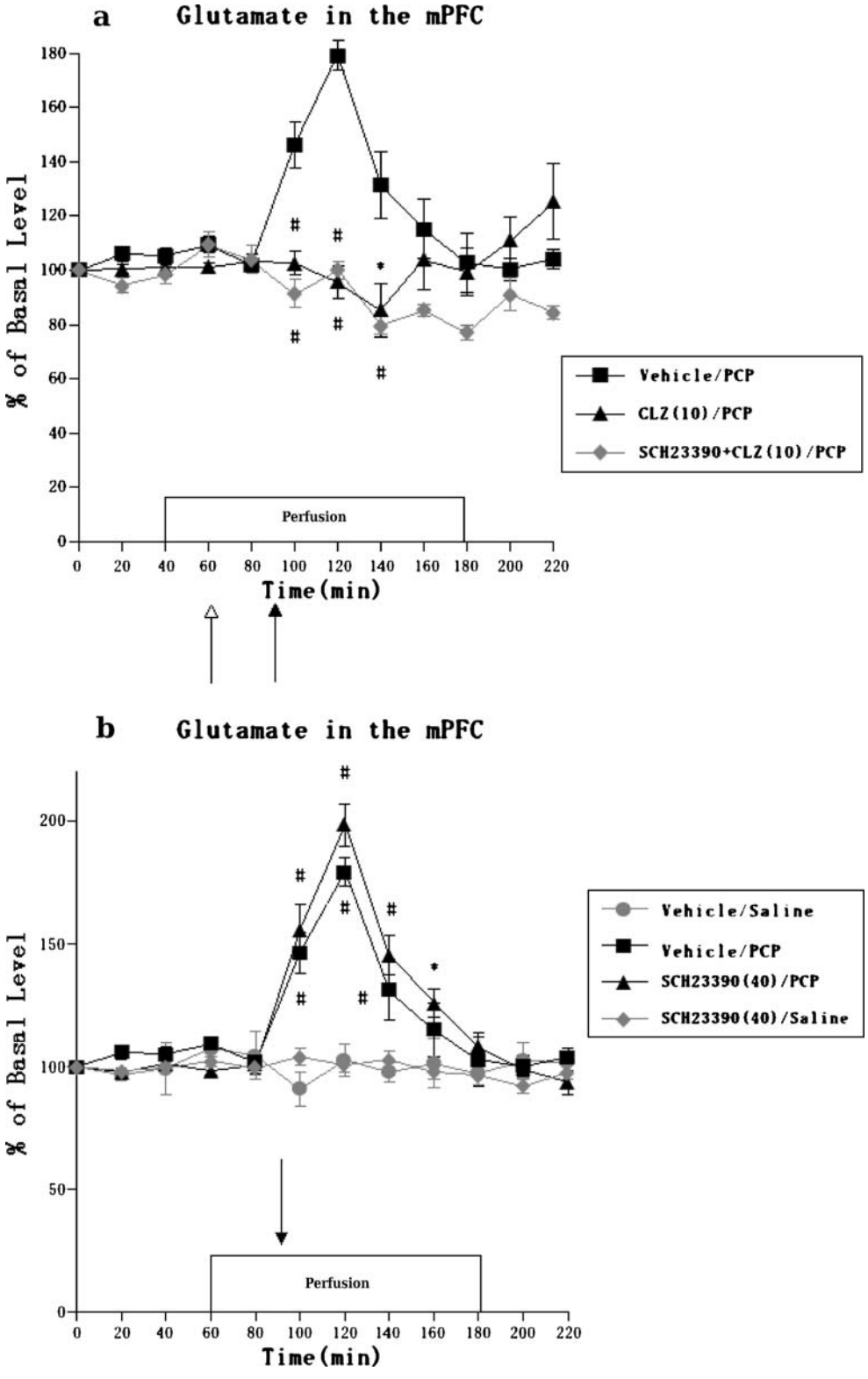
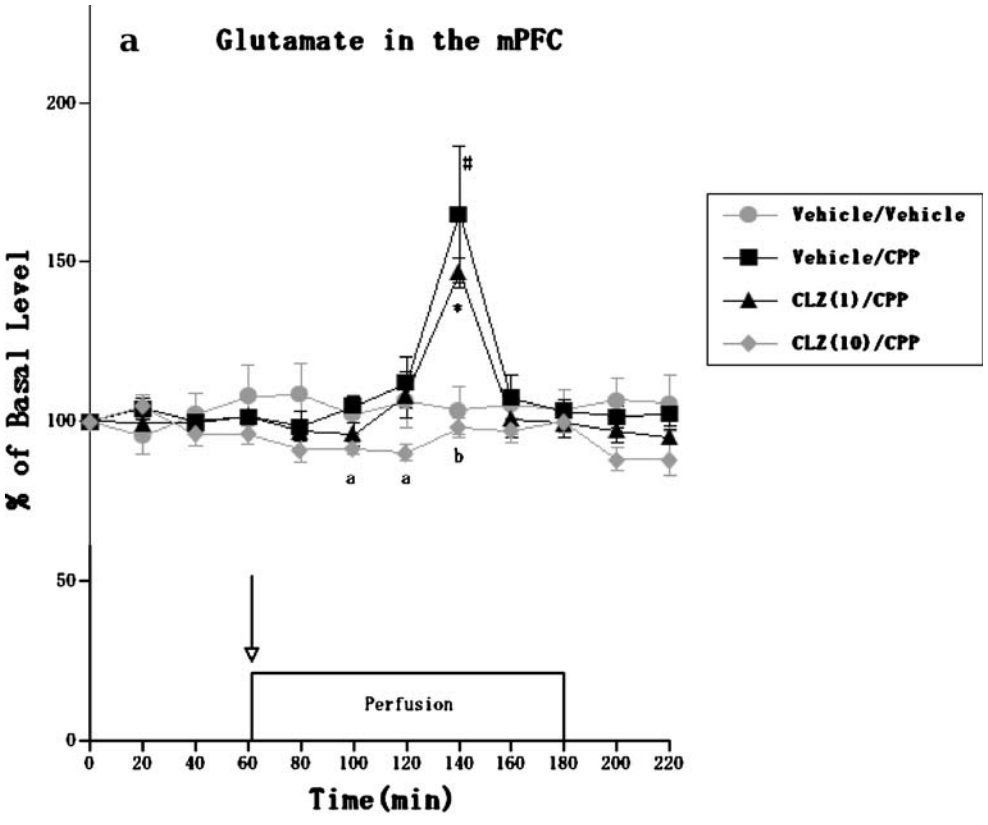


Fig. 3a, b Effect of locally administered SCH-23390 (40 μM) on the CLZ (10 mg/kg)-induced blockade of PCP (7.5 mg/kg)-induced acute increases in the mPFC, and PCP (7.5 mg/kg)-induced acute increases in glutamate levels in this region. **a** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(20, 170)=8.74$, $P<0.01$], an effect of group [$F(2, 17)=13.32$, $P<0.05$], and an effect of time [$F(10, 170)=4.36$, $P<0.01$]. * $P<0.05$ vs vehicle/PCP; # $P<0.01$, vs vehicle/PCP; *aP*<0.05, CLZ/PCP vs SCH23390+CLZ/PCP; *bP*<0.01, CLZ/PCP vs SCH23390+CLZ/PCP (post-hoc test). SCH-23390 (40 μM) was perfused in the mPFC from 40 min to 180 min. Clozapine (10 mg/kg, i.p.) and vehicle was injected at 60 min (*white arrow*). PCP (7.5 mg/kg, i.p.) was injected at 90 min (*black arrow*). **b**) A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30, 230)=9.90$, $P<0.01$], an effect of group [$F(3, 23)=18.82$, $P<0.01$], and an effect of time [$F(10, 230)=28.50$, $P<0.01$]. * $P<0.05$ vs vehicle/saline; # $P<0.01$ vs vehicle/saline (post-hoc test). SCH-23390 (40 μM) and vehicle (aCSF), were each perfused in the mPFC from 60 min to 180 min. PCP was injected at 90 min (*black arrow*)



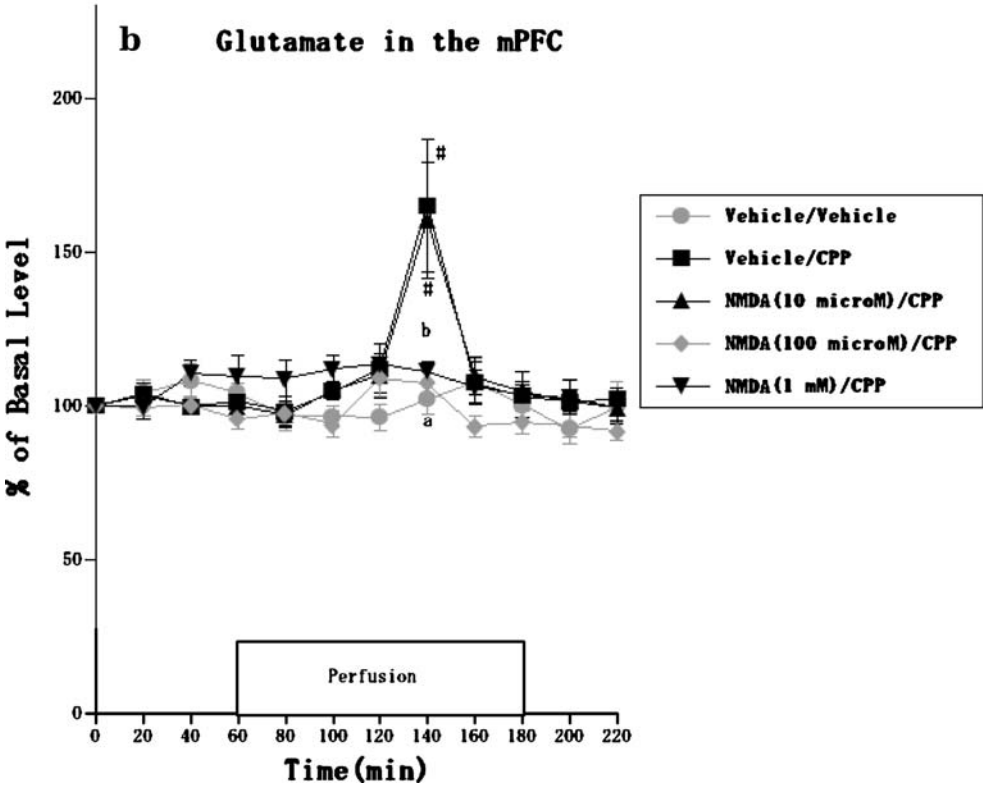


Fig. 4a, b Effect of systemically administered CLZ (1 and 10 mg/kg) or locally administered NMDA(10, 100 μM, and 1 mM) on locally applied CPP (200 μM)-induced acute increases in glutamate levels in the mPFC. **a** For CLZ data, a repeated two-way ANOVA revealed a significant effect of group×time interaction [F (30, 210)=3.52, $P<0.01$], an effect of group [F (3, 21)=3.96, $P<0.05$], and an effect of time [F (10, 210)=10.19, $P<0.01$]. * $P<0.05$ vs vehicle/vehicle; # $P<0.01$ vs vehicle/vehicle; $aP<0.05$, CLZ(10)/CPP vs vehicle/CPP; $bP<0.01$, CLZ(10)/CPP vs vehicle/CPP(post-hoc test). Clozapine (1 and 10 mg/kg, i.p.) and vehicle were each injected at 60 min (*white arrow*). CPP (200 μM) was perfused in the mPFC from 60 min to 180 min. **b** For NMDA data, a repeated two-way ANOVA revealed a significant effect of group×time interaction [F (40, 260)=2.84, $P<0.01$], an effect of group [F (4, 26)=4.35, $P<0.01$], and an effect of time [F (10, 260)=10.95, $p<0.01$]. # $P<0.01$ vs vehicle/vehicle; $aP<0.05$, NMDA (1 mM)/CPP vs vehicle/CPP; $bP<0.01$ NMDA (100 μM)/CPP vs vehicle/CPP (post-hoc test). NMDA (10, 100 μM, and 1 mM) and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. CPP (200 μM) was co-perfused with NMDA in the mPFC from 60 min to 180 min

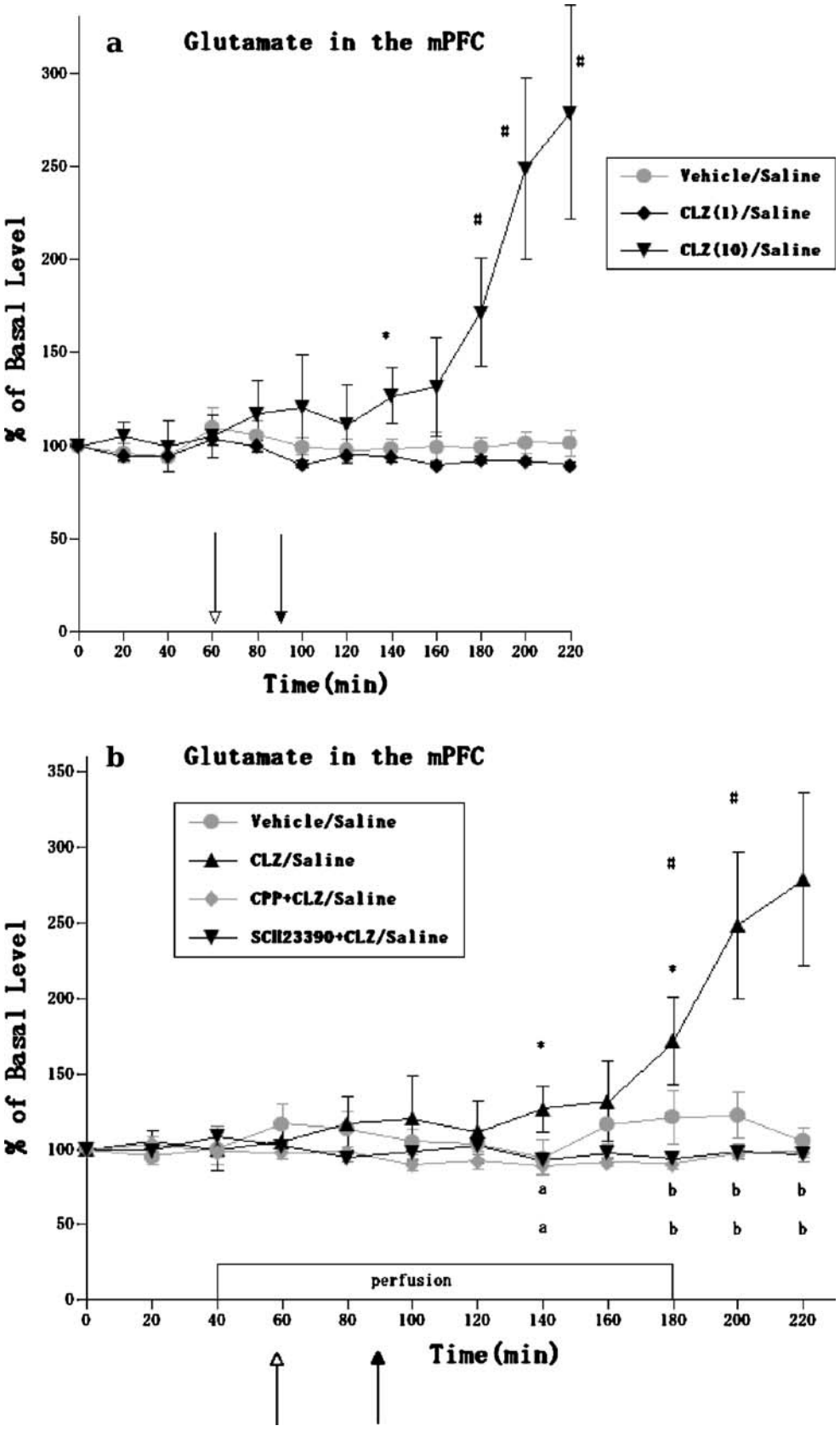
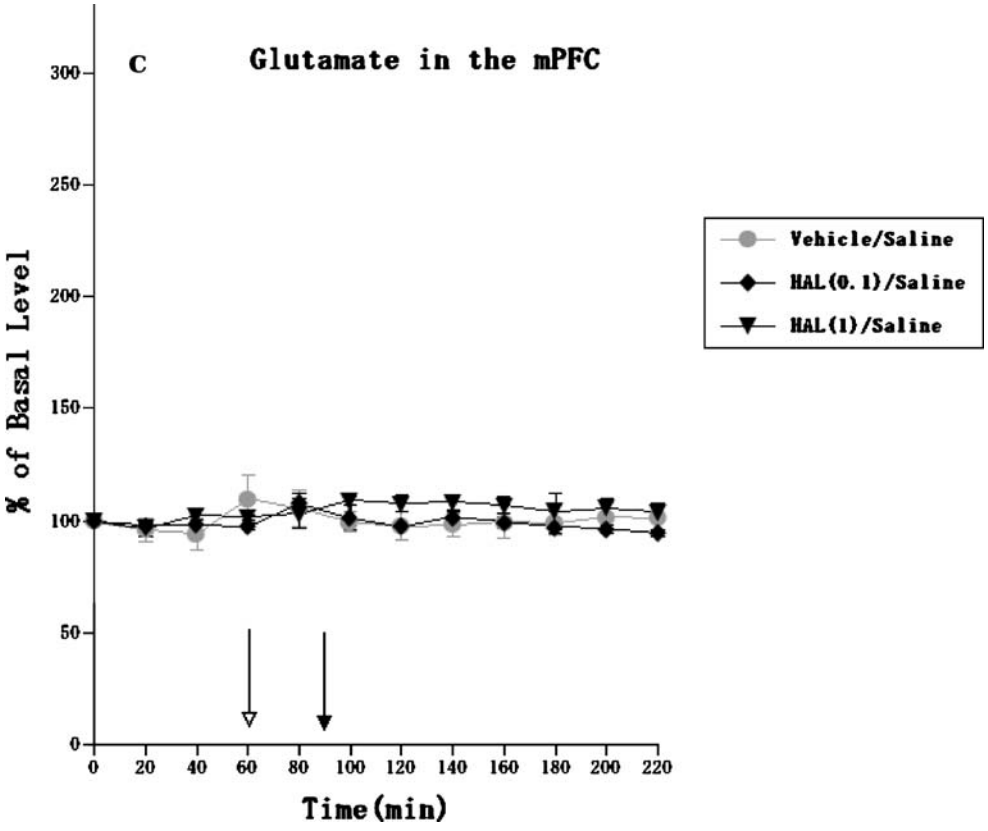


Fig. 5a–c Effect of systemically administered CLZ or HAL on glutamate levels in the mPFC emerging after saline injection, and effect of locally administered CPP or SCH-23390 on the CLZ-induced delayed increases in basal glutamate levels in this region **a** A repeated two-way ANOVA revealed a significant effect of group×time interaction [F (20, 160)=5.25, $P<0.01$], an effect of group [F (2, 16)= 14.92, $P<0.05$], and an effect of time [F (10, 160)=5.01, p<0.01].* $P<0.05$ vs vehicle/ saline; # $P<0.01$ vs vehicle/ saline; (post-hoc test). **b** A repeated two-way ANOVA revealed a significant effect of group×time interaction [F (30, 210)=4.57, $P<0.01$], an effect of group [F (3, 21)=10.69, $P<0.01$], and an effect of time [F (10, 210)=5.11, $P<0.01$]. * $P<0.05$ CLZ/saline vs vehicle/saline; # $P<0.01$, CLZ/saline vs vehicle/saline; $aP<0.05$, CPP+CLZ/saline vs CLZ/saline; SCH23390+CLZ/saline vs CLZ/saline; $bP<0.01$, CPP+ CLZ/saline vs CLZ/saline, SCH23390+CLZ/saline vs CLZ/saline(post-hoc test). **c** A repeated two-way ANOVA did not reveal a significant effect of group×time interaction [F (20, 170)=0.63, $P=0.89$], an effect of group [F (2, 17)=1.37, $P=$ 0.28], nor an effect of time [F (10, 170)=0.81, $P=0.62$]. CPP (200 μM) SCH-23390 (40 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. Clozapine (10 mg/kg, i.p.) and vehicle were each injected at 60 min (*white arrows*). Saline (1 ml/kg) was injected at 90 min (*black arrows*)

Fig. 5 (continued)



Effects of systemically administered CLZ or perfusion with NMDA in the mPFC on locally perfused CPP-induced acute increases in glutamate levels in this region

Perfusion with CPP (200 μM) in the mPFC acutely increased glutamate levels in this region. Systemic administration of CLZ at 10 mg/kg, but not 1 mg/kg, inhibited the CPP-induced acute increases in glutamate levels in the mPFC (Fig. 4a). Basal levels of glutamate (pmol/μl) for vehicle/vehicle (n=6), vehicle/CPP (n=6), CLZ (1)/CPP (n=7), CLZ (10)/CPP (n=7) groups were 0.31±0.056, 0.52±0.076, 0.32±0.054, and 0.27±0.060, respectively.

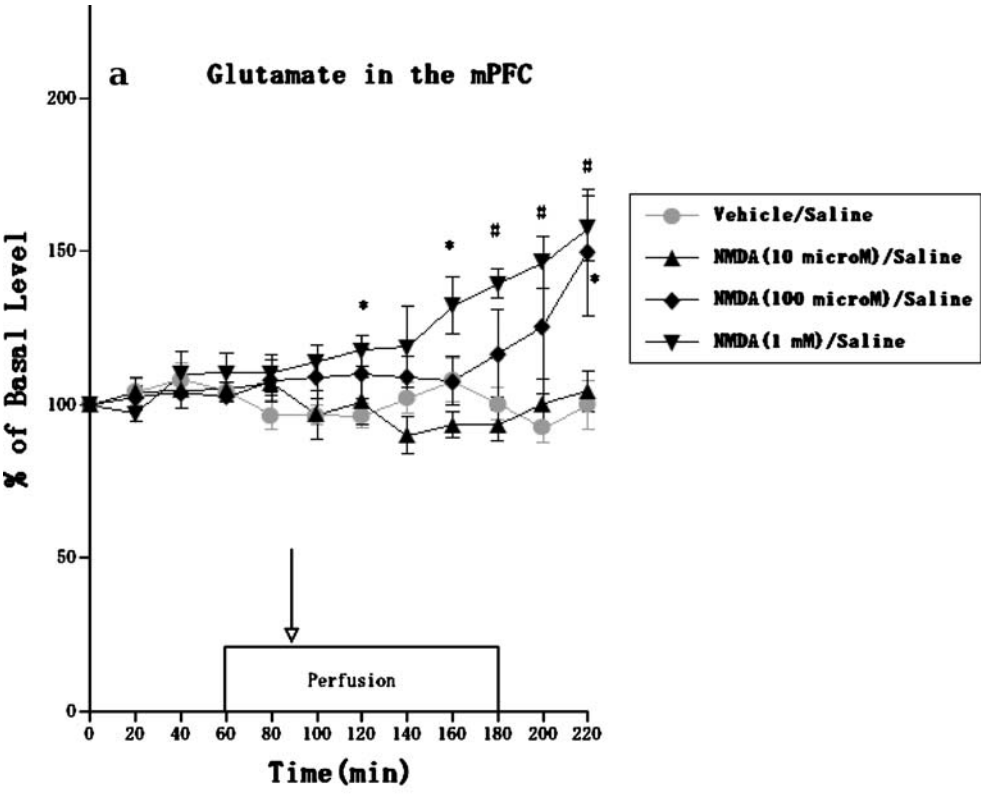
Perfusion with NMDA in the mPFC at a concentration of 1 mM or 100 μM inhibited CPP-induced acute increases in glutamate levels in the mPFC (Fig. 4b). Basal levels of glutamate (pmol/μl) for vehicle/vehicle (n=6), vehicle/CPP (n=6), NMDA (10 μM)/CPP (n=7), NMDA (100 μM)/CPP (n=6), and NMDA (1 mM)/CPP (n=6)groups were 0.53±0.074, 0.52±0.076, 0.52±0.065, 0.61±0.107, and 0.46±0.040, respectively.

Effects of systemically administered CLZ or HAL on glutamate levels emerging after saline injection, and effect of perfusion with CPP or SCH-23390 in the mPFC on systemically administered CLZ-induced delayed increases in glutamate levels in this region

Systemic administration of CLZ at 10 mg/kg, but not 1 mg/kg, induced delayed increases in glutamate levels in the mPFC (Fig. 5a). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=7), CLZ (1)/saline (n=6), and CLZ (10)/saline (n=6) groups are 0.31±0.056, 0.46±0.044, and 0.44±0.142, respectively.

Perfusion with CPP (200 μM) or SCH-23390 (40 μM) inhibited CLZ-induced delayed increases in glutamate levels in the mPFC (Fig. 5b). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), vehicle+CLZ/saline (n=6), CPP+CLZ/saline (n=6), and SCH23390+CLZ/saline (n=7) groups are 0.31±0.056, 0.44±0.142, 0.30±0.062, and 0.56±0.120, respectively.

HAL at 1 or 0.1 mg/kg had no effect on basal levels of glutamate in the mPFC (Fig. 5c). Basal levels of glutamate



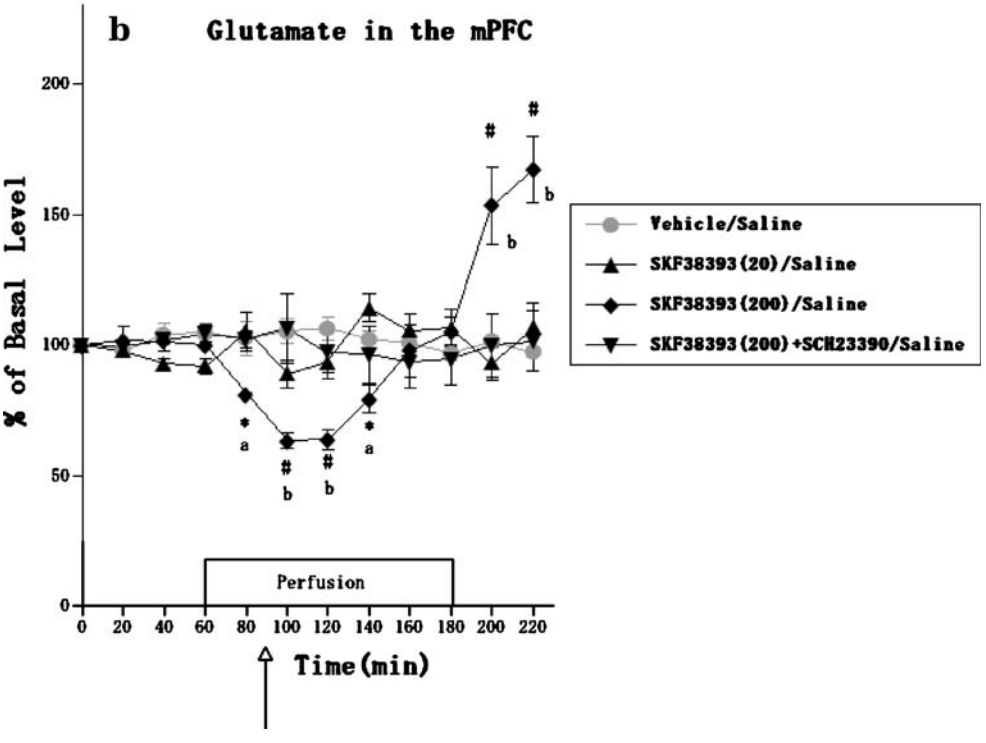
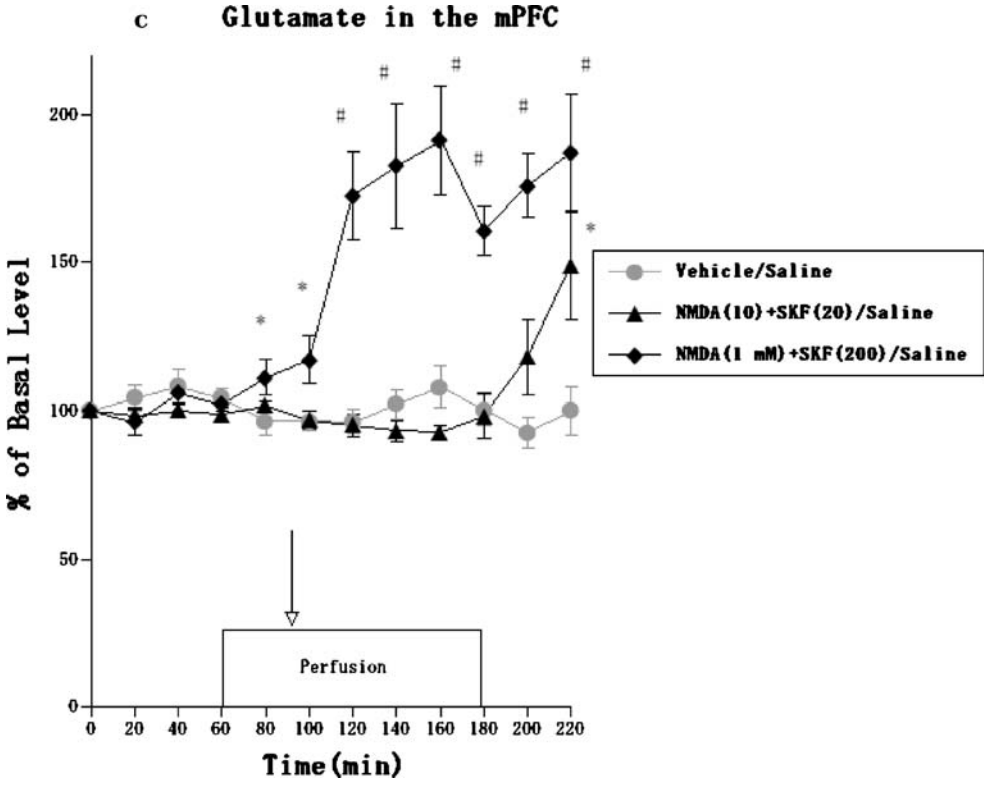


Fig. 6a–c Effect of locally administered NMDA (10, 100 μM, and 1 mM), SKF-38393 (20 and 200 μM), and co-perfusion with NMDA (10 μM, 1 mM) and SKF-38393 (20 and 200 μM) on basal glutamate levels in the mPFC. **a** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30, 232)=4.42, P<0.01$], an effect of group [$F(3, 22)=3.80, P<0.05$], and an effect of time [$F(10, 220)=8.46, P<0.01$]. * $P<0.05$, vs vehicle/saline; # $P<0.01$, vs vehicle/saline; *b*$P<0.01$, SKF38393(200)/PCP vs vehicle/PCP (post-hoc test). **b** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(30\ 210)=7.99, P<0.01$], and an effect of time [$F(10, 210)=7.22, P<0.01$]. * $P<0.05$, vs vehicle/saline; # $P<0.01$, vs vehicle/saline; *a*$P<0.01$, SKF38393 (200 μM)/saline vs SKF38393 (200 μM)+SCH23390/saline; *b*$P<0.01$, SKF38393(200 μM)/saline vs SKF38393 (200 μM)+SCH23390/saline (post-hoc test). **c** A repeated two-way ANOVA revealed a significant effect of group×time interaction [$F(20, 170)=9.45, P<0.01$], an effect of group [$F(2, 17)=23.81, P<0.01$], and an effect of time [$F(10, 170)=12.25, P<0.01$]. * $P<0.05$, NMDA (10)+SKF38393(10)/saline vs vehicle/saline; NMDA(1 mM)+SKF38393(200)/saline vs vehicle/saline; # $P<0.01$, NMDA (10)+SKF38393 (10)/saline vs vehicle/saline; NMDA(1 mM)+SKF38393(200)/saline vs vehicle/saline (post-hoc test). NMDA (10,100 μM, and 1 mM), NMDA (1 mM)+CPP (200 μM), SKF-38393 (20 and 200 μM), SKF-38393 (200 μM)+SCH-23390 (40 μM), NMDA (10 μM)+SKF-38393 (20 μM), NMDA (1 mM)+SKF-38393 (200 μM), and vehicle (aCSF) were each perfused in the mPFC from 60 min to 180 min. Saline (1 ml/kg, i.p.) was injected at 90 min (*white arrows*)

Fig. 6 (continued)



(pmol/μl) for vehicle/saline (n=7), HAL (0.1)/saline (n=6), and HAL (1)/saline (n=7) groups are 0.31±0.056, 0.31±0.056, and 0.37±0.102, respectively.

Effects of perfusion with NMDA, SKF-38393, or co-perfusion with NMDA and SKF-38393 in the mPFC on glutamate levels in this region emerging after saline injection

Perfusion with NMDA in the mPFC at a concentration of 1 mM or 100 μM, but not 10 μM, induced delayed increases in glutamate levels in the mPFC (Fig. 6a). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), NMDA (10 μM)/saline (n=6), NMDA (100 μM)/saline (n=7), NMDA (1 mM)/saline (n=7), and NMDA (1 mM)+CPP/saline (n=6) groups were 0.53±0.074, 0.37±0.052, 0.46±0.068, 0.62±0.063, and 0.46±0.040, respectively.

Perfusion with SKF-38393 at 200 μM, but not 20 μM, initially decreased extracellular glutamate levels in the mPFC, which was followed by delayed increases in glutamate levels at post-perfusion; these changes were blocked by co-perfusion with SCH-23390 (40 μM) (Fig. 6b). SKF-38393 (20 μM) and SCH-23390 (40 μM) had no effect on glutamate levels. Basal levels of glutamate (pmol/μl) for vehicle/saline (n=7), SKF38393 (20)/saline, (n=6) SKF38393 (200)/saline (n=6), and SKF38393 (200)+SCH23390 (40)/saline (n=6) groups were 0.40±0.079, 0.30±0.025, 0.42±0.060, and 0.48±0.057, respectively.

Co-perfusion with NMDA (10 μM) and SKF-38393 (20 μM), each of which alone had no effect on basal glutamate levels at these concentrations, induced delayed increases in glutamate levels in the mPFC. Furthermore, co-perfusion with NMDA (1 mM) and SKF-38393 (200 μM) markedly increased basal glutamate levels in the mPFC, and the higher levels persisted after co-perfusion (Fig. 6c). Basal levels of glutamate (pmol/μl) for vehicle/saline (n=6), NMDA (10)+SKF38393 (20)/saline (n=8), and NMDA (1 mM)+SKF38393 (200)/saline (n=6) groups were 0.53±0.075, 0.58±0.098, and 0.35±0.041, respectively.

Discussion

Origin of extracellular concentrations of glutamate levels measured by microdialysis

Rowley et al. (1995) demonstrated that about 40% of extracellular concentrations of glutamate measured by microdialysis are derived from exocytotic mechanisms, although a subsequent review (Timmerman and Westerink 1997) made the criticism that use of either the tetrodotoxin-

infusion or calcium-depletion method may not be able to accurately estimate the source of extracellular amino acid in dialysates. In our preliminary experiments, perfusion with 100 mM KCl in the mPFC acutely increased extracellular glutamate levels in this region (333±67.3% rise from basal levels) (data not shown). Ceglia et al. (2004) showed that TTX perfusion in the mPFC blocked a competitive NMDA receptor antagonist, CPP-induced acute increases in glutamate levels but not basal levels of glutamate in this region. These findings suggest that increases in extracellular concentrations of glutamate may, at least in part, reflect changes in the exocytotic release of glutamate from neurons.

Effects of CLZ on PCP-induced acute increases in glutamate levels in the mPFC by enhancing NMDA receptor-mediated neurotransmission

CLZ dose-relatedly inhibited PCP-induced acute increases in glutamate levels, with a weak effect on motor activity emerging after saline injection. Although HAL at 1 mg/kg blocked PCP-induced acute increases in glutamate levels, this dose of HAL completely blocked saline-induced motor activity. Therefore, CLZ is much more effective than HAL in blocking PCP-induced increases in glutamate levels in the mPFC.

As discussed in a previous study (Abekawa et al. 2003), a PCP-induced blockade of NMDA receptors on the GABAergic interneurons in the mPFC (Yonezawa et al. 1998) disinhibits the cortico-cortical glutamatergic neurons (Fonnum et al. 1981; Berendse et al. 1992), leading to increases glutamate levels in the mPFC (Adams and Moghaddam 1998; Moghaddam and Adams 1998; Krystal et al. 2003).

Considering that there are also several in vitro studies that have shown an effect by CLZ on NMDA receptors expressing on the glutamatergic pyramidal neurons (Arvanov et al. 1997; Ninan et al. 2003), we can speculate that CLZ may also be able to similarly enhance NMDA receptor-mediated neurotransmission in GABAergic interneurons in the mPFC. In fact, although there is a difference in anatomical location from the mPFC, CLZ potentiates NMDA receptor-mediated inhibitory neurotransmission in the nucleus accumbens in vitro (Wittmann et al. 2005).

In the present study, systemically administered CLZ dose-relatedly blocked locally applied CPP-induced acute increases in glutamate levels in the mPFC. Similarly, Ceglia et al. (2004) showed CPP-induced acute increases in glutamate levels in the mPFC. In addition, CLZ at 1 mg/kg inhibited PCP (7.5 mg/kg)- but not CPP (200 μM)-induced acute increases in glutamate levels. The inability to block the CPP-induced increases may be due to a larger blocking effect of CPP than of PCP on NMDA receptors. However, these findings at least suggest that CLZ enhances NMDA-mediated neurotransmission in the mPFC. Taken together, the results suggest that CLZ may enhance NMDA receptor-mediated GABAergic neurotransmission in the mPFC, leading to inhibition of PCP-induced acute increases in glutamate levels. Also, although the highest dose of HAL may activate NMDA-mediated neurotransmission, the ability of this typical antipsychotic to enhance NMDA-mediated transmission may be weaker than that of CLZ (ED50 in stimulating NMDA-mediated neurotransmission: CLZ: 14 nmol/l ; HAL: 38 nmol/l) (Arvanov and Wang 1998).

Acceleration of CLZ-induced enhancement of NMDA-mediated neurotransmission by dopamine D_1 receptor-mediated hyperdopaminergic state

In the mPFC, perfusion with NMDA concentration-dependently inhibited PCP-induced acute increases in glutamate levels, suggesting that stimulation of NMDA receptors blocks the PCP-induced acute effect. However, perfusion of the dopamine D_1 receptor agonist, SKF-38393 had no effect on the PCP-induced acute increases in glutamate levels. This suggests that stimulation of the dopamine D_1 receptor only has no effect on the PCP-induced effect. When NMDA and SKF-38393 were co-perfused, concentrations that individually had no effect on PCP-induced acute increases in glutamate levels, completely inhibited the increase in glutamate levels. Although different from the GABAergic neurons, the stimulation of dopamine D_1 receptors enhances NMDA-induced EPSCs in the glutamatergic pyramidal neurons in the mPFC in vitro (Wang and O'Donnell 2001; Gonzalez-Islas and Hablitz 2003). Taken together, the stimulation of the dopamine D_1 receptor may accelerate NMDA receptor-mediated GABAergic neurotransmission.

CLZ markedly increases dopamine levels in the mPFC (Koyama et al. 1994). As the efficacy of CLZ in blocking the dopamine D_1 receptors is weak (Matsubara et al. 1993), the CLZ-induced effect of increasing dopamine levels may overcome its weak blocking effect on dopamine D_1 receptors. As a result, CLZ may enhance dopamine D_1 receptor-mediated neurotransmission (Ahlenius 1999; Oerther and Ahlenius 2000). CLZ stimulates dopamine D_1 receptors, which then enhance the NMDA receptor-activating effect of this atypical antipsychotic (Chen and Yang 2001; Ninan and Wang 2003). In the nucleus accumbens, CLZ stimulates dopamine D_1 receptors, which enhances NMDA receptor-mediated neurotransmission in vitro (Wittmann et al. 2005). However, in the present study, perfusion with SKF-398393 did not reverse CLZ-mediated blocking of PCP-induced increases in glutamate levels. Taken together, the results suggest that, although dopamine D1 receptor stimulation enhances the inhibitory effect of NMDA on PCP-induced rise of glutamate levels, this

mechanism does not contribute to the ability of CLZ to prevent the effect of PCP on glutamatergic neurotransmission.

CLZ-induced delayed increases in glutamate levels

Similar to the study reported by Yamamoto et al. (1994), we observed that a 10 mg/kg dose of CLZ induced delayed increases in glutamate levels emerging after saline injection. These delayed increases were blocked by perfusion with either CPP or SCH-23390. NMDA stimulates presynaptic NMDA receptors on glutamatergic neuronal terminals to increase glutamate levels in the mPFC (Arco and Mora 2002). SKF-38393 stimulates presynaptic dopamine D_1 receptors on glutamatergic neuronal terminals to decrease glutamate levels in this region (Abekawa et al. 2000). In the present study, we found that delayed glutamate increases were followed by initial decreases in glutamate levels. Although there are differences between the mPFC and the ventral tegmental area (VTA), similar dopamine D_1 receptor agonist-induced biphasic changes in glutamate levels in the VTA have been previously reported (Wolf and Xue 1998). Based on these results, CLZ may have to stimulate not only dopamine D_1-mediated but also NMDA-mediated neurotransmission in order to induce the delayed increase in glutamate levels. In the present study, NMDA and SKF-38393 co-perfusion induced delayed increases in basal glutamate levels even though individual doses had no effect on basal glutamate levels.

Effects of CLZ and HAL on PCP-induced hyperlocomotion

The present study has reconfirmed the attenuating effect of CLZ on PCP-induced hyperlocomotion, with a much weaker decrease in saline-induced locomotor activity in response to CLZ than to HAL. Although small doses of CLZ and HAL may attenuate a stress-induced dopamine rise in the nucleus accumbens that directs locomotor activity (Kelly and Iversen 1976), HAL (1 mg/kg) completely blocked spontaneous movement even before PCP or saline injection (analysis of time course of locomotor activity—data not shown). Therefore, the blocking effect of HAL (1 mg/kg) on PCP-induced hyperlocomotion is likely to be influenced by extrapyramidal dysfunction.

Microinjection of PCP bilaterally into the PFC produces hyperlocomotion, and bilateral ibotenic acid lesions of the PFC sharply blunt this PCP-induced hyperlocomotion (Jentsch et al. 1998), suggesting that the primary site of pharmacological action of PCP is the PFC. Application of NMDA to the PFC was reported to inhibit PCP-induced increases in extracellular dopamine levels (Umino et al. 1998) and decreases in extracellular GABA levels (Yonezawa et al. 1998) in this region. Data from the present study suggest that PCP-induced hyperlocomotion depends on the ability of PCP to increase extracellular glutamate levels in the mPFC. To support this suggestion, we need to show in future work, using microinjection techniques, that all conditions (microinjection of NMDA or NMDA+SKF-38393 to the bilateral mPFC) preventing the effect of PCP on extracellular glutamate rise also prevent PCP-induced hyperlocomotion.

Previously, our group (Abekawa et al. 2003) showed that 5-HT_{2A} receptor blockade may inhibit PCP-induced acute increases in glutamate levels in the mPFC. Consistently, Ceglia et al. (2004) reported that a selective 5-HT_{2A} receptor antagonist, M100907, blocks CPP-induced increases in glutamate levels in the mPFC. Therefore, CLZ might block 5-HT_{2A} receptors to inhibit PCP-induced increases in glutamate levels.

Adams and Moghaddam (2001) have shown that CLZ has no effect on PCP-induced increases in glutamate levels in the PFC, nor does it have any effect on PCP-induced hyperlocomotion. These results differ quite remarkably from our findings. Such differences between previous studies and the present work could be attributed to the definition of mPFC, recovery and size of the probe.

In conclusion, both CLZ and HAL dose-relatedly attenuated PCP-induced hyperlocomotion, and concentration-relatedly blocked PCP-induced acute increases in glutamate levels in the mPFC, with a much weaker decrease in saline-induced locomotor activity by CLZ than HAL. CLZ dose-relatedly blocked an acute increase in glutamate levels in the mPFC induced by local perfusion with a competitive NMDA receptor antagonist, CPP, in this region. Although perfusion with a dopamine D_1 receptor agonist, SKF-38393, enhanced the blocking effect of a subthreshold concentration of NMDA perfusion in this region on PCP-induced acute increases in glutamate, perfusion with SKF-38393 did not reverse CLZ-mediated blocking of PCP-induced acute increases in glutamate levels. Therefore, CLZ might block the PCP-induced acute increases in glutamate levels in the mPFC by enhancing NMDA receptor-mediated neurotransmission in this region that is not accelerated by enhanced dopaminergic transmission via dopamine D_1 receptors. CLZ and novel agents that enhance both dopamine D_1 and NMDA receptor-mediated transmission can improve TRS-related pathophysiology.

Acknowledgements This study was supported in part by Grant-in-Aid No 14370287 and No 15591207 for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

Abekawa T, Ohmori T, Ito K, Koyama T (2000) D_1 dopamine receptor activation reduces extracellular glutamate and GAB concentrations in the medial prefrontal cortex. Brain Res 867:250–254

Abekawa T, Honda M, Ito K, Koyama T (2003) Effects of NRA0045, a novel potent antagonist at dopamine D_4, 5-HT_{2A}, and α_1 adrenaline receptors, and NRA0160, a selective D_4 receptor antagonist, on phencyclidine-induced behavior and glutamate release in rats. Psychopharmacology 169:247–256

Adams B, Moghaddam B (1998) Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci 18:5545–5554

Adams BW, Moghaddam B (2001) Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. Biol Psychiatry 50:750–757

Ahlenius S (1999) Clozapine: dopamine D_1 receptor agonism in the prefrontal cortex as the code to decipher a Rosetta stone of antipsychotic drugs. Pharmacol Toxicol 84:193–196

Arco AD, Mora F (2002) NMDA and AMPA/kainite glutamatergic agonists increase the extracelluar concentrations of GABA in the prefrontal cortex of the freely moving rat: modulation by endogenous dopamine. Brain Res Bull 57:623–630

Arvanov VL, Wang RY (1998) M100907, a selective 5-HT_{2A} receptor antagonist and a potential antipsychotic drug, facilitates N-methyl-D-aspartate receptor mediated neurotransmission in the rat medial prefrontal cortical neurons in vitro. Neuropsychopharmacology 18:197–209

Arvanov VL, Liang X, Schwartz J, Grossman S, Wang RY (1997) Clozapine and Haloperidol modulate N-methyl-D-aspartate- and non-N-methyl-D-aspartate receptor-mediated neurotransmission in rat prefrontal cortical neurons in vitro. J Pharmacol Exp Ther 283:226–234

Berendse HK, Galis DE, Graaf Y, Gronewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. J Comp Neurol 316:314–347

Bitter I, Dosssenbach MR, Brook S, Feldman PD, Metcalfe S, Gagiano CA, Furedi J, Bartko G, Janka Z, Banki CM, Kovacs G, Breier A, Olanzapine HGCK Study Group (2004) Olanzapine versus clozapine in treatment-resistant or treatment-intolerant schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 28:173–180

Ceglia I, Carli M, Baviera M, Renoldi G, Calcango E, Invernizzi RW (2004) The 5-HT_{2A} receptor antagonist M100,907 prevents extracellular glutamate rising in response to NMDA receptor blockade in the mPFC. J Neurochem 91:189–199

Chen L, Yang CR (2001) Interaction of dopamine D_1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. J Neurophysiol 87:2324–2336

Fonnum F, Mathisen-SJ, Divac I (1981) Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibers in rat brain. Neuroscience 6:863–873

Gonzalez-Islas C, Hablitz JJ (2003) Dopamine enhances EPSCs in layer II–III pyramidal neurons in rat prefrontal cortex. J Neurosci 23:867–875

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301–1308

Jentsch JD, Tran A, Taylor JR, Roth RH (1998) Prefrontal cortical involvement in phencyclidine-induced activation of mesolimbic dopamine system: behavioral and neurochemical evidence. Psychopharmacology 138:89–95

Kane J, Honigfeld G, Singer J, Meltzer H, The Clozaril Collaborative Study Group (1988) Clozapine for the treatment-resistant schizophrenic: a double-blind comparison with chlorpromazine. Arch Gen Psychiatry 45:789–796

Kelly PH, Iversen SD (1976) Selective 6 OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol 40:45–56

Koyama T, Matsubara S, Matsubara R, Inoue T, Yamashita I (1994) Prefrontal dopaminergic activation by clozapine in the medial prefrontal cortex; an in vivo microdialysis study. In: Morogi T, Yamamoto K (eds) The biology of schizophrenia. Elsevier, Amsterdam, pp 233–243

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Charney DS Jr (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine in humans. Arch Gen Psychiatry 51:199–214

Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R (2003) NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. Psychopharmacology 169:215–233

Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neurpsychopharmacology 13:9–19

Lindroth P, Mopper K (1979) High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *o*-phthaldialdehyde. Anal Chem 51:1667–1674

Luisada PV (1978) The phencyclidine psychosis. In: Peterson RC et al (eds) Phencyclidine (PCP) abuse; an appraisal. NIDA Research Monograph 21. National Institute of Drug Abuse, Division of Research, Rockville, pp 241–253

Malhotra AK, Pinals DA, Weinberger H, Sirocco K, Missar CD, Pickar D, Breier A (1996) NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. Neuropsychopharmacology 14:301–307

Malhotra AK, Adler CM, Kennison SD, Elman I, Picker D, Breier A (1997) Clozapine blunts N-methyl-D-aspartate antagonist-induced psychosis: a study with ketamine. Biol Psychiatry 42:664–668

Matsubara S, Matsubara R, Kusumi I, Koyama T, Yamashita I (1993) Dopamine D_1, D_2 and Serotonin$_2$ receptor occupation by typical and atypical antipsychotic drugs in vivo. J Pharmacol Exp Ther 265:498–508

Moghaddam B, Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281:1349–1352

Ninan IPE, Wang RY (2003) Modulation of the ability of clozapine to facilitate NMDA- and electrically evoked responses in pyramidal cells of the rat medial prefrontal cortex by dopamine: pharmacological evidence. Eur J Neurosci 17:1306–1312

Ninan IPE, Jardemark KE, Wang RY (2003) Differential effects of atypical and typical antipsychotic drugs on N-methyl-D-aspartate- and electrically evoked responses in the pyramidal cells of the rat medial prefrontal cortex. Synapse 48:66–79

Oerther S, Ahlenius S (2000) Atypical antipsychotics and dopamine D_1 receptor agonism: an in vivo experimental study using core temperature measurements in the rat. J Pharmacol Exp Ther 292:731–736

Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates, 3rd edn. Academic Press, New York

Rowley HL, Martin KF, Marsden CA (1995) Determination of in vivo amino acid neurotransmitters by high-performance liquid chromatography with o-phthalaldehyde-sulphite derivatization. J Neurosci Methods 57:93–99

Sturgeon RD, Fessler RG, Meltzer HY (1979) Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. Eur J Pharmacol 59:169–179

Timmerman WLA, Westerink BHC (1997) Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27:242–261

Umino A, Takahashi K, Nishikawa T (1998) Characterization of the phencyclidine-induced increase in prefrontal cortical dopamine metabolism in the rat. Br J Pharmacol 124:377–385

Volavka J, Czobor P, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, Cooper TB, Chakos M, Lieberman JA (2002) Clozapine, olanzapine, risperidone, and haloperidol in The treatment of patients with chronic schizophrenia and schizoaffective disorder. Am J Psychiatry 159:255–262

Wang J, O'Donnell P (2001) D(1) dopamine receptors potentiate NMDA-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. Cereb Cortex 11:452–462

Wolf ME, Xue CJ (1998) Amphetamine and D_1 dopamine receptor agonists produce biphasic effects on glutamate efflux in rat ventral tegmental area: modification by repeated amphetamine administration. J Neurochem 70:198–209

Wittmann M, Marino MJ, Henze DA, Seabrook GR, Conn PJ (2005) Clozapine potentiation of N-methyl-D-aspartate receptor currents in the nucleus accumbens: role of NR_{2B} and protein kinase A/Src kinase. J Pharma Exp Ther 313:594–603

Yamamoto BK, Pehek EA, Meltzer HY (1994) Brain region effects of clozapine on amino acid and monoamine transmission. J Clin Psychiatry 55:8–14

Yonezawa Y, Kuroki T, Kawahara T, Tashiro N, Uchimura H (1998) Involvement of γ-aminobutyric acid neurotransmission in phencyclidine-induced dopamine release in the medial prefrontal cortex. Eur J Pharmacol 341:45–56