Neuropharmacology 58 (2010) 632–639

Contents lists available at ScienceDirect

Neuropharmacology

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

Activation of metabotropic glutamate (mGlu)2 receptors suppresses histamine release in limbic brain regions following acute ketamine challenge

Matthew J. Fell*, Jason S. Katner, Bryan G. Johnson, Albert Khilevich, Jeffrey M. Schkeryantz, Kenneth W. Perry, Kjell A. Svensson

Neuroscience Discovery Research, Eli Lilly and Company, Indianapolis, IN 46285, USA

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
30 October 2009
Accepted 18 November 2009

Keywords:
In vivo microdialysis
Ketamine
mGlu2/3 receptors
Histamine
LY379268
CBiPES

ABSTRACT

In the present study we demonstrated that ketamine, an NMDA antagonist and possible psychotomimetic, increases extracellular histamine (HA) in the rat brain. We then examined the ability of the group II mGlu receptor agonist LY379268 to modulate the ketamine evoked increases in HA release in three limbic brain regions. Ketamine (25 mg/kg) increased HA in the medial prefrontal cortex (mPFC), ventral hippocampus (vHipp) and the nucleus accumbens (NAc) shell. LY379268 administered alone was without effect on basal HA efflux in the mPFC or vHipp but modestly decreased HA efflux in the NAc shell. Administration of LY379268 (3 and 10 mg/kg) prior to ketamine significantly attenuated the HA response in the mPFC, vHipp and the NAc shell. The inhibitory effects of LY379268 in the mPFC were mimicked by the systemic administration of the mGlu2 receptor positive allosteric modulator CBiPES (60 mg/kg). Finally, local perfusion experiments revealed that the effects of LY379268 on ketamine evoked HA efflux appear to be mediated by mGlu2 receptors outside the PFC as the intra-mPFC perfusion of LY379268 (100 μM or 300 μM) failed to attenuate ketamine evoked increases in HA efflux. Together, these novel observations reveal an effect of ketamine on histaminergic transmission in limbic brain areas and provide further insight into the possible antipsychotic mechanism of action of mGlu2/3 receptor agonists.

1. Introduction

The acute administration of non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists (phencyclidine, ketamine and MK-801) is a commonly used experimental model of schizophrenia because in humans these drugs induce symptoms common to schizophrenia (Javitt and Zukin, 1991). In rodents, the effects of NMDA receptor antagonists manifest as increases in locomotor activity, stereotypies (Danysz et al., 1994; Moghaddam et al., 1997), impaired cognition and attentional deficits (Danysz et al., 1988; Parada-Turska and Turski, 1990). Evidence suggest that the behavioral effects of NMDA receptor antagonists may be mediated by increased limbic forebrain release of glutamate (Moghaddam et al., 1997), dopamine (DA) (Moghaddam et al., 1997), norepinephrine (NE) (Kubota et al., 1999) and serotonin (5-HT) (Lindefors et al., 1997) and an inhibition of GABAergic neurotransmission (Coyle, 2004; Yonezawa et al., 1998).

Although studied to a lesser extent, a role for the histaminergic system in the effects of NMDA receptor antagonists has been suggested. NMDA receptor antagonists such as PCP and MK-801 enhance levels of the major histamine metabolite N^{T}-methylhistamine (t-MeHA) in limbic brain regions in mice (Faucard et al., 2006; Itoh et al., 1985). Clinically, evidence exists linking a hyperactive histaminergic system to the pathophysiology of schizophrenia. Firstly, elevated t-MeHA levels were detected in the cerebrospinal fluid of patients with schizophrenia when compared to healthy controls (Prell et al., 1995). Secondly, decreases in cortical and subcortical histamine H_1 receptors have been observed at postmortem (Nakai et al., 1991) and in a PET study of patients with schizophrenics (Iwabuchi et al., 2005). Together, these data imply a role for increased histamine neuron activity in schizophrenia and suggest that reducing histaminergic transmission may be beneficial in schizophrenia.

Group II metabotropic glutamate (mGlu) receptors (mGlu2 and mGlu3) are located in forebrain and limbic brain regions and function as presynaptic autoreceptors to limit excessive glutamatergic neurotransmission (Schoepp, 2001). As excessive glutamatergic neurotransmission has been implicated in the pathophysiology of schizophrenia, stimulation of group II mGlu

* Corresponding author. Neuroscience Discovery Research, Lilly Research Laboratories, Lilly Corporate Center, DC0510, Indianapolis, IN 46285, USA. Tel.: +1 317 433 1096; fax: +1 317 276 7600.
E-mail address: Fell_matthew@lilly.com (M.J. Fell).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.11.014

receptors may represent a potential target for the treatment of the disorder (Schoepp and Marek, 2002). In the clinic, LY2140023 (the prodrug of the mGlu2/3 agonist LY404039) demonstrated efficacy in the positive and negative symptoms in patients with schizophrenia (Patil et al., 2007). In pre-clinical animal models, agonists of mGlu2/3 receptors (LY379268, LY345740 and LY404039) block many of the behavioral effects of NMDA receptor antagonists. Moreover, mGlu2/3 receptor agonists have been shown to normalize increased neurotransmitter efflux in limbic brain regions after NMDA receptor antagonism (Lorrain et al., 2003a, 2003b; Moghaddam and Adams, 1998; Schoepp and Marek, 2002; Swanson and Schoepp, 2003).

In the present study, we use in vivo microdialysis to evaluate the effect of ketamine on histamine release in limbic brain regions implicated in the pathophysiology of schizophrenia; mPFC, vHipp and NAc Shell. As previous pre-clinical studies have shown mGlu2/3 receptor agonists to attenuate increases in neurotransmitter efflux after NMDA receptor antagonism, we sought to determine if the mGlu2/3 receptor agonist LY379268 could attenuate the histamine response to NMDA receptor antagonism. Additionally, we investigated whether the effects of LY379268 on ketamine evoked histamine release could be mimicked by selective mGlu2 receptor positive allosteric modulator (PAM) CBIPES (*N*-(4′-cyano-biphenyl-3-yl)-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride). Finally, as the ability of LY379268 to attenuate neurotransmitter efflux after NMDA receptor antagonism may be linked to reduced glutamatergic transmission within the mPFC, we conducted local perfusion studies to determine the role of the mPFC in mediating the effects of LY379268 on ketamine evoked histamine efflux.

2. Methods

2.1. Animals

All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Eli Lilly Institutional Animal Care and Use Committee. Subjects were male Sprague–Dawley rats (Taconic Germantown, NY) weighing between 250 and 350 g. Rats were singly housed with standard laboratory chow and water available ad libitum and maintained on a 12-h light/dark cycle (lights on at 06:00, lights off at 18:00). All efforts were made to minimize the discomfort and the number of animals used. All experiments were performed between 8 am and 4 pm using appropriate vehicle controls.

2.2. Microdialysis procedures

Parts of this microdialysis technique have been previously described (Perry et al., 2008). However for these experiments all surgeries were carried out by Taconic (Germantown, NY) 5–7 days before the experiment. In brief, the rats were anesthetized with isoflurane gas and mounted in a stereotaxic frame. A 2 cm midsagittal skin incision was made in the scalp in order to visualize the skull landmarks: bregma and lambda. Blunt dissection was used to separate and retract the underlying fascia. Three holes were drilled to accept three anchoring screws, while a fourth hole was drilled to accept the implant. A microdialysis guide and stylet (BAS, West Lafayette, IN) were stereotaxically placed in the locations given below. The guide cannula was then bonded to the skull and screws via cranioplastic cement. The stereotaxic coordinates for the PFC cannula were: A (anterior to bregma), 3.2 mm; L (lateral from the midsagittal suture) 0.8 mm; and V (ventral from the dura surface), −2 mm. The coordinates for the vHipp and nucleus accumbens shell were A −5.3 mm; L 4.6 mm; V, −3.5 mm; A, 1.7 mm; L, 0.8 mm; V, −6 mm respectively (Paxinos and Watson, 1986). A concentric type probe (BR-4 or BR-2) from BAS (West Lafayette, IN) to match the implanted cannula with a 4 mm (mPFC and vHipp) or 2 mm (NAc shell) membrane tip extending below the cannula was flushed with water and carefully inserted through the cannula 16 h before the experiment began. Immediately after insertion of the probe the rat was placed in the cage where the experiment was to be conducted in order to acclimate to the new cage overnight.

On the morning of the experiment the rat was connected to a fraction collection system for freely moving animals (BioAnalytical Systems (BASi), West Lafayette, IN). The input tube of the dialysis probe was connected to a syringe pump (BeeHive and BabyBee, BASi) which delivered an artificial cerebrospinal fluid containing 150 mM NaCl, 3 mM KCl, 1.7 mM $CaCl_2$ and 0.9 mM $MgCl_2$ (pH 6.0) to the probe at a rate of 1.5 μl/min. The output tubes from the rats were attached to a refrigerated fraction collector (BASi). After a period of 2 h for equilibration of the probe and establishment of stable monoamine baseline levels, collection of 20 min fractions was started. The flow from the output lines was collected in 400 μl plastic tubes which contained 10 μl of an antioxidant solution (3 mM L-cysteine, 10 nM EDTA and 50 nM isoproterenol as an internal standard, in 0.5 M acetic acid). The antioxidant serves to acidify the dialysate and prevents degradation of the amines and metabolites during the 24 h period that it takes to collect the samples and complete the HPLC assays. Typically the total sample volume was 40 μl. Three baseline samples were collected before injection of any drugs. All microdialysis data were calculated as percent change from dialysate basal concentrations with 100% defined as the average of the three drug pre-injection values and each group had 5–8 rats.

2.3. Biochemical determinations

2.3.1. Histamine analysis

Histamine was analyzed using a modified version of the method previously described by Westerink et al. (2002). Histamine was separated on an HPLC column and quantified by fluorimetric detection after a postcolumn derivatization with an o-phthaldialdehyde-containing reagent as follows. The mobile phase consisted of 0.16 M KH2PO4, 0.1 mM sodium octanesulfonic acid and 0.1 mM EDTA; the pH was adjusted to 4.6. The flow rate of the mobile phase was 0.7 ml/min. The eluent line was connected by a T-piece for mixing with a reagent line through which a 0.02% solution of o-phthaldialdehyde (OPA) in 0.15 M NaOH was delivered at 0.6 ml/min. The OPA reagent was mixed with the eluent in a mixing coil of Teflon tubing (OD 1.1 mm, ID 0.55 mm; length 1 m) which was insulated to allow the derivatization reaction to proceed at ambient temperature. The OPA reagent solution was prepared fresh daily, protected from light, and kept cooled in ice. Histamine was separated on a reverse-phase column, a BDS Hypersil, 3 μm, C18 analytical column (4.6 × 100 mm from Thermo-Fisher). The fluorescence of the reaction product was measured by a fluorimeter (Jasco FP-920; excitation: 350 nm; emission: 450 nm) set at the highest sensitivity. The sensitivity for histamine was about 20 fmol per sample (20 μl).

2.4. Drugs

LY379268 and CBiPES (*N*-(4′-cyano-biphenyl-3-yl)-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride)] were synthesized at Lilly Research Laboratories. LY379268 was dissolved in sterile 0.9% NaCl by the drop-wise addition of 5 N NaOH. CBiPES was suspended in a solution consisting of 1% carboxymethylcellulose, 0.25% Tween 80, and 0.05% Dow antifoam. S-(+)-Ketamine hydrochloride was obtained from Sigma Aldrich (St. Louis, MO) and dissolved in sterile water. LY379268 or its vehicle and ketamine or its vehicle was administered subcutaneously (s.c.) in a volume of 1 ml/kg. CBiPES was administered via the intraperitoneal route in a volume of 2 ml/kg. LY379268, CBiPES or vehicle for LY379268/CBiPES was given 40 min prior to ketamine (25 mg/kg, s.c.) or vehicle. The doses of LY379268 (3 and 10 mg/kg), CBiPES (60 mg/kg) and ketamine (25 mg/kg) were selected based on preliminary studies conducted in our laboratory and previously published in vivo behavioral and neurochemical studies. At these doses LY379268 prevents ketamine (25 mg/kg) evoked increases in locomotor activity and increases in glutamate and norepinephrine efflux in the mPFC and vHipp respectively (Lorrain et al., 2003a,b; Moghaddam et al., 1997; Swanson and Schoepp, 2003). For central administration LY379268 and 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX, Tocris) were dissolved in aCSF and the pH adjusted to 7.4 with the drop-wise addition of 5 N NaOH. Both compounds were delivered via the microdialysis probe by manually switching the inlet tubing from a syringe containing aCSF to one containing the drug.

2.5. Statistical analysis

All dialysis probe placements were checked by perfusing a tetra red solution through the dialysis probe for subsequent histological examination. Only results derived from rats with correctly positioned probes were included in the data analysis. All microdialysis data were calculated as percent change from dialysate basal concentrations with 100% defined as the average of the final three drug pre-injection values ($N = 5–7$). Time course data represent the percent change from baseline and were analyzed by two-way analysis of variance (ANOVA) followed by *Post-hoc* Bonferroni corrected *t*-test. The summary data represent the average (±SEM) percent change over a 100 min period post injection of ketamine or vehicle. For the effect of LY379268 on basal histamine the summary data represent average (±SEM) percent change over a 180 min period post injection of LY379268 or vehicle. Summary data were subjected to a one-way analysis of variance (ANOVA) followed by *Post-hoc* analyses using Dunnett's corrected *t*-test. The level of significance was set at $P < 0.05$. All analyses were performed using the GraphPad PRISM statistical program (GraphPad, San Diego, CA).