Neuropharmacology 112 (2017) 198–209

Contents lists available at ScienceDirect

Neuropharmacology

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

Ketamine treatment involves medial prefrontal cortex serotonin to induce a rapid antidepressant-like activity in BALB/cJ mice

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ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form
9 May 2016
Accepted 15 May 2016
Available online 17 May 2016

Keywords:
Ketamine
Serotonin
Rapid antidepressant-like activity
Medial prefrontal cortex
Dorsal raphe nucleus
Antidepressant drug
Highly anxious BALB/cJ mice
Microdialysis

ABSTRACT

Unlike classic serotonergic antidepressant drugs, ketamine, an NMDA receptor antagonist, exhibits a rapid and persistent antidepressant (AD) activity, at sub-anaesthetic doses in treatment-resistant depressed patients and in preclinical studies in rodents. The mechanisms mediating this activity are unclear. Here, we assessed the role of the brain serotonergic system in the AD-like activity of an acute sub-anaesthetic ketamine dose. We compared ketamine and fluoxetine responses in several behavioral tests currently used to predict anxiolytic/antidepressant-like potential in rodents. We also measured their effects on extracellular serotonin levels $[5\text{-HT}]_{ext}$ in the medial prefrontal cortex (mPFCx) and brainstem dorsal raphe nucleus (DRN), a serotonergic nucleus involved in emotional behavior, and on 5-HT cell firing in the DRN in highly anxious BALB/cJ mice. Ketamine (10 mg/kg i.p.) had no anxiolytic-like effect, but displayed a long lasting AD-like activity, i.e., 24 h post-administration, compared to fluoxetine (18 mg/kg i.p.). Ketamine (144%) and fluoxetine (171%) increased mPFCx $[5\text{-HT}]_{ext}$ compared to vehicle. Ketamine-induced AD-like effect was abolished by a tryptophan hydroxylase inhibitor, *para*-chlorophenylalanine (PCPA) pointing out the role of the 5-HT system in its behavioral activity. Interestingly, increase in cortical $[5\text{-HT}]_{ext}$ following intra-mPFCx ketamine bilateral injection (0.25 μg/side) was correlated with its AD-like activity as measured on swimming duration in the FST in the same mice. Furthermore, pre-treatment with a selective AMPA receptor antagonist (intra-DRN NBQX) blunted the effects of intra-mPFCx ketamine on both the swimming duration in the FST and mPFCx $[5\text{-HT}]_{ext}$ suggesting that the AD-like activity of ketamine required activation of DRN AMPA receptors and recruited the prefrontal cortex/brainstem DRN neural circuit in BALB/c mice. These results confirm a key role of cortical 5-HT release in ketamine's AD-like activity following the blockade of glutamatergic NMDA receptors. Tight interactions between mPFCx glutamatergic and serotonergic systems may explain the differences in this activity between ketamine and fluoxetine *in vivo*.

This article is part of the Special Issue entitled 'Ionotropic glutamate receptors'.

1. Introduction

Ketamine, a non-competitive, glutamatergic *N*-methyl-D-aspartate receptor (NMDA-R) antagonist that binds to the phencyclidine site within this ionotropic Ca^{2+} channel, has been found to relieve symptoms within hours when administered at sub-anaesthetic doses in treatment-resistant depressed patients (Berman et al., 2000). Since this discovery, many studies have confirmed ketamine's efficacy in humans as well as in animals. However, the mechanism of action underpinning this rapid antidepressant response in animal models still remains largely unknown.

Preclinical studies with ketamine mainly focused on the glutamatergic system. Thus, ketamine was described as a powerful antagonist at NMDA receptors (elimination half-life < 1 h; *in vitro* $EC_{50} = 760$ nM; *in vivo* $ED_{50} = 4.4$ mg/kg) (Lord et al., 2013; Murray et al., 2000). Antagonism of NMDA-R could be the key pharmacological feature underlying the rapid antidepressant effect of a low dose of ketamine (Krystal et al., 2013). However, the neurochemical mechanisms underlying this response are likely to be more

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http://dx.doi.org/10.1016/j.neuropharm.2016.05.010
0028-3908/

Abbreviations

$[5\text{-HT}]_{ext}$	extracellular serotonin level
5-HT	serotonin
8-OHDPAT	8-Hydroxy-*N*,*N*-dipropyl-2-aminotetralin
aCSF	artificial cerebrospinal fluid
BDNF	brain-derived neurotrophic factor
DA	dopamine
DRN	dorsal raphe nucleus
EPM	elevated plus maze
FST	forced swim test
i.p.	intraperitoneal
mPFCx	medial prefrontal cortex
MDD	major depressive disorder
mTOR	mammalian target of rapamycin
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*] quinoxaline-2,3-dione
NSF	novelty suppressed feeding
OF	open field
PCP	phencyclidine
PCPA	*para*-chlorophenylalanine
TPH	tryptophan hydroxylase
SERT	serotonin transporter
SSRIs	selective serotonin reuptake inhibitors
NMDA-R	glutamatergic NMDA receptor
NMDR-2A/2B	glutamatergic NMDA receptor subunit 2A/2B
VTA	ventral tegmental area
WAY100635	*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)-cyclohexanecarboxamide

complex than a selective blockade of NMDA-R (Naughton et al., 2014). Its pharmacology has shown affinities (*and functional activity*) for PCP-site located on NMDA-R (0.5 μM; *antagonist*), NMDR-2A, NMDR-2B binding sites, but also for non-glutamatergic neurotransmitter receptors [sigma-1 receptor (*agonist*), muscarinic, μ opioid receptor, dopamine D_2 receptor (0.5 μM), 5-HT_2 receptor (*in vitro* 15 μM)] (Kapur and Seeman, 2002). Thus, the fast antidepressant effect of ketamine may involve non-selective multi-system changes, including the serotoninergic system, *via* direct and indirect effects (Kapur and Seeman, 2002). Indeed, recently, an *in vivo* microdialysis study performed in the prefrontal cortex of awake monkeys showed an increase in extracellular serotonin (5-HT) levels after acute ketamine injection (Yamamoto et al., 2013). Although functional interactions between glutamate and monoamines are well documented, surprisingly, an acute ketamine administration did not affect the firing activity of serotonin and dopamine neurons in rats (El Iskandrani et al., 2015).

Although ketamine antidepressant-like effects have been assessed, no study performed a behavioral characterization from the antidepressant-like effects to the anxiolytic-like effects.

The medial prefrontal cortex (mPFCx) plays a key role in ketamine's pharmacological effects, because NMDA-R, the main target with highest affinity to ketamine (Murray et al., 2000), is widely expressed in this brain region (Kamiyama et al., 2011; Sanz-Clemente et al., 2013). Artigas's group demonstrated that 5-HT release in the mPFCx depends on the excitatory glutamatergic transmission (Lopez-Gil et al., 2012). Moreover, it has been shown that mPFCx projections to the dorsal raphe nucleus (DRN) control stressful behavior (Amat et al., 2016). Indeed, a recent study demonstrated that the mPFCx is an important brain region in which deep brain stimulation produced the most profound antidepressant effects on a variety of depressive-like behavioral tests in rats (Lim et al., 2015). Thus, here, we hypothesized that serotonergic efflux in the mPFCx/DRN circuit can play a role, at least partially, in ketamine-induced rapid/long lasting antidepressant-like activity in rodents.

First, our study aimed to perform a behavioral characterization of putative long lasting anxiolytic/antidepressant-like effects ketamine (3 or 10 mg/kg, i.p., 24hr before testing), in comparison to fluoxetine (18 mg/kg, i.p, 24hr before testing) in male BALB/cJ mice using different behavioral paradigms predictive of anxiolytic- or antidepressant-like activity. Second, we investigated the role of the serotoninergic component in ketamine −induced changes in behavioral activity using *para*-chlorophenylalanine (PCPA)-induced serotonin depletion in the DRN and also following serotonin release as measured in the mPFCx using *in vivo* microdialysis under the same experimental conditions as behavioral tests. Finally, we challenged the effects of local intra-mPFCx ketamine administration in the microdialysis and the FST measured in the same animals, and then extending to a combination with intra-DRN administration of AMPA receptor antagonist NBQX to clarify the implication of mPFCx/DRN neural circuit. The present experimental strategy offers the possibility of linking ketamine's antidepressant/anxiolytic activity to the serotonergic system in regard to the behavioral and neurochemical levels, giving furthermore persuasive evidence for the implication of a serotonergic pathway in ketamine's antidepressant mechanism.

2. Materials and methods

2.1. Animals

Male BALB/cJ mice (7–8-weeks old) weighing 23–25 g at the beginning of the experiments were purchased from Janvier Labs (Le Genest-Saint-Isle). The BALB/cJ strain of mice was chosen for its baseline anxiety phenotype (Dulawa et al., 2004). They were housed in groups of five in a temperature (21 ± 1 °C) controlled room with a 12 h light: 12 h dark cycle (lights on at 06:00 h). Food and water were available *ad libitum* except during behavioral observations. Particular efforts were made to minimize the number of mice used in the experiments. Protocols were approved by the Institutional Animal Care and Use Committee in France (Council directive # 87–848, October 19, 1987, "Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92–196" to A.M.G.) as well as with the European directive 2010/63/EU.

2.2. Drugs and treatments

Ketamine (3 or 10 mg/kg) purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and fluoxetine hydrochloride (18 mg/kg) purchased from Anawa Trading (Zurich, Switzerland) were dissolved in vehicle (NaCl 0.9%) and administered 24 h prior to the behavioral tests. Drug doses and pre-treatment times were based on previous studies performed either in our laboratory for fluoxetine (David et al., 2009) or in the literature for ketamine (Li et al., 2010; Liu et al., 2012; Iijima et al., 2012; Koike et al., 2013; Zanos et al., 2015). Diazepam (1.5 mg/kg, i.p., 30 min before testing) was used as a positive control, in animal paradigm predictive of anxiolytic-like effects (David et al., 2007). *Para*-chlorophenylalanine methyl ester