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Pharmacological blockade of GluN2B-containing NMDA receptors induces antidepressant-like effects lacking psychotomimetic action and neurotoxicity in the perinatal and adult rodent brain

Juan M. Lima-Ojeda [a], Miriam A. Vogt [a], Natascha Pfeiffer [a], Christof Dormann [a], Georg Köhr [b,c], Rolf Sprengel [c], Peter Gass [a], Dragos Inta [a,*]

[a] RG Animal Models in Psychiatry, Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany
[b] RG Physiology of Neural Networks, Psychiatry/Psychopharmacology, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany
[c] Department of Molecular Neurobiology, Max Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany

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ABSTRACT

NMDA receptor (NMDAR) antagonists like ketamine and MK-801 possess remarkable antidepressant effects with fast onset. However, they over-stimulate the retrosplenial cortex, evoking psychosis-like effects and neuronal injury, revealed by de novo induction of the heat shock protein 70 (Hsp70). Moreover, early in the development MK-801 triggers widespread cortical apoptosis, inducing extensive caspase-3 expression. Altogether these data raise strong concerns on the clinical applicability of NMDAR antagonist therapies. Therefore, the development of novel therapeutics targeting more specifically NMDAR to avoid psychotomimetic effects is necessary. Here we investigated a GluN2B (NR2B) antagonist in behavioral and neurotoxicity paradigms in rats to assess its potential as possible alternative to unspecific NMDA receptor antagonists. We found that treatment with the GluN2B specific antagonist Ro 25-6981 evoked robust antidepressant-like effects. Moreover, Ro 25-6981 did not cause hyperactivity as displayed after treatment with unspecific NMDAR antagonists, a correlate of psychosis-like effects in rodents. Additionally, Ro 25-6981, unlike MK-801, did not induce caspase-3 and HSP70 expression, markers of neurotoxicity in the perinatal and adult brain, respectively. Moreover, unexpectedly, in the adult retrosplenial cortex Ro 25-6981 pretreatment significantly reduced MK-801-triggered neurotoxicity. Our results suggest that GluN2B antagonists may represent valuable alternatives to unspecific NMDAR antagonists with robust antidepressant efficacy and a more favorable side-effect profile.

1. Introduction

Monoaminergic antidepressants represent the main class of drugs used in the treatment of depression. These substances show, however, a delayed onset of action of weeks and a partially incomplete efficacy. Therefore, there is a need for novel alternative drugs with antidepressant potential. Pharmacological agents acting on the glutamate system, especially those blocking ionotropic NMDAR, are intensively studied as alternative treatments (Autry et al., 2011; Li et al., 2010). NMDAR antagonists, such as ketamine, induce robust antidepressant effects with fast onset, both in animal models of depression (Maeng et al., 2008) and clinical studies (Berman et al., 2000; Zarate et al., 2006). The mechanism by which NMDAR antagonists exert their fast antidepressant action is only partly understood, recent evidence indicating rapid synthesis of brain-derived neurotrophic factor and rapid synapse formation by NMDAR antagonists (Autry et al., 2011; Li et al., 2010). Despite these encouraging results, the clinical use of NMDAR antagonists as antidepressants is strongly hampered by severe side effects, particularly by psychotic symptoms in humans (Krystal et al., 1994). Moreover, in rodents, NMDAR antagonists determine cortical neurotoxicity, causing neuronal injury in layers III and IV of the cingulate and retrosplenial cortex (Fix et al., 1993; Olney et al., 1989; Tomitaka et al., 2000a). The neurotoxic effect is the result of overstimulation of this cortical area by NMDA receptor antagonists as indicated by prolonged expression of the immediate early gene c-Fos (de Olmos et al., 2009; Gass et al., 1993). The induction of the heat shock protein 70 (Hsp70) in the retrosplenial cortex is also a consequence of intense overstimulation of this area by NMDAR antagonists and represents a reliable immunohistochemical marker for quantifying cortical injury (Sharp et al., 1992).

Abbreviations: Hsp70, heat shock protein 70; NMDAR, n-methyl-D-aspartate receptors; P7, Postnatal Day 7; i.p, intraperitoneal; DG, dentate gyrus.

* Corresponding author at: Central Institute for Mental Health Mannheim, University of Heidelberg, J 5, 68159 Mannheim, Germany. Tel.: +49 621 1703 2933; fax: +49 621 1703 6205.
E-mail address: Dragos.Inta@zi-mannheim.de (D. Inta).

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On the other hand, NMDAR antagonists induce also at earlier, perinatal stages strong neurotoxic effects. This results in a widespread cortical apoptosis as shown by caspase-3 expression after administration of NMDAR antagonists at postnatal day 7 (P7) (Ikonomidou et al., 1999). Perinatal treatment with MK-801 results in protracted morphological, gene expression and behavioral changes at pre- and postpubertal stages, showing similarities to alterations observed in schizophrenia (Harris et al., 2003; Uehara et al., 2009).

Despite the data obtained in pharmacological and numerous genetic models of NMDAR inactivation, the contribution of different NMDAR subunits to the development of deleterious side-effects remains unclear (for review see Inta et al., 2010). However, the functional involvement of different NMDAR subtypes is important for the development of more selective therapeutics with fewer side effects. NMDA receptors are tetramers composed of two obligatory GluN1 (NR1) and two GluN2 (NR2) subunits, with GluN2A (NR2A) and GluN2B (NR2B) as the predominant subunits expressed in the forebrain (Monyer et al., 1994).

In order to avoid deleterious side effects of non-specific NMDAR antagonists, subunit-specific NMDAR antagonists were developed. Recently, a GluN2B antagonist displayed in a clinical trial similar antidepressant potency as global NMDAR antagonists (Preskorn et al., 2008). Here we analyzed potential antidepressant-like effects of the GluN2B antagonist Ro 25-6981 in a standard rodent test (Porsolt forced swim test). Additionally, we investigated if Ro 25-6981 induces hyperactivity similarly as MK-801, as animal correlate of psychosis-like symptoms, in the openfield test. We have previously reported that, in contrast to MK-801, Ro 25-6981 does not induce a significant cortical c-Fos expression (Inta et al., 2009; Inta et al., 2012a, 2012b). To further analyze mechanisms potentially underlying or influencing the side-effect profile of Ro 25-6981, we compared the neurotoxicity induced by Ro 25-6981 and MK-801, alone or in combination, in the developing postnatal and adult retrosplenial rat cortex by analyzing at these stages the expression of well-established markers for NMDAR-triggered neurotoxicity, caspase-3 (Ikonomidou et al., 1999; Hanslick et al., 2009) and Hsp70 (Dickerson and Sharp, 2006; Sharp et al., 1992, 1994; Tomitaka et al., 2000a, 2000b).

2. Materials and methods

2.1. Animals

For evaluation of neurotoxicity in the adult retrosplenial cortex we used an established marker, heat shock protein 70 (Hsp70) expression (Sharp et al., 1992) in 3-months-old female Sprague–Dawley rats (Janvier, Le Genest Saint Isle, France). Females were used, because NMDAR-induced neurotoxicity is much stronger in females than in males (Sharp et al., 1992). Rats were used instead of mice due to the specificity of the anti-Hsp70 antibody for rats, and because MK-801 triggers a significantly stronger cortical neurotoxicity in adult rats compared to mice (Bender et al., 2010). For analysis of apoptosis at perinatal stages by caspase-3 expression we used P7 C57BL/6N mice (Charles River, Sulzfeld, Germany), kept in macrolon type II cages. Rats were kept 2 weeks before and during the experimental phase single-housed in macrolon type III cages in a dark-light cycle with lights on at 7 am.

For the assessment of behavioral changes due to MK-801 and Ro 25-6981 treatment as well as the synergistic effects of both substances, male C57BL/6N (Ch. River, 11 weeks old) were tested in either a openfield ($n = 32$) or a Porsolt Forced Swim Test ($n = 32$). All behavioral tests were conducted during the dark phase. Mice were kept 2 weeks before and during the experimental phase single-housed in macrolon type II cages in a reversed dark-light cycle with lights on at 7 pm. Animals were housed in standard conditions, humidity 40–60%, temperature 22 ± 0,5 °C, supplied with standard rodent food (SSNIFF) and water ad libitum. Food was autoclaved before using.

All experiments had been approved by the German Committee on Animal Care and Use, according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drug treatment

For Hsp70 experiments, adult female rats ($n = 6$ for each group) were injected with (1) Ro 25-6981 (10 mg/kg i.p.) or (2) MK-801 (2 mg/kg i.p.) or (3) Ro 25-6981 (10 mg/kg i.p.) 30 min before MK-801 (2 mg/kg i.p.), or MK-801 (2 mg/kg i.p.) alone, and perfused 24 h later. For determination of caspase-3 expression, 7-days-old mice ($n = 6$ for each drug) were treated with (1) Ro 25-6981 (10 mg/kg i.p.) or (2) MK-801 (0.5 mg/kg i.p.) or with (3) Ro 25-6981 (10 mg/kg i.p.) 30 min before MK-801 (0.5 mg/kg i.p.) respectively, and sacrificed 8 h later.

For both behavioral tests, animals were injected intraperitoneally with saline (4 ml/kg), Ro 25-6981 (10 mg/kg), MK-801 (0.5 mg/kg) or Ro 25-6981 (10 mg/kg) in combination with MK-801 (0.5 mg/kg) ($n = 8$ for each treatment group in each test). All substances were dissolved in saline and injected diluted in a volume of 4 ml/kg bodyweight. For the Porsolt forced swim test, saline, MK-801 and Ro 25-6981 were injected 30 min before testing. For the combined treatment with Ro 25-6981 and MK-801, Ro 25-6981 was injected 1 h before testing, while MK-801 was subsequently injected 30 min before testing. For the openfield test, saline, MK-801 and Ro 25-6981 were injected directly before introducing the animals into the openfield arena. For the treatment with Ro 25-6981 and MK-801, Ro 25-6981 was injected 30 min before testing, while MK-801 was injected directly before testing.

2.3. Immunohistochemistry

50 μm coronal free floating vibratome sections were washed in 0.1 M phosphate buffer saline containing 0.3% Triton X-100 (PBST), pH 7.4, immersed in 0.3% hydrogen peroxidase for 20 min, and washed again before being incubated for 24 h at 4 °C with the respective primary antibody: anti-Hsp70 monoclonal rat (Enzo Life Sciences, Germany), 1:1000 or anti-caspase 3 polyclonal rabbit (Cell Signalling, Beverly, MA, USA) 1:1000. Afterwards, sections were incubated with secondary antibody (biotinylated anti-rabbit IgG for the caspase-3 and biotinylated anti-mouse IgG for the Hsp70 staining, Vector Laboratories, diluted 1:400) and processed for the DAB reaction, as described previously (Böttiger et al. 1999; Gass et al., 1995).

2.4. Cell counting

Cell counting was performed by an investigator blind to the treatment using a light microscope (LEICA TCS-NT). Quantification of Hsp70-positive cells was performed within a 0.1 mm^2 area centered over layer III of the retrosplenial cortex, bilaterally, every third section being counted (Inta et al., 2012a). Quantification of caspase-3 expressing cells was done in the retrosplenial cortex of P7 mice in analogy.

2.5. Behavioral testing procedures

The openfield test examines the locomotion and activity by monitoring the mouse in a square shaped, white Open field, measuring 50 × 50 cm^2, illuminated from above by 25 lx (Chourbaji et al., 2008; Hellweg et al., 2007). Mice were placed individually into the arena and monitored for 90 min by a Video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision X8 (Noldus Information Technology, Wageningen, the Netherlands). For each sample, the system recorded position, object area and the status of defined events. Parameters assessed for the present study were total distance moved, velocity, and time in center, which was defined as the area 10 cm distant from the walls.