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SHORT COMMUNICATION

Role of the nitric oxide donor sodium nitroprusside in the antidepressant effect of ketamine in mice

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Abstract
Ketamine may represent an efficient alternative antidepressant with rapid therapeutic onset; however, the clinical use of ketamine is hampered by psychosis-like side-effects. Recent studies suggest that the nitric oxide (NO) donor sodium nitroprusside (SNP) prevents psychosis-like abnormalities triggered by ketamine or another NMDA receptor (NMDAR) antagonist, phencyclidine (PCP) in rats. SNP was shown to elicit antipsychotic effects also in humans. Considering the tight interrelation between NMDAR activation and neuronal NO synthesis, we evaluated the effect of pre-treatment with SNP on the antidepressant action of ketamine. We found that SNP (0.5–1 mg/kg, i.p.) did not alter the antidepressant effect of ketamine (30 mg/kg) in the Porsolt Forced Swim Test (FST) in mice. Additionally, SNP by itself produced no effect in the FST or in the openfield. This suggests indirectly a differential involvement of the nitrinergic system in the antidepressant vs. psychotomimetic effect of ketamine, although an influence of species-specific differences cannot be excluded in this interpretation.

1. Introduction

Classic monoaminergic drugs represent the main class of antidepressants used in treating major depression. Main drawbacks of these substances are the delayed onset of action with onset only weeks after treatment initiation and the incomplete efficacy. Glutamatergic drugs like the NMDAR antagonist

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ketamine may represent valuable alternative drugs, showing fast onset within few hours and sustained therapeutic action lasting up to 2 weeks (Berman et al., 2000). Three main targets for the anti-depressive action of ketamine have been identified: mammalian target of rapamycin (mTOR), eukaryotic elongation factor 2 (eEF2), and glycogen synthase kinase-3 (GSK-3) (Niciu et al., 2014). However, it remains unclear, if only these molecular pathways are responsible for the rapid, long-lasting antidepressant effect of ketamine. NMDAR are interconnected with numerous proteins in the postsynaptic density and activate complex intracellular pathways. Of similar complexity are the behavioral effects induced by ketamine, including also psychotic symptoms.

Glutamate acting through NMDAR provides the main activation signal for neuronal nitric oxide (NO) synthesis. The NO donor SNP blocks in rats schizophrenia-like abnormalities induced by ketamine (Maia-de-Oliveira et al., 2015) and another NMDAR antagonist, phencyclidine (PCP) (Bujas-Bobanovic et al., 2000). Recent clinical studies reported significant antipsychotic action of a single dose of SNP in humans with schizophrenia (Hallak et al., 2013), an effect that was long-lasting over weeks comparable with the antidepressant effect of ketamine. These results suggest a role of nitrinergic pathways in the psychotomimetic effects of NMDAR antagonists and possibly in schizophrenia.

Here we aimed to clarify if SNP plays as well a role in the antidepressant effect of ketamine. Of note, the possible role of SNP in depression-like paradigms was not examined up to date.

2. Experimental procedures

2.1. Animals

For the assessment of behavioral changes due to ketamine or SNP treatment as well as the synergistic effects of both substances, male C57BL/6 N (Charles River, Sulzfeld, Germany, 12 weeks old) were used. 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 p.m., the behavioral testing was conducted during the dark phase (9 a.m.–1 p.m.). Animals were housed in standard conditions, humidity 40–60%, temperature 22±0.5 °C, supplied with standard rodent food (SSNIFF) and water ad libitum, as done previously (Inta et al., 2012). 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

Animals were divided in two groups, one group was assigned to the openfield, and the other was dedicated to perform the Porsolt Forced Swim Test. For the openfield, mice were treated (n=6 per treatment group) with either Saline (4 ml/kg), SNP (0.5 mg/kg), SNP (1 mg/kg) or ketamine (30 mg/kg) to test for locomotion effects of the single substances. For the Porsolt Forced Swim Test, the animals were divided in 6 treatment groups and injected as follows with (1) saline (4 ml/kg), (2) SNP (0.5 mg/kg), (3) SNP (1 mg/kg), (4) ketamine (30 mg/kg), (5) SNP (0.5 mg/kg) in combination with ketamine (30 mg/kg), or (6) SNP (1 mg/kg) in combination with ketamine (30 mg/kg) (n=12 per treatment group). All substances were dissolved in saline and intraperitoneally injected diluted in a volume of 4 ml/kg bodyweight. At day 2 in the Forced Swim Test, mice were treated with the same substance as at day 1 to avoid possible substance interferences. We were guided in choosing the dosage for the SNP treatment by data showing the effectiveness of 0.25–0.5 mg/kg SNP i.p. in increasing the pain threshold in mice (Jain et al., 2001). Indeed, the doses used in the presented study in mice are lower than those used in rats (2–6 mg/kg SNP) which that did not elicit behavioral changes. However, in mice severe deleterious effects were reported when more than 2 mg/kg SNP were applied, including ataxia (Sani et al., 2011), severe hypolocomotion and even catalepsy (Dall'Igna et al., 2001).

2.3. Behavioral testing procedures

2.3.1. Porsolt forced swim test

The FST represents a paradigm for the assessment of despair behavior by analyzing immobility scores in an inescapable aversive situation. Mice were placed into a glass cylinder (23 cm height, 13 cm diameter), which was filled with water (21 °C) up to a height of 12 cm. A testing period of 6 min was used to determine the onset and the percentage of time spent immobile. Mice were monitored by a Video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision XT. For each sample, the system recorded position, object area and the status of defined events. Parameters assessed were latency to become immobile and immobility time.

2.3.2. Openfield test

The openfield test examines the locomotor and explorative characteristics of an animal placed into an unknown arena. Activity monitoring was conducted in a square shaped, white arena, measuring 50 × 50 cm^2 and illuminated from above by 25 lx. Mice were placed individually into the arena and monitored for 10 min by a Video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision XT (Noldus Information Technology, Wageningen, the Netherlands). For each sample, the system recorded position, object area and the status of defined events. Parameters assessed were total distance moved, velocity, and time in center, which was defined as the area 10 cm distant from the walls, as earlier described (Fuss et al., 2010; Berkel et al., 2012).

2.4. Statistical analysis

Statistical analysis was performed using the program SPSS 20. The mean of the parameters assessed in the behavioral tests were calculated for each treatment group and differences between treatment groups were determined by repeated measurement ANOVA with the treatment factors ketamine and SNP for the Porsolt Forced Swim Test or by one-way ANOVA with treatment as factor followed by Bonferroni-post-hoc tests for the openfield data, with $p<0.05$ considered as statistically significant.

3. Results

In the FST the latency to become immobile was enhanced as expected when the animals were treated with ketamine (Figure 1A) (repeated measurement ANOVA factor ketamine: $F(1,66)=33.334$ $p<0.001$). SNP had no effect on latency to become immobile (factor SNP: $F(2,66)=0.993$ $p=0.376$) and did not influence the effect of ketamine on latency (SNP x ketamine: $F(2,66)=0.602$ $p=0.551$). In general, the latency was decreased at the second day of testing (factor time: $F(1,66)=66.341$ $p<0.001$) (Figure 1A). Concerning the immobility time, treatment with ketamine induced clear antidepressant-like effects by decreasing the despair-like

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