Ketamine is antidepressant and enhances neuropeptide Y expression in the prefrontal cortex and hippocampus of a serotonin transporter knock out rat model

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INTRODUCTION

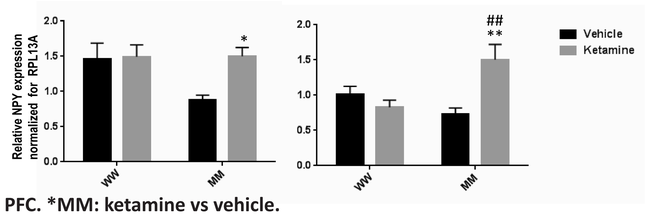
Subanesthetic ketamine exerts rapid antidepressant effect both after a single and repeated administrations. However, the molecular mechanisms of action have not been fully elucidated (Krystal et al 2013; Lapidus et al 2014; Wan et al 2015). Based on our work with rodent models of depression, PTSD and anxiety, in this study focusing on NPY, we hypothesized that: (1) The NPYergic system is altered in a rat model of deficient serotonergic system (SERTKO) that displays depression-like behavior; (2) Ketamine will have antidepressant effect in that model; (3) In similarity to other antidepressant treatment modalities, ketamine will also modify the NPYergic system.

METHODS

Procedures. 35-45 week old male rats, 7-8/group, were injected S-ketamine (ip, 15mg/kg in 0.9% NaCl) or placebo 60 min preceding the 5 min open field test (OFT) in a 60 x 60 x 60 cm box. Ten min forced swim test (FST) using 50 cm high cylinders filled to 35cm with 24o C water was done 10 min after OFT. OFT was analyzed using an automated video tracking system (Noldus Ethovision XT 8, Wageningen, The Netherlands) and FST was video recorded and manually scored in Ethovision XT8 by an investigator blind to the experimental condition. Brains were harvested 30 min after the FST, snap frozen in isopentane and stored at -80o C. Coronal sections, 14 µm thick, were collected at -18o C using a cryostat sectioner (Leica, Kista, Sweden); 20-50 mg of hippocampus and PFC punches were homogenized in microtubes with disposable pestles and total RNA isolated using RNeasy kit (Qiagen, Hilden, Germany). cDNA was prepared by reverse transcription of total RNA with SuperScript III (Invitrogen, Carlsbad, CA, USA) and amplified by gene specific primers in RT-PCR reactions performed on an Applied Biosystems 7300 instrument with SYBR green or TaqMan mix (Applied Biosystems, Foster City, CA, USA). Normalization of target genes expression NPY, NPY-Y2R and NPY-Y1R was performed with RPL13A and Ppia reference genes for each sample.

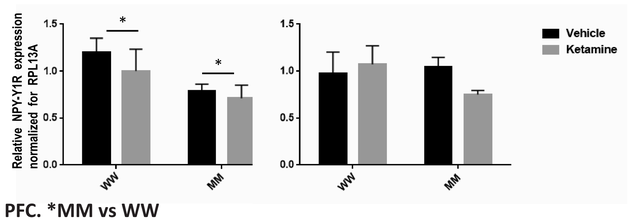
Prefrontal cortex

Hippocampus

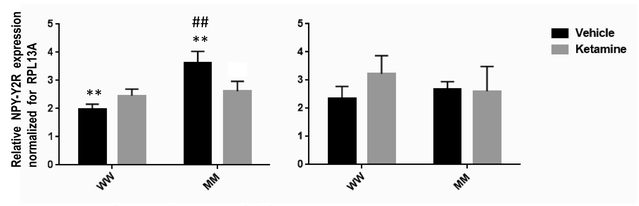


PFC. *MM: ketamine vs vehicle.

Hippocampus. ** MM: ketamine vs vehicle; Ketamine: ## MM vs WW



PFC. *MM vs WW



PFC. **MM: ketamine vs vehicle; ## MM vs WW

Rats received a single ketamine or vehicle ip injection.
WW, wild type; MM, homozygous;
Data were analyzed by a two-way ANOVA followed by a post-hoc test.

RESULTS

BEHAVIOR.

(1) OFT Neither genotype nor treatment had a significant effect on any of the measured parameters in the OFT, that is SERT MM showed normal locomotor behavior and ketamine did not acutely affect it.

(2). FST Vehicle-treated SERT MM rats showed statistically significant higher immobility compared to the WW confirming previous findings that SERT MM rats display a depressive-like phenotype (Neumann et al 2011). Ketamine reduced significantly immobility in the FST.

GENE EXPRESSION NPY, NPY-Y1R, NPY-Y2R.

(1) Prefrontal Cortex

i. NPY: a trend to lower NPY in MM vs WW. Ketamine increased NPY expression in MM (p<.01) but had no effect in WW.

ii. NPY-Y1R: a trend to a decreased expression in MM vs WW (p<.06) Ketamine had no effect.

iii. NPY-Y2R: in placebo treated animals NPY-Y2R was higher in MM compared to the WW (p<.01). Ketamine had no effect in WW but reduced NPY-Y2 in MM (p<.01).

(2) Hippocampus

i. NPY was reduced in MM vs WW. Ketamine increased NPY in MM (p<.01).

ii. NPY-Y1R: no MM vs WW differences. Ketamine had no effect.

iii. NPY-Y2R: no significant differences.

COMMENT

*Dysregulation of the monoaminergic systems is a sufficient but likely not a necessary factor in etiology and pathophysiology of affective disorders. Ample evidence indicates that neuropeptides, glutamatergic signaling, neurotrophic factors and altered neuroplasticity play a role.

*We and others have demonstrated changes in the NPYergic system in depression, PTSD, and anxiety, both in patients and in translational animal models (Cohen et al 2012; Heilig et al 2004; Sah et al 2009; Wu et al 2011) and in this study further explored if ketamine would also affect the NPYergic system by assessing NPY, NPY-Y1 and NPY-Y2 gene expression in the SERTKO model.

The most salient findings were:

i. lower NPY expression in SERT homozygous rats compared to the wild type animals which is in line with our hypothesis that one of the hallmarks of depression is reduced NPY in brain regions relevant for the disorder. Whether due to decreased NPY-Y1R or increased NPY-Y2R effects, or alternative splicing of Npy (Caberlotto, Hurd 2001; Melas et al 2012) is the subject of further investigations. Of note, while it is usually assumed that compounds increasing NPYergic function act by agonistic action on NPY-Y1R, in this study we found that ketamine significantly reduced the inhibitory presynaptic NPY-Y2R thereby increasing effects of NPY, a result consistent with our previous experiment (Bacchi et al 2006).

ii. Our findings indicate that intact serotonergic system is not a prerequisite for the antidepressant effect of ketamine.

iii. Our data add to the consistent findings in humans as well as in animal models that the NPYergic system is altered in states of dysregulated emotionality and that all so far investigated antidepressants increase NPY, thereby adding weight to our hypothesis that it is a candidate for a final common pathway for antidepressant effects (Wu et al 2011).

Study weakness.

The N was small and effects of only a single injection of one ketamine dose were investigated.

Study strength.

First demonstration that
i. ketamine has differential effects in a model of depression (SERTKO MM) vs control animals (wild type).

ii. ketamine increases NPY and decreases NPY-Y2 receptor expression, findings that are likely relevant for its antidepressant effect.

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