ANTIDEPRESSANT-LIKE EFFECTS OF THE AMPA POTENTIATOR S47445 ARE ASSOCIATED WITH CHANGES IN HIPPOCAMPAL BDNF AND mTOR SIGNALING IN BULBECTOMY MICE MODEL

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Purpose of the study
The positive allosteric modulator of glutamate AMPA-type receptors S47445 increases neurotrophic levels, enhancing synaptic plasticity and proconvulsive functions in aged rodent models [1–4]. Moreover, the chronic administration of this compound induces antidepressant- and anxiolytic-like effects in a wide variety of animal models of depression [5]. Here, we have assessed the behavioral effects on depression and anxiety paradigms after the chronic administration of S47445 in the bilateral olfactory bulbectomy (OBX) mice model of depression. In addition, we have evaluated the changes in neurotrophic (BDNF) and neuroplastic markers (mTOR pathway) in the hippocampus, which are associated to the antidepressant effect.

Methods
Animals: Adult C57Bl/6J male mice were submitted to OBX and received S47445 at 3 doses (1-3-10mg/kg/day, i.p.) or fluoxetine (18mg/kg/day, i.p.) for 4 weeks (n=15/group). OBX mice were behaviorally assessed following 3 and 4 weeks of treatment.

Open-field test (locomotor activity): Open-field was performed under high luminance conditions (400 lx), and the peripheral and central locomotor activities were video-recorded and analyzed using ANYMAZE® (ANY-maze®, Stoelting Co., IL).

Western Blot: At the end of behavioral testing (day 28), and after a 24h washout period, mice were sacrificed and the hippocampi were dissected. Whole protein homogenates were extracted using Trisure® Isolation Reagent (Sigma-Aldrich) according to the manufacturer’s instructions. Protein quantification was performed according to the Lowry method (Lowry et al., 1951). About 50-60 µg of protein were resolved on 8.0% SDS-PAGE and transferred to PVDF or nitrocellulose membranes. Membranes were incubated in rabbit anti-BDNF (Santa Cruz Biotechnology), mTOR (Sigma-Aldrich), phospho-mTOR, 4EBP1 and phospho-4EBP1 (Cell Signalling). After washing in TBS-T (1X0.05% Tween 20), membranes were incubated with horseradish peroxidase conjugated anti-mouse secondary antibody. Secondary antibodies were detected with ECL Advance Kit (GE Healthcare Europe GmbH, Munich, Germany). Blot quantifications were performed by densitometric scanning using Scion Image Software. The densitometry values were normalized with respect to the values obtained with anti-glyceraldehyde-3-phosphate dehydrogenase antibody (Sigma-Aldrich). Data are expressed as a percentage of the WT group values (100%) and are presented as the mean ± S.E.M.

Data analysis: Statistical analysis was made using an unpaired Student’s t-test, and one-way ANOVA test. The significance level was set at p<0.05.

Results

Figure 1. Open field test. Reversion of OBX-induced hyperactivity after 21 and 28 days of treatment with S47445 at 1 and 3 mg/kg/day. Student’s t-test, ***p<0.001 vs sham-veh, one-way ANOVA, *p<0.05, #p<0.01 vs OBX-veh.

Figure 2. Open field test. Anxiolytic-like effect in the OBX model after 21 days of treatment with S47445 at 1 and 3 mg/kg/day. No effect was observed after chronic fluoxetine treatment (18 mg/kg/day). One-way ANOVA, #p<0.01 vs OBX-veh.

Figure 5. 4EBP1 (A), p-4EBP1 (B) and ratio p-4EBP1/4EBP1 (C). Increased expression of 4EBP1 with S47445 at 3 mg/kg/day and fluoxetine at 18 mg/kg/day (A). Increased expression of the phosphorylated form after 28 days of treatment with S47445 at 3 mg/kg/day (B). One-way ANOVA, *p<0.05 vs OBX-veh, #p<0.05 vs OBX-Fx.

Figure 3. BDNF. A trend to increased BDNF was observed in OBX model. This was reverted after 28 days of treatment with S47445 3 mg/kg/day. No effect was observed with fluoxetine 18 mg/kg/day. One-way ANOVA, *p<0.05 vs OBX-veh, #p<0.05 vs OBX-Fx.

Figure 4. p-mTOR (A) and ratio p-mTOR/mTOR (B). A trend to increased activated mTOR was present in the hippocampus of OBX mice, that was reverted after 28 days of treatment with S47445 at 3 mg/kg/day. No effect was observed after chronic fluoxetine administration (18 mg/kg/day). One-way ANOVA.

CONCLUSION
- Chronic administration of S47445 induced an antidepressant-like effect in the OBX mice model at low-medium doses (1 and 3 mg/kg/day).
- S47445 counteracts OBX-induced trend of changes by decreasing BDNF and mTOR activation, and by increasing p4EBP1 expression in the hippocampus. Fluoxetine had no effect on these parameters.
- Thus, the antidepressant effects of S47445 were associated with the modulation of hippocampal BDNF/mTOR/p-4EBP1 signaling-pathway in the OBX mice model of depression, in contrast to that observed with fluoxetine.

References

Disclosure
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