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Effects of ketamine and stress on the neurotrophin receptors expression

E. V. Shaburova[✉1, 2], D. A. Lanshakov[1, 2]

[1] Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, 10 Academician Lavrentyev Avenue, Novosibirsk 630090, Russia
[2] Novosibirsk State University, 1 Pirogova Str., Novosibirsk 630090, Russia

Authors
Elizaveta V. Shaburova, SPIN: 4612-4997, Scopus AuthorID: 57209880329, ORCID: 0000-0001-7795-2534, e-mail: sliza.nsk@gmail.com

Dmitriy A. Lanshakov, SPIN: 7041-4461, Scopus AuthorID: 34872521600, ORCID: 0000-0002-8482-1302, e-mail: dmitriylanshakov@gmail.com

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Abstract. Effects of a subanesthetic dose of ketamine and acute stress on the major (*p75*, *Trkb*, *Trkc*) and novel (*Sorcs1*, *Sorcs2*, *Sorcs3*) neurotrophin receptors and *Bdnf* gene expression in rats were examined. Adult male Wistar rats were treated with ketamine (15 mg/kg) or saline and half of each group was subjected to tail suspension test for 6 minutes, which was interpreted as an acute mild stressor. After an hour the total RNA was isolated from prefrontal cortex, midbrain and brainstem regions of each animal and mRNA expressions were analysed by real-time RT-PCR. The results showed different significant interactions between factors which were strongly dependent on the structure of the brain. Both subanesthetic doses of ketamine and acute mild stress caused changes in neurotrophin receptors and *Bdnf* gene expression in examined brain areas associated with depression. The obtained results allow to surmise that BDNF, SORCS1, SORCS3 and p75 receptors are involved in the ketamine-induced neuroplasticity and antidepressant activity.

Keywords: ketamine, neurotrophin, SorCS1, SorCS3, BDNF, p75.

Introduction & Aims

Ketamine, a glutamate NMDA (N-Methyl-D-Aspartate) receptor antagonist, exhibits a rapid antidepressant activity and is involved in structural and synaptic plasticity (Kavalali, Monteggia 2015), but the precise mechanism of these effects remains unknown. There are many pieces of evidence suggesting neurotrophins and their receptors, such as TRK (tyrosine kinases) and p75NTR (p75 neurotrophin receptor), are involved in ketamine effects (Pałucha-Poniewiera et al. 2019). Members of Vps10p family of sorting receptors SORCS1, SORCS2 and SORCS3 (Sortilin Related VPS10 Domain Containing Receptors 1–3) are another neurotrophin receptors, but their functions are still poorly understood. Several researchers demonstrated an impact of these receptors on the maintenance of energy balance (Subkhangulova et al. 2018) and structural plasticity (Savas et al. 2015; Glerup et al. 2016; Christiansen et al. 2017). In this pilot study we investigated whether ketamine and stress affect mRNA expression of BDNF (brain-derived neurotrophic factor) and neurotrophin receptors SORCS1-3, p75, TRKB and TRKC in different brain areas associated with depression.

Methods

Adult male Wistar rats were injected intraperitoneally with ketamine (15 mg/kg body weight, i. p.) or saline. Two hours later half of the animals from each group were subjected to stress exposure (tail suspension test) for 6 minutes. One hour later tissue samples were harvested from different brain areas (prefrontal cortex, midbrain and stem) of all animals for total RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction (Lanshakov et al. 2016). 5 μg of total RNA for each sample were reverse transcribed with RT mixtures containing 50 U M-MuLV (SybEnzyme), 1× RT-buffer, and 200 ng of oligo(dT)-primer in a total volume of 20 μl for 90 min at 42°C. The reaction was terminated by heating at 70°C for 15 min. The cDNA samples were then used for real-time PCR measurement with mRNA-specific primers and TaqMan probes for *Bdnf, Sorcs1-3, p75, Trkb, Trkc* and *β-actin*. Primers and TaqMan probes were designed to span specific exon boundaries with IDT Oligoanalyzer tool (https://eu.idtdna.com/pages/tools/oligoanalyzer), using mRNA sequences that were obtained from rat Ensemble genome database (Rnor_6.0; https://www.ensembl.org/index.html). Relative mRNA expression level of each gene was found by using ΔΔCt method and normalized to *β-actin.* Statistical differences were determined by two-way ANOVA followed by Fisher's least significant difference *post hoc* analysis.

Results

We observed significant additive effect of both stress and ketamine on *Bdnf* mRNA expression in the cortex ($F(1, 18) = 6.273$, $p < 0.022$) and in the midbrain ($F(1, 17) = 4.581$, $p < 0.047$); however, in the brainstem, the *Bdnf* expression was increased only after stress ($F(1, 18) = 7.105$, $p < 0.016$). In the prefrontal cortex, ketamine ($p < 0.047$) and stress ($p < 0.009$) both suppressed expression of *Sorcs3* mRNA without summing of these effects. The levels of *Sorcs1* ($F(1, 18) = 4.437$, $p < 0.049$) and *p75* ($F(1, 18) = 5.586$, $p < 0.030$) mRNA in the midbrain decreased significantly after ketamine treatment (with stress and without stress, respectively). However, *p75* mRNA expression in the cortex decreased significantly after ketamine administration or tail suspension test, but not after their combination (ketamine X stress interaction: $F(1, 14) = 5.586$, $p < 0.022$). No significant effect of either ketamine or stress on the *Sorcs2, Trkb* and *Trkc* mRNA expression was observed.

Conclusion

We have found that acute stress and ketamine at subanesthetic doses have additive up-regulating

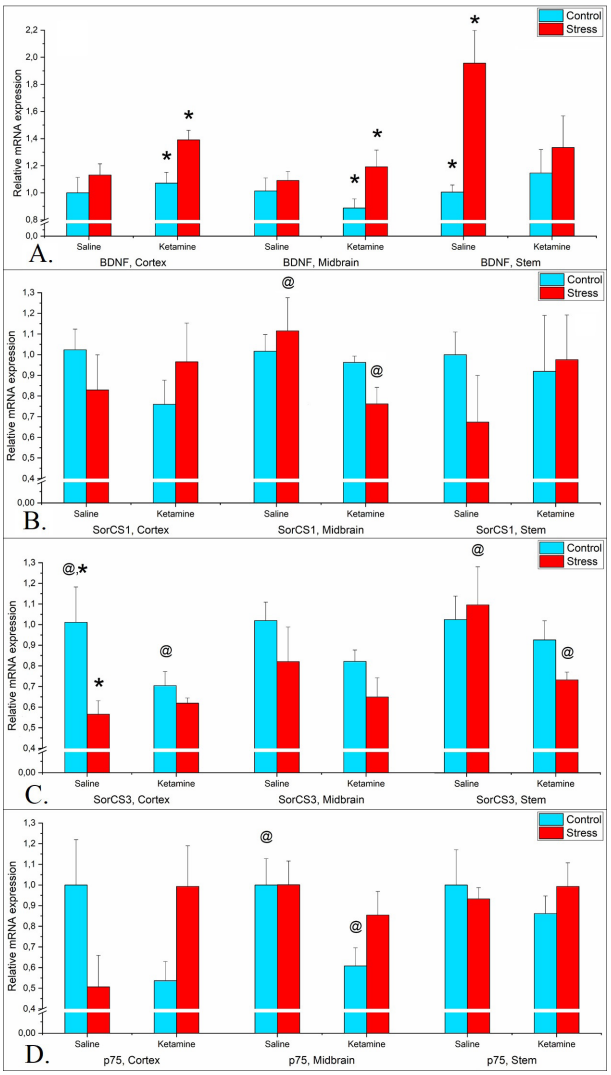

Fig. 1. Relative *Bdnf* (A), *Sorcs1* (B), *Sorcs3* (C), *p75* (D) mRNA expression (comparing to *β-actin*) in cortex, midbrain and stem. Data are presented as the means ± SE of 5–6 rats. Significant difference at $p < 0.05$; * between groups with or without stress exposure, @ between groups with saline or ketamine treatment

effects on *Bdnf* mRNA expression. This suggests the contribution of two different molecular pathways (NDMA receptors — PKD (Yu et al. 2016); GR receptors (Shishkina et al. 2015)), induced by these factors, in the genes expression increasing. However, in the brainstem region, we observed a different pattern of gene expression changes that can be explained by the small amount of NMDA receptors there (Monyer et al. 1994).

Furthermore, we observed additive down-regulating effects of both factors on the *Sorcs3* mRNA expression in the prefrontal cortex and in the midbrain, but not in stem, where only stress produced an impact. Levels of expression there might be mostly downregulated by signals from glutamatergic projections.

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The impact of ketamine on *Sorcs1* mRNA expression decreasing was detected only in midbrain after stress exposure, which indicates the possible involvement of this protein in the mechanism of ketamine acute antidepressant effect.

In the prefrontal cortex, there were antagonistic relationships between stress exposure and ketamine treatment on *p75* mRNA expression, so ketamine negates the effect of acute stress. This could be interpreted as an action induced by stress exposure molecular cascade which takes place only in the cortex after subanesthetic ketamine injection.

In conclusion, pretreatment with ketamine and acute stress both caused changes in neurotrophin receptors and *Bdnf* gene expression in examined brain areas. These findings suggest that SORCS1, SORCS3 and p75 receptors are involved in the ketamine-induced neuroplasticity and antidepressant activity. Further research is needed to confirm these suggestions.

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