DOI: 10.4149/BLL_2018_078

Bratisl Med J 2018; 119 (7)
429 – 433

EXPERIMENTAL STUDY

Melatonin modulate the expression of α_1- and β_2-adrenoceptors in the hippocampus of rats subjected to unpredictable chronic mild stress

Stefanovic B, Spasojevic N, Jovanovic P, Ferizovic H, Dronjak S

Institute of Nuclear Sciences "VINCA", Laboratory of Molecular Biology and Endocrinology, University of Belgrade, Belgrade, Serbia. **sladj@vinca.rs**

ABSTRACT

OBJECTIVE: This study investigated the effects of chronic melatonin treatment on gene expression of α_1-, α_2-, β_1- and β_2-adrenoceptors in the hippocampus of rats subjected to chronic unpredictable mild stress (CUMS).

BACKGROUND: Preclinical studies have also shown that melatonin prevented short- and long-term memory impairments and exhibited antidepressant-like actions.

METHODS: For this study, we used 24 animals, which were divided into four groups, and the experiment lasted 4 weeks. We quantified the changes in mRNA and protein levels of α_1-, α_2-, β_1- and β_2-adrenoceptors in the hippocampus after melatonin treatment.

RESULTS: Our results demonstrated a decreased gene expression of α_1-, α_2- and β_2-adrenoceptors in the hippocampus of rats subjected to unpredictable chronic mild stress, while there was no change in gene expression of β_1-adrenoceptors. Melatonin treatment in the CUMS rats prevented the stress-induced decrease in mRNA and protein levels of α_1-and β_2-adrenoceptors, whereas did not affect either on mRNA or protein level of β_1-and α_2-adrenoceptors.

CONCLUSION: Our data suggest that melatonin, by increasing reduced levels of α_1- and β_2-adrenoceptors mRNA and protein in the hippocampus of chronic stressed rats, may be beneficial in conditions such as chronic stress and provides an experimental opportunity to probe into further molecular mechanisms underlying the regulation of these receptor subtype *(Fig. 2, Ref. 28)*. Text in PDF *www.elis.sk.*

KEY WORDS: melatonin, hippocampus, catecholamines, α-adrenoceptors, β-adrenoceptors, chronic stress.

Introduction

Melatonin is a neurohormone primarily synthesized by the pineal gland during darkness (1) with a well-established role in regulating seasonal and circadian rhythms. Renewed attention has been given to the role of melatonin in modulating behavior, immune system, and responses to stress, cancer and aging (2). Also, it has been proved that melatonin had anti-inflammatory and antioxidant action (3). Preclinical studies have also shown that melatonin prevented short- and long-term memory impairments induced by chronic sleep deprivation and exhibited antidepressant-like actions (4, 5). Of the many brain regions affected in depression, the hippocampus is well known for its role in cognition stress sensitivity to emotional and memory impairments (6, 7). Hippocampus is sensitive to stress, which activates noradrenaline terminals deriving from the locus coeruleus (8). Activation of the noradrenergic system may play an important integrative function in coping with and adapting to stress. Adrenergic receptors (α- and β-subtypes) are the basic targets of noradrenaline, especially in mediating sympathetic activation in central nervous system. It has been proposed that these receptors trigger similar changes in the CNS during a successful adaptation to chronic stress and antidepressant therapy to yield adaptive changes in neural output or plasticity (6).

There are reports concerning the changes in the density of adrenoceptors in depressed individuals. Postmortem studies on brains of suicide victims indicated an increase in cortical α_1- and α_2-adrenoceptors (9, 10). β-adrenoceptors have also been implicated in the pathophysiology of depression. Several studies support the thesis that the activation of β_1- and β_2-adrenoceptors in the CNS leads to antidepressant-like effect (11, 12). De Paermentier and co-workers (13) showed a decreased density of cortical β-adrenoceptors in the antidepressant-free depressed suicide victims. There is no information regarding the direct action of melatonin on the expression of α- and β-adrenergic receptors in hippocampus of chronically stressed rats. CUMS is currently regarded by many investigators as one of the most naturalistic and predictive animal models of depression.

Institute of Nuclear Sciences "VINCA", Laboratory of Molecular Biology and Endocrinology, University of Belgrade, Belgrade, Serbia

Address for correspondence: S. Dronjak, PhD, Institute of Nuclear Sciences "Vinca", Laboratory of Molecular Biology and Endocrinology, P.O.B. 522-090, 11000 Belgrade, Serbia.
Phone/Fax: +381112455561

Acknowledgement: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract nos. 173044.

In this context, we investigated the effects of chronic melatonin treatment on gene expression of α_1-, α_2-, β_1- and β_2-adrenergic receptors in the hippocampus of rats subjected to CUMS.

Methods

The experiment was performed on male Wistar rats (11 weeks old, 250–310 g). The care was taken to minimize the pain and discomfort of the animals according to the guidelines of the Ethical Committee for the use of laboratory animals of the "Vinca" Institute based on EU directive 2010/63EU.

For this study, we used 24 animals, which were divided into four groups. First group was control (unstressed). animals received injection of vehicle daily (0.9 % NaCl containing 5 % ethanol). Second group was control (unstressed) and animals received melatonin. Third group was chronic unpredictable mildly stressed group. The animals from this group also received vehicle. In the fourth group were chronic unpredictable mildly stressed animals, also receiving melatonin. Melatonin was given in dose of 10 mg/kg body weight by intraperitoneal (i.p.) route, between 2:00 and 3:00 p.m.

The chronic unpredictable mild stress (CUMS) procedure was a variation of methods described by Grippo et al (14). The day after the CUMS procedure, all animals were sacrificed by decapitation, hippocampus was quickly removed on ice, frozen in liquid nitrogen and stored at –70 °C until it was analyzed.

Total RNAs from hippocampal tissue was extracted using TRIzol® Reagent (Thermo Fischer Scientific, MA USA) according to the manufacturer's instructions. Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Bead (GE

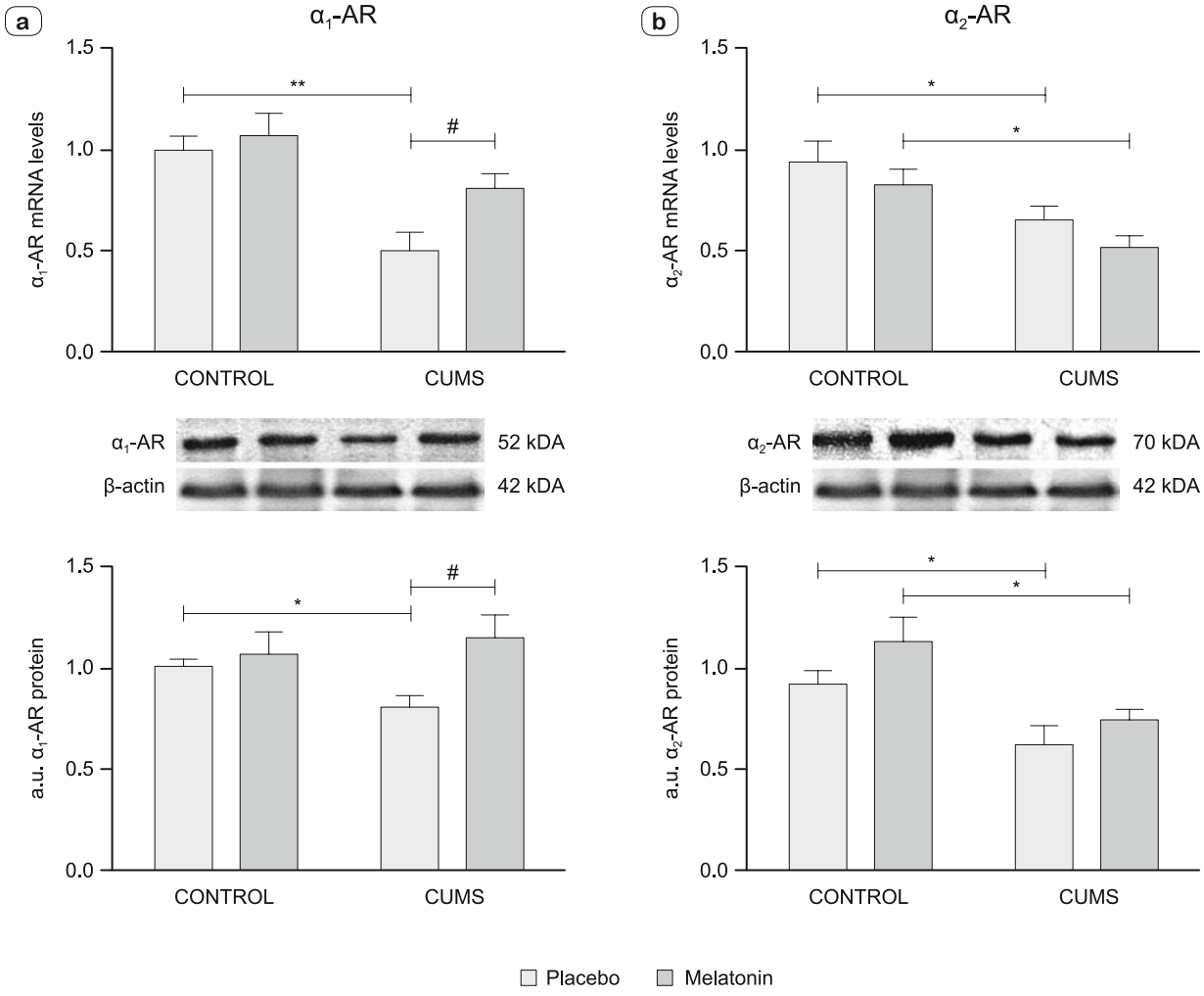

Fig. 1. Effect of chronic melatonin treatment on a) alpha 1 adrenoceptor (α_1-AR) and b) alpha 2 adrenoceptor (α_2-AR) gene expression in the hippocampus of rats exposed to CUMS for 28 days. The relative mRNA levels for α_1-AR and α_2-AR were determined by applying RT-PCR. The final result was expressed as fold change relative to the calibrator and normalized to cyclophilin A for variation between samples. α_1-AR and α_2-AR protein levels were determined by Western immunoblotting. The final result was expressed in arbitrary units and normalized in relation to β-actin. The values are expressed as the mean ± S.E.M. of 6 rats. Statistical significance: $^{*}p < 0.05$; $^{**}p < 0.05$ CUMS vs control; $^{\#}p < 0.05$ placebo vs melatonin.

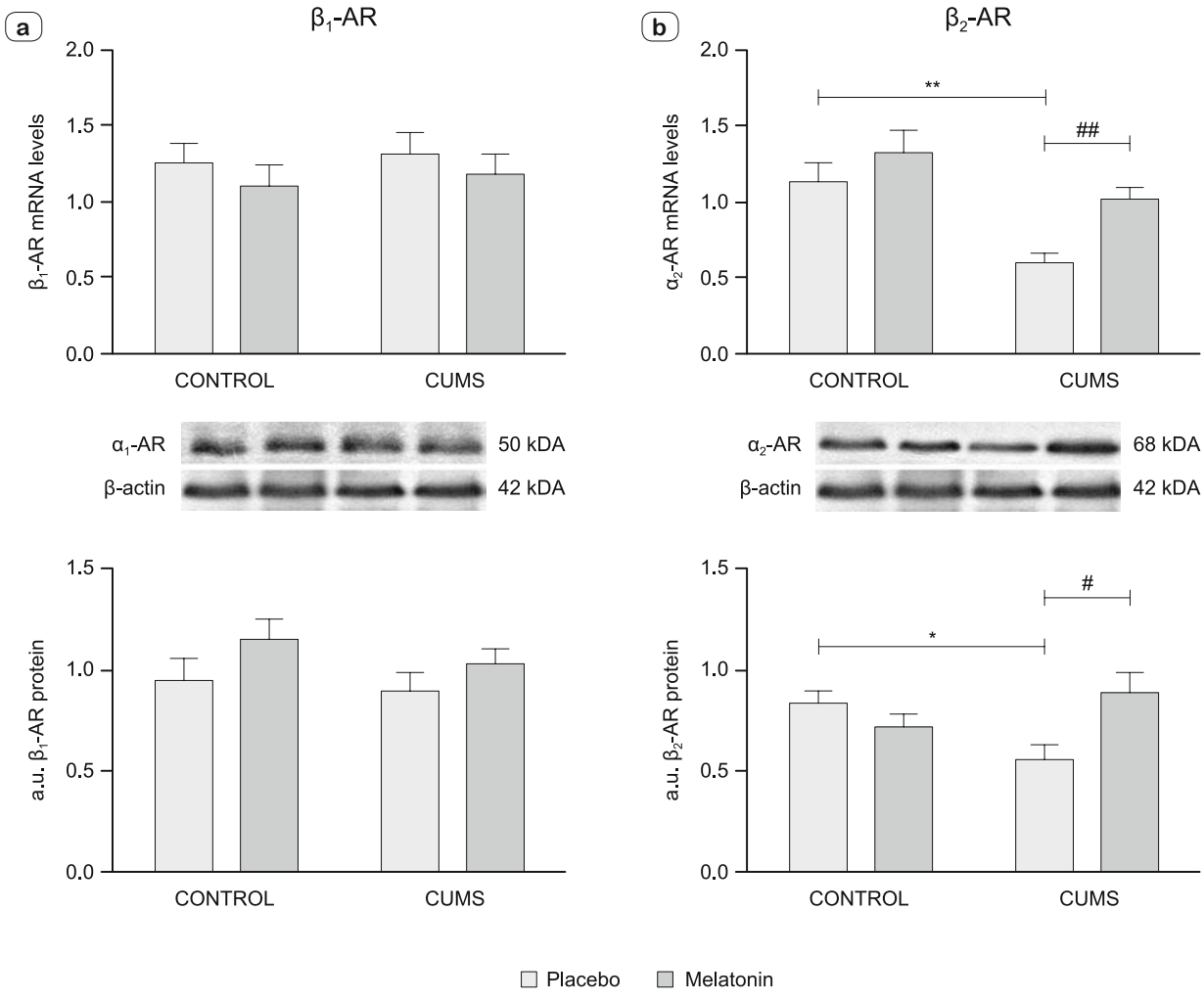

Fig. 2. Effect of chronic melatonin treatment on a) beta 1 adrenoceptor (β_1-AR) and b) beta 2 adrenoceptor (β_2-AR) gene expression in the hippocampus of rats exposed to CUMS for 28 days. The relative mRNA levels for β_1-AR and β_2-AR were determined by applying RT-PCR. The final result was expressed as fold change relative to the calibrator and normalized to cyclophilin A for variation between samples. β_1-AR and β_2-AR protein levels were determined by Western immunoblotting. The final result was expressed in arbitrary units and normalized in relation to β-actin. The values are expressed as the mean ± S.E.M. of 6 rats. Statistical significance: * p < 0.05; ** p < 0.05 CUMS vs control; $^{\#}$ p < 0.05; $^{\#\#}$ p < 0.01 placebo vs melatonin.

Healthcare Life Sciences, PA USA) and pd $(N)_6$ primer according to manufacturer's protocol. Real-Time RT-PCR assay was done exactly as previously described by Gavrilovic et al (15). TaqMan PCR reaction was carried out using Assay-on-Demand Gene Expression Products (Thermo Fischer Scientific, MA USA) for β_1 (ID:Rn00824536_s1) and for β_2 (ID:Rn005606650_s1), for α_1 (ID:Rn00567876_m1), and for α_2 (ID:Rn00562488_s1). A reference endogenous control was included in each analysis to correct the differences in the inter-assay amplification efficiency and all the transcripts were normalized to cyclophyline A (ID:Rn00690933) expression. Hippocampal tissue wase homogenized in RIPA Lysis Buffer System (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, sc-24948). After centrifugation (12 000rpm, 20 min at 4 °C), the supernatant was taken and protein concentration was determined by the method of Lowry et al (16). For measuring α_1, α_2, β_1 and β_2 protein levels, a polyclonal anti-α_1 primary antibody, rabbit (sc28982, dilution 1:500, Santa Cruz Biotecnology, California, USA), a polyclonal anti-α_2 primary antibody, rabbit (sc28983, dilution 1:500, Santa Cruz Biotechnology, California, USA), polyclonal anti-β_1 primary antibody, rabbit (ab3442, dilution 1:1000, Abcam, Cambridge, UK) and polyclonal anti-β_2 primary antibody, rabbit (ab182136, dilution 1:1000 Abcam, Cambridge, UK) were used respectively. Rabbit polyclonal anti-β-actin (dilution 1:1000; Abcam, Cambridge, UK) was used to detect β- actin as a loading control. Washed membrane was further incubated in the horseradish peroxidase conjugated secondary anti-rabbit antibody for luminol based detection (ab6721, dilution 1:5000, Abcam, Cambridge, UK). Secondary antibody was then visualized by Immobil-

ion Western Chemiluminescent HPR Substrate (Merck Millipore, Massachusetts, USA). Western blot analysis was performed as previously described by Gavrilovic et al (15).

Statistical analysis

The results are reported as the mean ± S.E.M. Significance of the differences in gene expression levels of the examined α_1, α_2, β_1, and β_2-adrenergic receptors in hippocampus of rats subjected to CUMS and melatonin treatment were estimated by Two-Way ANOVA test. The Tuckey post hoc test was used to evaluate the differences between the groups. Statistical significance was accepted at $p < 0.05$. Results processing was done in the OriginPro, version 8.0 software program (OriginLab Corporation, Massachusetts, USA).

Results

The α_1-adrenoceptor ($F_{(1,23)} = 16.04$, $p < 0.001$) and α_2-adrenoceptor ($F_{(1,23)} = 14.64$, $p < 0.01$) mRNA levels were significantly influenced by stress. Compared to the control group, a decrease was observed in α_1-adrenoceptor (by 50 %, $p < 0.001$) and α_2-adrenoceptor (by 69 %, $p < 0.05$) mRNA levels in the rats exposed to CUMS. Melatonin increased gene expression of α_1-adrenoceptor in CUMS group (60 %, $p < 0.05$) (Fig. 1a). Exposure of the rats to CUMS decreased protein levels of α_1-adrenoceptor (by 80 %, $p < 0.05$) and α_2-adrenoceptor (by 69 %, $p < 0.05$). Melatonin treatment induced an increase of α_1-adrenoceptor protein levels (by 43 %, $p < 0.05$) in the hippocampus of stressed rats, without affecting α_2-adrenoceptor protein content (Fig. 1b).

No significant differences in the levels of mRNA β_1-adrenoceptors and protein levels between the placebo and stressed groups were found. Chronic treatment with melatonin did not change β_1-adrenoceptors gene expression and protein levels (Fig. 2a).

Two-way ANOVA demonstrated a significant effect of both CUMS ($F_{(1,23)} = 15.55$, $p < 0.001$) and the melatonin treatment ($F_{(1,23)} = 7.93$, $p < 0.05$) on β_2-adrenoceptors mRNA. Stress decreased mRNA (by 49 %, $p < 0.01$) levels of this adrenergic receptor. On the other hand, melatonin treatment increased β_2-adrenoceptor mRNA (by 74 %, $p < 0.01$) in the stressed rats. Two-way ANOVA also showed a significant interaction between treatment and stress ($F_{(1,23)} = 8.78$, $p < 0.01$) on β_2-adrenoceptors protein levels. Post hoc testing showed that while stress produced a significant reduction in protein content of this adrenergic receptor (by 33 %, $p < 0.05$), melatonin treatment prevented that CUMS-induced decrease (Fig. 2b).

Discussion

Since α- and β-adrenoceptors are involved in the regulation of a variety of mental and bodily functions, it can be assumed that stress-induced changes in the central adrenoceptors system constitute a molecular basis for physiological changes in stressed individuals. α_1- and α_2-adrenoceptors are supposed to be important regulatory elements in responses to stress. Previous studies in male tree shrews showed that chronic psychosocial stress down-regulated biding sites for α_2-adrenergic ligands in several brain stem nuclei such as: reduced α_{2A}-adrenoceptor mRNA expression in locus coeruleus, solitary tract neurons and neurons of lateral reticular nucleus (17). Similarly, repeated stress significantly decreased mRNA levels for α_1-adrenoceptors in midbrain and hypothalamus (18). Our results are consistent with previous work demonstrating a decreased gene expression of α_1- and α_2-adrenoceptors in the hippocampus of rats subjected to unpredictable chronic mild stress.

The mammalian hippocampus receives noradrenergic innervation and hippocampal neurons express β_1- and β_2-adrenoceptors. Both type of receptors are localized to the cell membrane and cytoplasm, however only β_2-adrenoceptors are found in the nucleus (19). The data presented here showed that CUMS caused a decrease of β_2-adrenoceptors mRNA and protein levels, while there was no change in gene expression of β_1- in the hippocampus. Our results are consistent with Flügge et al (20), who reported that repeated exposure to subordination stress down-regulated β-adrenoceptors expression in the hippocampus. α_1- and α_2-adrenoreceptors and their signaling system were important targets of antidepressant drugs (21). Kreiner et al (22) reported that in contrast to imipramine and electroconvulsive shock, which produce up-regulation of the α_{1A}-adrenoceptors mRNA expression, chronically administered citalopram did not affect the α_{1A}-adrenoceptors mRNA level in the prefrontal cortex. Our observations indicated that melatonin treatment in the CUMS rats prevented the stress-induced decrease in α_1-mRNA and protein levels. On the other hand, melatonin treatment did not affect either mRNA or protein level of α_2-adrenoceptors in the hippocampus of rats exposed to CUMS. Given that Stone and co-workers (23) reported that brain α_1-adrenoceptors were impaired or inhibited in depressed patients or after stress in animal models, and were restored by a number of antidepressants, it is possible that melatonin mediated antidepressant-like effect by modulating the gene expression of α_1-adrenoceptors in the hippocampus of CUMS rats.

Although α-adrenoceptors can influence hippocampal function, largely through regulating neuronal excitability, β -adrenoceptors exert very specific effects on synaptic information encoding (24). Our data revealed that melatonin treatment prevented stress-induced decrease in the expression of β_2-adrenoceptors in hippocampus of CUMS rats. β-adrenoceptors in hippocampus play an important role in regulating synaptic plasticity and memory consolidation. The activation of these adrenoceptors results in the stimulation of extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) that is a key step in the activation of the cAMP response element-binding protein (CREB) that mediates protein transcription and thus strongly supports persistent synaptic plasticity and long-term memory (25). Maity et al (26) reported that norepinephrine activating β-adrenoceptors could boost long-term potentiation, a putative cellular mechanism for memory formation. The hippocampus-dependent memory is impaired following a reduction of norepinephrine or after blockade of β-adrenoceptors (27). Taken together, it gives rise to the possibility that melatonin-induced up-regulation of gene expression of β_2-adrenoceptors in the hippocampus of CUMS rats could enhance long-term potentiation and learning and memory. Our findings here

provide novel targets for exploring the molecular mechanisms by which melatonin is improving mental health modulating through dysfunctional noradrenergic synaptic transmission. Recently, we reported that reduced noradrenaline content was increased in the hippocampus of stressed rats treated with melatonin (28). The present results suggested that repeated treatment with melatonin might improve a reduced expression of α_1- and β_2-adrenoreceptors, and it was in a good correlation with increased noradrenaline in the hippocampus. Although the molecular basis of the gene regulation of α- and β-adrenoceptors in the hippocampus of rats exposed to CUMS during melatonin treatment remains to be elucidated, increased gene expression of α_1- and β_2-adrenoceptors provides an experimental opportunity to probe into further molecular mechanisms underlying the regulation of these receptor subtype. Further experiments on transcriptional activation and mRNA stability will be required to unravel the complexity of stress- and melatonin-dependent regulation of α- and β-adrenoceptor gene expression.

References.

1. Pandi-Perumal SR, Srinivasan V, Maestroni GJ, Cardinali DP, Poeggeler B, Hardeland R. Melatonin: nature's most versatile biological signal? FEBS J 2006; 273 (13): 2813–2838.

2. Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. Front Neuroendocrinol 2004; 25 (3–4): 177–195.

3. Barlas AM, Sadic M, Atilgan HI et al. Melatonin: a hepatoprotective agent against radioiodine toxicity in rats. Bratisl Med J 2017; 118 (2): 95–100.

4. Cardinali DP, Srinivasan V, Brzezinski A, Brown GM. Melatonin and its analogs in insomnia and depression. J Pineal Res, 2012; 52 (4): 365–375.

5. Ramirez-Rodriguez G, Vega-Rivera M, Oikawa-Sala J, Gómez-Sánchez A, Ortiz-López L, Estrada-Camarena E. Melatonin synergizes with citalopram to induce antidepressant-like behavior and to promote hippocampal neurogenesis in adult mice. J Pineal Res 2014; 56 (4): 450–461.

6. Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 1999; 46 (9): 1181–1191.

7. Saygin M, Ozguner MF, Onder O, Doguc DK, Ilhan I, Peker Y. The impact of sleep deprivation on hippocampal-mediated learning and memory in rats. Bratisl Med J 2017; 118 (7): 408 – 416.

8. Carter AJ. Hippocampal noradrenaline release in awake, freely moving rats is regulated by alpha-2adrenoceptors but not by adenosine receptors. J Pharmacol Exp Ther 1997; 281 (2): 648–654.

9. Arango V, Ernsberger P, Sved AF, Mann JJ. Quantitative autoradiography of alpha 1- and alpha 2-adrenergic receptors in the cerebral cortex of controls and suicide victims. Brain Res 1993; 630 (1–2): 271–282.

10. Meana JJ, Barturen F, Garcı´a-Sevilla JA. Alpha 2-adrenoceptors in the brain of suicide victims: increased receptor density associated with major depression. Biol Psychiatry 1992; 31 (5): 471–490.

11. Gu L, Liu YJ, Wang BY, Yi LT. Role for monoaminergic systems in the antidepressant-like effect of ethanol extracts from Hemerocallis citrina. J Ethnopharmacol 2012; 139 (3): 780–787.

12. Zhang HT, Huang Y, O'Donnell JM. Antagonism of the antidepressant-like effects of clenbuterol by central administration of beta-adrenergic antagonists in rats. Psychopharmacology (Berl) 2003; 170 (1): 102–107.

13. De Paermentier F, Cheetham SC, Crompton MR, Katona CL, Horton RW. Brain beta-adrenoceptor binding sites in antidepressant-free depressed suicide victims. Brain Res 1990; 525 (1): 71–77.

14. Grippo AJ, Moffitt JA, Johnson AK. Cardiovascular alterations and autonomic imbalance in an experimental model of depression. Am J Physiol Regul Integr Comp Physiol 2002; 282 (5): R1333–1341.

15. Gavrilovic L, Spasojevic N, Tanic N, Dronjak S. Chronic isolation of adult rats decreases gene expression of catecholamine biosynthetic enzymes in adrenal medulla. Neuroendocrinol Lett 2008; 29 (6): 1015–1020.

16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193 (1): 265–275.

17. Meyer H, Palchaudhuri M, Scheinin M, Flügge G. Regulation of alpha(2A)-adrenoceptor expression by chronic stress in neurons of the brain stem. Brain Res 2000; 880 (1–2): 147–158.

18. Miyahara S, Komori T, Fujiwara R, Shizuya K, Yamamoto M, Ohmori M, Okazaki Y. Effects of single and repeated stresses on the expression of mRNA for alpha1-adrenoceptors in the rat hypothalamus and midbrain. Eur J Pharmacol 1999; 379 (1): 111–114.

19. Guo NN, Li BM. Cellular and subcellular distributions of beta1- and beta2-adrenoceptors in the CA1 and CA3 regions of the rat hippocampus. Neuroscience 2007; 146 (1): 298–305.

20. Flügge G, Ahrens O, Fuchs E. Beta-adrenoceptors in the tree shrew brain. I. Distribution and characterization of [125I]iodocyanopindolol binding sites. Cell Mol Neurobiol 1997; 17 (4): 401–415.

21. Nalepa I, Vetulani J. The responsiveness of cerebral cortical adrenergic receptors after chronic administration of atypical antidepressant mianserin. J Psychiatry Neurosci 1994; 19 (2): 120–128.

22. Kreiner G, Bielawski A, Zelek-Molik A, Kowalska M, Nalepa I. Chronic treatment with citalopram does not affect the expression of alpha1-adrenergic receptor (alpha1-AR) subtypes. Pol J Pharmacol 2004; 56 (6): 831–836.

23. Stone E, Lin Y, Rosengarten H, Kramer KH, Quartermain D. Emerging evidence for a central epinephrine-innervated a1-adrenergic system that regulates behavioral activation and is impaired in depression. Neuropsychopharmacology. 2003; 28 (8): 1387–1399.

24. Segal M, Markram H, Richter-Levin G. Actions of norepinephrine in the rat hippocampus. Prog Brain Res 1991; 88: 323–330.

25. Kandel ER. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol Brain 2012; 14: 5–14.

26. Maity S, Rah S, Sonenberg N, Gkogkas CG, Nguyen PV. Norepinephrine triggers metaplasticity of LTP by increasing translation of specific mRNAs. Learn Mem 2015; 22 (10): 499–508.

27. Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA. A distinct role for norepinephrine in memory retrieval. Cell 2004; 117 (1): 131–143.

28. Stefanovic B, Spasojevic N, Jovanovic P, Jasnic N, Djordjevic J, Dronjak S. Melatonin mediated antidepressant-like effect in the hippocampus of chronic stress-induced depression rats: Regulating vesicular monoamine transporter 2 and monoamine oxidase A levels. Eur Neuropsychopharmacol 2016; 26 (10): 1629–1637.

Received February 1, 2018.
Accepted March 13, 2018.