Hippocampal asymmetry in expression of catecholamine synthesizing enzyme and transporters in socially isolated rats

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Abstract

OBJECTIVES: Right-left asymmetry of human brain function has been known for a century. Brain asymmetry and lateralization has been observed at the neurochemical level. At the neurochemical level, it is important to further correlate changes in monoaminergic activity with the synthesis and reuptake of these monoamines. The aim of the present study was to analyze the effect of social isolation on catecholamine stores as well as on the regulation of catecholamine synthesis and uptake in the right and left hippocampus.

METHODS: We examined changes in protein levels of dopamine-β-hydroxylase (DBH), norepinephrine transporter (NET) and vesicular monoamine transporter 2 (VMAT 2) in the right and left hippocampus of socially isolated adult male rats during 12 weeks by Western blot analysis.

RESULTS: Chronic isolation stress reduced norepinephrine content in the right hippocampus. No changes were observed in protein levels of DBH and NET in the right hippocampus, whereas expression of this norepinephrine synthetizing enzyme and transporter were elevated in the left hippocampus. On the other hand, chronic isolation stress caused reduction of VMAT2 protein in the right hippocampus.

CONCLUSION: Our results reveale not only the lateralization of stress regulatory system but they also show that long-term isolation stress produces right-left asymmetry of the hippocampus norepinephrine, different regulation of the catecholamines synthesis and reuptake.

ORIGINAL ARTICLE

INTRODUCTION

Stressful life events cause a variety of conditions affecting cerebral and neuroendocrine functions. Repeated stressful events also may determine sensitisation leading to an increased responsiveness to stress stimuli (Bob 2008). Brain monoamine systems play a crucial role in mediating social behaviors. Social relationships are an important modulator of mental health. Social isolation might produce several negative long-term consequences for the animal. It has been demonstrated that isolated rats exhibit impairments in learning and memory tasks (Larsson *et al.* 2002) as well as changes in the brain neurochemistry (Brenes *et al.* 2008). It should be noted that there are many data regarding neurochemical alterations such as depletion of norepinephrine, dopamine and serotonin. Lapiz and co-workers (2003) shown that isolation-reared rats have reduced presynaptic noradrenergic function in the hippocampus. Cerebral lateralization is one of the well-known asymmetries (Gerendai & Halász 1997). Right-left asymmetries are now known to be present in a variety of species, including rodents and humans. Numerous brain regions show right-left asymmetries including hippocampus. A study of healthy individuals pointed out that regarding hippocampal volume asymmetry the right hippocampus was larger than the left (Preussner *et al.* 2000). Also, in male rats, some portions of the right hippocampus are larger than the left (Diamond *et al.* 1983). Recent studies suggest that hippocampal excitatory synapse are asymmetrically arranged (Shinohara *et al.* 2008). Brain asymmetry and lateralization has also been observed at the neurochemical level. In normal rats an asymmetrical right-left distribution of the norepinephrine content in neocortex and hippocampus was found (Hecht *et al.* 1982). Hui and co-workers (2011) shown that the effects of maternal separation on hippocampal neurochemistry were most significant in the right hippocampus. At the neurochemical level, it is important to further correlate changes in monoaminergic activity with synthesis and reuptake of these monoamines. Therefore, we hypothesized that differential expression of synthesizing enzyme and transporters in the right and left hippocampus might provide the basis for altered monoaminergic activity.

This prompted us to investigate catecholamine stores and changes in protein levels of dopamine-β-hydroxylase (DBH), norepinephrine transporter (NET) and vesicular monoamine transporter 2 (VMAT 2) in the right and left hippocampus of socially isolated adult male rats during 12 weeks, by Western blot analysis.

MATERIAL AND METHODS

Animals

Adult, 11-week-old Wistar rat males, maintained under standard laboratory conditions with water and food *ad libitum* in the groups of four individuals *per* cage were used. The care was taken to minimize the pain and discomfort of the animals according to the recommendations of the Ethical Committee of the "Vinca" Institute, Belgrade based on the Guide for Care and Use of Laboratory Animals of the National Institute of Health (Bethesda, MD, U.S.A). In the experiment we used 14 animals, which were divided into two groups. One group of animals was subjected to social isolation with a single animal *per* cage, while second group was naive controls which were group housed. After 12 weeks rats were decapitated, the right and left hippocampi rapidly dissected, frozen in liquid nitrogen and stored at –70 °C until analyzed.

Radioenzymatic assay

Hippocampi were immersed into cold (4 °C) perchloric acid (0.3 μg of tissue per 30 μl of 0.1 N $HClO_4$), homogenized in a motor-driven homogenizer, the homogenates centrifuged (20 000 r.p.m., 20 min, 4 °C) and the supernatants (30 μl) used for determination of catecholamines. Content of catecholamines in the tissues was determined using modified chromatographic method of Peuler and Johnson (1977) based on the conversion of catecholamines into the corresponding O-methylated derivatives by purified catechol-O-methyl-transferase (COMT) in the presence of S-adenosyl-l-(^{3}H-methyl)-methionine. The O-methylated derivatives were extracted and oxidized into ^{3}H-vaniline. Radioacitivites were measured using toluene-based scintillation cocktail in a LKB-Wallac model 1219 scintillation counter at an efficiency of 40% for tritium.

Western blot analysis

Hippocampal tissue were homogenized in 0.05 M sodium phosphate buffer pH 6.65. 15 μg of hippocampal protein extract separated by 10% SDS-polyacrylamide gel electrophoresis were transferred to a supported nitrocellulose membrane (Hybond™ C Extra, Amersham Bioscience, GE Healthcare, Buckinghamshire, UK). The membrane was blocked in 5% non-fat dry milk in Tris-buffered saline-Tween (TBST). All following washes and antibody incubations were also performed in TBST at ambient temperature on a shaker. For measuring DBH, NET and VMAT 2 protein levels, a polyclonal anti-DBH (N-Terminal) antibody, sheep (dilution 1:1000, Sigma-Aldrich, United States), a polyclonal anti-NET primary antibody rabbit (dilution 1:1 000, Abcam, Cambridge, UK) and polyclonal anti-VMAT 2 primary antibody, rabbit (dilution 1:5 000, Abcam, Cambridge, UK) respectively, were used. Washed membrane was further incubated in the horseradish peroxidase conjugated secondary anti-mouse or anti-rabbit antibody for luminol based detection (dilution 1:5 000, GE Healthcare, Buckinghamshire, UK). Secondary antibody was then visualized by Amersham ECL Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). Western blot analysis was performed as previously described (Gavrilovic *et al.* 2008).

Statistical analyses

The results are reported as means ± S.E.M. Significance of the differences in dopamine and norepinephrine content and protein levels of the examined catecholamine biosynthetic enzyme and transporters in the right and left hippocampi subjected to chronic social isolation were estimated by two-way ANOVA test. The Tukey post hoc test was used to evaluate the differences between the groups. Statistical significance was accepted at $p<0.05$.

RESULTS

In our study, we tested hippocampal tissue samples from rats exposed to social isolation for 12 weeks. In post-hoc analyses (Tukey-test) we have found a significant change of norepinephrine (31%, $p<0.01$) only in right hippocampus of the rats exposed to chronic psychosocial stress. Dopamine levels were decrease by 20% without significant effect in in the right hippocampus of stressed animals (Figure 1).

DBH protein content was markedly increased only in the left hippocampus of stressed rats (by 11%, $p<0.05$), as compared to those found in the control animals. (Figure 2).

The results presented in Figure 3. indicate that NET protein content was markedly increased in the left hippocampus of stressed rats (by 104%, $p<0.05$), as compared to those found in the control animals. Stress procedure did not induce any change in NET protein levels in right hippocampus.

The exposure of animals to social isolation for 12 weeks did not induce any change in VMAT 2 protein levels in left hippocampus. However, this stress procedure induced a VMAT 2 protein level decrease in the right (by 28%, $p<0.05$) hippocampal tissue (Figure 4).

DISCUSSION

The hippocampus plays an important role in the response to stress, especially in adjusment to repeated stressful experience. Asymmetries in hippocampal structure and function have been reported in antisocial groups using PET (Chesterman *et al.* 1994). The results of the present study show that 12 weeks of social isolation caused a significant depletion of norepinephrine stores in the right hippocampus only. Cerebral hemispheric lateralization alludes to the localization of the brain function on either the right or left side of the brain and is an important factor in the progress of the depression. Our present results, which indicate that isolated housing of adult rats resulted in the decreased of norepinephrine in the right hippocampus, support the findings of Mandal *et al.* (1996) that the right hemisphere dysfunction is characteristic of depressive illness. Similar to that obtained for norepinephrine, we observed a 28% decrease of dopamine in the right hippocampus, however, this reduction failed to achieve a

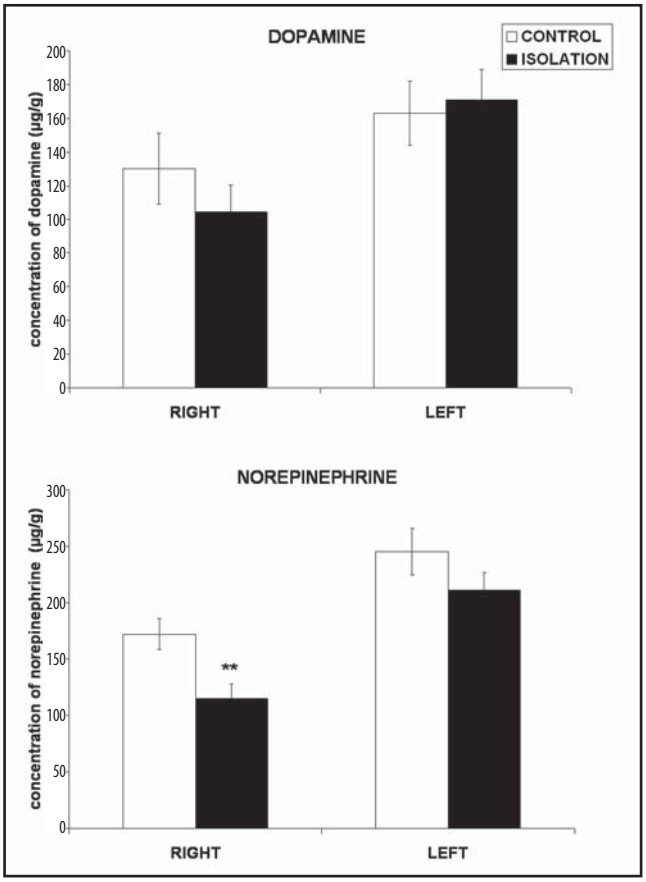

Fig. 1. Effects of chronic isolation stress on concentration of dopamine and norepinephrine in right and left hippocampus of group- and individually housed rats. The values expressed in µg/g fresh tissue are the means ± S.E.M. of 5–7 rats. Statistical significance: $^{**}p<0.01$ individually housed *vs.* group-housed control (Tukey-test).

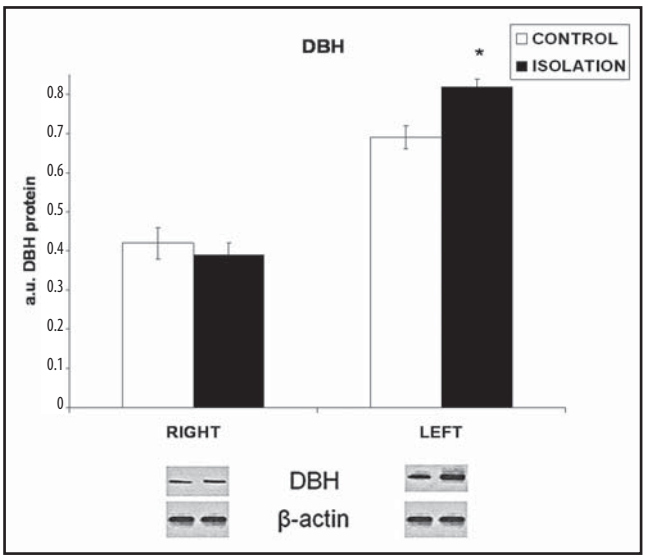

Fig. 2. Effects of chronic isolation stress on protein levels of dopamine-ß-hydroxylase (DBH) in right and left hippocampus of group- and individually housed adult rat males. Protein levels were expressed in arbitrary units normalized in relation to β-actin. The values are means ±S.E.M. of 5–7 rats. Statistical significance: $^{*}p<0.05$ individually housed *vs.* group-housed control (Tukey-test).

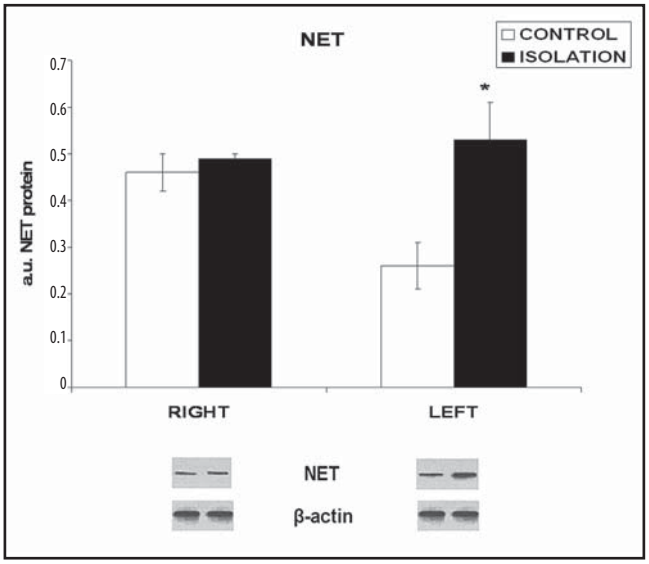

Fig. 3. Effects of chronic isolation stress on protein levels of norepinephrine transporter (NET) in right and left hippocampus of group- and individually housed adult rat males. Protein levels were expressed in arbitrary units normalized in relation to β-actin. The values are means ±S.E.M. of 5-7 rats. Statistical significance: **p*<0.05 individually housed *vs.* group-housed control (Tukey-test).

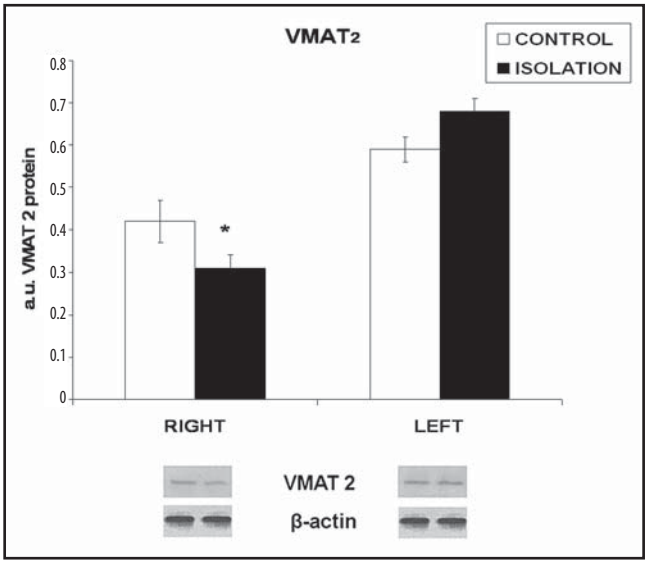

Fig. 4. Effects of chronic isolation stress on protein levels of vesicular monoamine transporter 2 (VMAT 2) in right and left hippocampus of group- and individually housed adult rat males. Protein levels were expressed in arbitrary units normalized in relation to β-actin. The values are means ±S.E.M. of 5-7 rats. Statistical significance: **p*<0.05 individually housed *vs.* group-housed control (Tukey-test).

statistically significant difference. The maintenance of norepinephrine stores is reflection of the efficiency of both reuptake of released transmitter from the synapse and mechanisms regulating its synthesis. Therefore, we decided to examine protein levels of DBH, NET and VMAT 2. Our results show that chronic isolation stress increased DBH protein levels in the left hippocampus. However, no significant alternations in protein levels of DBH were observed in the right hippocampus.

Almost 90% of the norepinephrine released into the synaptic cleft is recaptured by uptake into presynaptic neurons. The reuptake is maneuvered by the NET. Most of the uptaken norepinephrine is translocated into synaptic vesicles by VMAT 2 (Zheng *et al.* 2006; Onoa *et al.* 2010; Wimalasena 2011). The data show that NET protein levels in the right hippocampus of isolated rats remain unchanged compared with controls. However, in the left hippocampus NET protein levels were significantly elevated. On the other hand, VMAT2 protein was reduce in the right hippocampus. Schwartz and co-workers (2003) found a decreased limbic VMAT 2 in a genetic rat model of depression and Caudle and co-workers (2007) reported that mice with decreased VMAT 2 have aging-associated decreases in strial dopamine. The higher levels of DBH and NET expression in the left hippocampus observed after exposure to chronic isolation stress, suggest that these parameters may be increased in response to a higher demand for synthesis and uptake maintaining transmitter stores constant. The decrease in norepinephrine stores and VMAT 2 as well as unchange expression of DBH and NET found in our present study point to reduced stress-reactivity of the right hippocampal noradrenergic neurons and may indicate that this neurotransmitter system has become desensitized in these animals due to their chronic state of stress-hyperreactivity. These our results are consistent with the view that the right hemisphere has a special role in the control of autonomic functions essential for survival. Data show that the right hemisphere is more vulnerable to traumatic influences than the left one (Henry 1993; 1997). Though the lateralized regulation of stress responses of the hippocampus indicates a close relationship between stress and the right brain mechanisms, there nevertheless are some limitations that require further investigation. Considering that VMAT 2 is not responsible only for the translocation of catecholamine but also, for serotonin a reduced VMAT 2 in the right hippocampus can also indicate alternations in the hippocampal serotonin. Thus, further studies, to be concerned with the analysis of serotonin content are needed.

Based on these results, it may be concluded that norepinephrine stores in the hippocampus of chronic psychosocially stressed rats are asymmetrical which seem to have resulted from lateralized synthesis and reuptake.

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