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Hippocampus remodeling by chronic stress accompanied by GR, proteasome and caspase-3 overexpression

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ABSTRACT

Chronic stress is a threat to homeostasis for many brain regions. While hippocampal formation is one of the most stress-sensitive areas of the cortex, molecular changes occurring as a result of increased glucocorticoid neurotoxicity in hippocampus are largely unknown. The aim of these studies was to investigate mRNA expression of mineralocorticoid and glucocorticoid receptors (MR, GR), proteasome subunits β5 (constitutive subunit) and β1i (inducible immunoproteasome subunit), mTOR (mammalian target of rapamycin), bcl-2; as well as caspase-3 immunoreactivity (confocal microscopy) in adult Wistar rat hippocampus following 10-day restraint stress (plastic restrainers, 6 h daily). Chronic restraint led to a significant reduction in number of neuronal and astroglial cells in hippocampal regions CA1–3. This reaction was combined with substantial increase in GR and decrease in MR mRNA levels with the greatest response – 1.5-fold amplitude increase – observed in dentate gyrus and CA3 correspondingly. Stress did not change the expression of constitutive β5 subunit but dramatically enhanced expression of inducible β1i subunit and increased mTOR, and bcl-2 mRNA expression. Multiple scattered cells demonstrating caspase-3^{+} profile were found in hippocampus of stressed animals. The study demonstrates that hippocampal remodeling induced by chronic restraint stress is associated with GR, immunoproteasome, mTOR, caspase-3 and bcl-2 overexpression in hippocampus.

1. Introduction

Chronic stress produces consistent changes within the hippocampus including decreased dendritic length, reduced branch number (Fuchs et al., 2006) and decreased NCAM expression (Touyarot and Sandi, 2002). These changes have traditionally corresponded to hippocampus-dependent spatial memory deficits (Fuchs et al., 2006) and depression-like disorders (Höschl and Hajek, 2001). Furthermore there is an evidence from the clinical studies that individuals having disorders with elevated plasma glucocorticoids (GC), such as major depressive disorder and Cushing's syndrome, are also associated with smaller hippocampal volume (Tata et al., 2006).

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Considerable experimental data suggests that GC are the main effectors of hippocampal damage caused by different types of stress (Höschl and Hajek, 2001; Sekita-Krzak et al., 2003). It is also suggested, that hippocampal atrophy induced by corticosteroids may play an important role in the pathogenesis of a range of neuropsychiatric disorders (Höschl and Hajek, 2001). Despite this, the molecular mechanisms mediating glucocorticoid neurotoxicity in hippocampus remain obscure (Nitta et al., 1999; Touyarot and Sandi, 2002). It has been shown that GC treatment induces synapse loss (Tata et al., 2006) and neurodegeneration in CA3 and GD area—effects believed to be mediated by GR type of receptors (Datson et al., 2010; Hassan et al., 1996; Sekita-Krzak et al., 2003). These receptors are activated only by a high GC levels usually observed under stress conditions (Conway-Campbell et al., 2007). The other corticosteroid receptors (MR) have a much greater affinity for GC, remain activated by GC levels observed in basal conditions (Conway-Campbell et al., 2007) and are important for maintenance of the granule cell layer (Almeida et al., 2000; Sloviter et al., 1993; Sousa et al., 1998).

Among GR-dependent genes there are a number of factors related to cell survival and death. First of all, it is shown that GR can activate immunoproteasome expression (Mishto et al., 2003), known to be involved in pathogenesis of some neurodegenerative disorders (Díaz-Hernández et al., 2003; Eleftheriadis et al., 2006). Also, GC can significantly change activity of translation factors (such as mTOR) (Gu et al., 2010; Kfir-Erenfeld et al., 2010) and increase expression of pro-apoptotic agents (Djordjevic et al., 2009), however these changes have not been investigated in neuronal tissue under stress. In the present paper we have studied neuronal and glial reaction following chronic restraint stress, as well as level of mRNA expression of GR and MR receptors; constitutive (β5) and inducible (β1i, LMP2) proteasome subunits; translation regulation factor mTOR and antiapoptotic gene bcl-2, together with protein level of pro-apoptotic enzyme caspase-3.

2. Results

2.1. Animal response on chronic restraint stress

Chronic restraint stress resulted in a significant ($P<0.05$) body weight loss (about 5%) unlike control animals, maintained on the same diet, did not loose their mass (see Table 1). Adrenal glands of stressed rats were significantly ($P<0.05$) hypertrophied with average mass increased 1.5 times as compared to control. At the same time, mass of the thymus gland was decreased four times—from 1.92 ± 0.01 g in control down to 0.58 ± 0.05 g in stressed group ($P<0.01$). Thymo-adrenal weight relation was correspondingly shifted from control 2.61 ± 0.23 to 12.27 ± 0.60 ($P<0.01$; Table 1). Gastric mucosa observed during animal killing was intact for control animals and ulcerated for stressed rats. These changes are classically associated with chronic stress, providing an evidence for the efficacy of the restraint stress model.

2.2. Neuronal and glial reaction

Confocal microscopy of hippocampal sections reveled a decrease in the number of NeuN-labelled cells in chronically stressed animals (Fig. 1). NeuN is a widely used neuronal-specific marker and cells labelled with NeuN were mainly localized within *stratum pyramidale* of CA1, CA2, CA3 and *gyrus dentatus* (GD) regions corresponding to the sites of neuronal localization. Thus, the reduction in number of $NeuN^+$-cells suggests neuronal loss happening across hippocampus *stratum pyramidale*. In CA1 the number of labelled neurons decreased from 229.6 ± 7.2 per mm of stratum pyramidale length (mm^{-1}) to $204.6\pm6.2\ mm^{-1}$ ($P<0.05$), in CA2 from $239.2\pm6.1\ mm^{-1}$ to $141.6\pm4.7\ mm^{-1}$ ($P<0.001$) and in CA3 from $181.4\pm7.0\ mm^{-1}$ to $104.9\pm3.6\ mm^{-1}$ ($P<0.001$). Thus, the neuronal response induced by the chronic stress was most prominent in CA2 and CA3 areas (Fig. 1).

Table 2 – Primer sequences used for qRT-PCR.

mRNA	TaqMan kit
Proteasome subunit β5	TaqMan Gene Expression Assay 7500 Rn01488742_m1
Proteasome subunit β1i	Custom TaqMan Gene Expression Assay 7500
Mtor	TaqMan Gene Expression Assay 7500 Fast Real-time PCR System Custom № 443137
bcl-2	TaqMan Gene Expression Assay 7500 Fast Real-time PCR System Custom № 443138
GADPH	TaqMan Rodent GADPH Control Reagent (VICTMProbe)

Table 1 – Changes in body mass and adrenal and thymus glands weights during the term of experiments.

	Control		Restraint stress	
	Beginning of the experiments	End of the experiments	Beginning of the experiments	End of the experiments
Body mass, gr	211.1 ± 2.5	215.7 ± 4.3	217.2 ± 4.3	$207.3\pm4.2^*$
Adrenal glands weight, ‰	–	0.158 ± 0.007	–	0.227 ± 0.010
Thymus weight, ‰	–	1.92 ± 0.01	–	$0.58\pm0.05^*$
Thymo-adrenal coefficient	–	2.61 ± 0.23	–	$12.27\pm0.60^*$

* Indicate significant differences between groups of stressed and control animals. Statistical significance was determined using ANOVA test followed by a Tukey's post-hoc HSD test. $P<0.05$ was considered to be statistically significant.