Distinct Effect of Stress on 11β-Hydroxysteroid Dehydrogenase Type 1 and Corticosteroid Receptors in Dorsal and Ventral Hippocampus

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Summary

Multiple lines of evidence suggest the participation of the hippocampus in the feedback inhibition of the hypothalamus-pituitary-adrenal axis during stress response. This inhibition is mediated by glucocorticoid feedback due to the sensitivity of the hippocampus to these hormones. The sensitivity is determined by the expression of glucocorticoid (GR) and mineralocorticoid (MR) receptors and 11β-hydroxysteroid dehydrogenase type 1 (11HSD1), an enzyme that regulates the conversion of glucocorticoids from inactive to active form. The goal of our study was to assess the effect of stress on the expression of 11HSD1, GR and MR in the ventral and dorsal region of the CA1 hippocampus in three different rat strains with diverse responses to stress: Fisher 344, Lewis and Wistar. Stress stimulated 11HSD1 in the ventral but not dorsal CA1 hippocampus of Fisher 344 but not Lewis or Wistar rats. In contrast, GR expression following stress was decreased in the dorsal but not ventral CA1 hippocampus of all three strains. MR expression was not changed in either the dorsal or ventral CA1 region. These results indicate that (1) depending on the strain, stress stimulates 11HSD1 in the ventral hippocampus, which is known to be involved in stress and emotion reactions whereas (2) independent of strain, stress inhibits GR in the dorsal hippocampus, which is predominantly involved in cognitive functions.

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Introduction

Stressful situations generate profound physiological and behavioral disturbances. Firstly, the stressor-related information from the sensory systems is conveyed to the brain where the situation is processed and forwarded to the hypothalamus. These signals lead to the activation of two output systems – the sympatho-adrenomedullary and hypothalamic-pituitary-adrenocortical (HPA) axes. The HPA axis is controlled by the parvocellular neurons in the paraventricular nucleus but also by stress excitatory and inhibitory circuits that are activated by stressors in various brain structures including the hippocampus, amygdala and prefrontal cortex (Ulrich-Lai and Herman 2009). Activation of the HPA axis induces the release of corticosteroids that self-regulate the activation and responsiveness of the HPA axis through a negative feedback mechanism (Kolber *et al.* 2008). Once released, corticosteroids are able to influence the brain through rapid non-genomic (Groeneweg *et al.* 2011) and slower, long-lasting genomic pathways in which they bind to high-affinity mineralocorticoid (MR) and lower-affinity glucticoid receptors (GR) (de Kloet *et al.* 1998, Herbert *et al.* 2006). Whereas GRs are expressed throughout the brain, MRs have a distribution that is predominantly restricted to the central amygdala, gyrus dentatus and hippocampal subregions CA3 and especially CA1 (de Kloet *et al.* 1998).

The response of the target cells to corticosteroids depends not only on their plasma level and receptor density, but also on the pre-receptor metabolism, which determines the intracellular concentration of the

biologically active hormone. This metabolism converts cortisol and corticosterone from their inert 11-oxo forms (cortisone, 11-dehydrocorticosterone) and thus amplifies the local action of the hormone (for a review, see Herbert *et al*. 2006, Wyrwoll *et al*. 2011). This amplification depends on the activity of 11β-hydroxysteroid dehydrogenase type I (11HSD1), an enzyme that is highly expressed in various brain structures, including the hippocampus (Rajan *et al*. 1996).

Numerous studies indicate that a number of stress-associated conditions affect hippocampal memory and plasticity, that the hippocampus inhibits the activity of the HPA axis and that corticosteroids play an important role in these processes (Herman *et al*. 2005, Herbert *et al*. 2006, Kim *et al*. 2006). For example, mice carrying a deletion of the GR in the cerebral cortex and hippocampus but retaining GR expression in the paraventricular nuclei have a delayed shut-off of HPA axis responses to stressors (Furay *et al*. 2008). Chronic stress in the hippocampus influences the number and functioning of GR (Joël *et al*. 2007) and acute stress modulates the expression of GR and MR genes (Paskitti *et al*. 2000). However, it is unknown whether the hippocampus modulates the expression of 11HSD1 when a situation is perceived as stressful. The aim of this study was therefore to determine the effect of stress on the expression of hippocampal 11HSD1 and to find out whether the genetic background can modulate the response of 11HSD1 to stress. Three different rat strains with differing levels of stress responsivity were used: Fisher 344 (F344), Lewis (LEW) and Wistar (WIS) rats. LEW rats display a markedly smaller HPA reactivity to a wide range of physical, psychological and immunological stressors and different expression of GR and MR compared with the F344 rats, which have a very reactive HPA axis (Sternberg *et al*. 1989, Smith *et al*. 1994, Dhabhar *et al*. 1995, Oitzl *et al*. 1995, Monček *et al*. 2001). Since LEW rats are inbreeds derived from WIS rats, we chose WIS rats as the appropriate comparative strain.

Materials and Methods

Animals and stress procedure

Naïve male WIS (Inst. of Physiology, Prague), LEW and F344 rats (both Charles River, Germany), 6-8 weeks of age were initially housed in groups of three or four in polypropylene cages for three weeks to acclimatize to the laboratory prior to any manipulations.

The animals were kept under controlled conditions (23±1 °C, lights on between 6:00 and 18:00 h) with free access to rat chow pellets and tap water. To elicit a stronger stress response, we employed a variable stress protocol consisting of 3 days of tandem exposures to various stressors according to Tsoory and Richter-Levin (2006). On the first day, the animals were exposed to a forced swim for 10 min that was carried out in an opaque cylindrical water tank (diameter 0.3 m; height 0.5 m; water depth 0.3 m; water temperature 22±1 °C), on the second day they were placed on an elevated platform (15 x 15 cm at a height of 70 cm above floor level) for three 30 min trials with a 60 min inter-trial interval and on the third day the rats were immobilized for 2 h in a plastic box. After the last stress session, the rats were immediately anesthetized with isoflurane and blood was collected by cardiac puncture, then the animals were killed by decapitation, the brain removed and promptly frozen. All animal experiments and tissue collections were carried out between 9 a.m. and 1 p.m., the rats of the various strains being evenly distributed across this period. The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology.

Brain sampling and processing

Brain sampling and processing was performed as previously described (Švec *et al*. 2010) with some modifications. Briefly, serial 12 μm frozen brain sections were prepared on a cryostat. Selected sections were adhered onto slides coated with polyethylene naphthalate membrane (Leica Microsystems, Wetzlar, Germany), placed in 95 % ethanol, stained with 4 % cresyl violet acetate and washed three times in 95 % ethanol. The dorsal and ventral parts of the CA1 subregion (dCA1, vCA1) of the hippocampus were dissected using a LMD6000 Laser Microdissection System (Leica) and captured into the caps of the microcentrifuge tubes (average area of brain tissue: 0.25 mm^2). The stereotaxic coordinates for the hippocampus were –4.92 mm from the bregma (mediolateral axis: ±4.0 for dCA1 and ±5.0 for vCA1; dorsoventral axis: ±3.0 for dCA1 and ±8.0 for vCA1) as defined by Paxinos and Watson (2007). The dissected tissue was homogenized in 75 μl of RTL buffer (Qiagen, Hilden, Germany) and stored at –80 °C.

Extraction of total RNA was performed using a RNeasy Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction and RNA was evaluated with a NanoDrop spectrophotometer

(NanoDrop Products, Wilmington, DE, USA). Reverse transcription was performed with Enhanced Avian Reverse Transcriptase (Sigma-Aldrich, St. Louis, MO, USA). The cDNA samples were analyzed by real-time PCR in a Viia 7 Sequence Detection System (Life Technologies, Foster City, CA, USA) using TaqMan Gene Expression Master Mix and TaqMan Assays (Life Technologies, Carlsbad, CA, USA) specific for rat 11HSD1 (cat. no. Rn01461862_m1) and mineralocorticoid (cat. no. Rn00565562_m1) and glucocorticoid receptors (cat. no. Rn00561369_m1). The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; TaqMan Endogenous Control, cat. no. 4352338), was used to normalize mRNA expression. This gene was chosen because its transcript is not changed in the rat brain during stress (Porterfield *et al*. 2011). The data were analyzed using the standard curve method.

Plasma corticosterone measurement

Blood was centrifuged, plasma collected and stored at –20 °C until analysis. Plasma concentrations of corticosterone were quantified using a commercially available rat corticosterone radioimmunoassay kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. Assays were conducted in a single run to prevent inter-assay variability; the intra-assay variability was less than 10 %.

Statistical analysis

All data are expressed as means ± SEM. The data were analyzed with a two-way ANOVA (Statistica 6.1., StatSoft Inc., Tulsa, OK, USA) for the effect of genotype (between-subject factor) and treatment (within-subject factor) and their interactions. As no interactions between these two factors were found in any studied parameter (11HSD1, MR, GR, plasma corticosterone), the *p* values are not given in Results. *Post-hoc* analyses were performed using the Student-Newman-Keuls test. The value p<0.05 was considered statistically significant.

Results

To account for possible differences in the effect of our stress paradigm in WIS, LEW and F344 rats, plasma corticosterone levels were determined in control unstressed animals and in stressed rats immediately after the last stress session. Two-way ANOVA revealed a significant effect of stress (F[1,36]=54.72; p<0.001) but

there was no significant effect of genotype (F[2,36]=1.36, n.s.). A *post-hoc* analysis indicated that the plasma corticosterone of stressed WIS, LEW and F344 rats was significantly higher than that of the control unstressed group (Table 1).

Table 1. Effect of stress on plasma levels of corticosterone in Wistar, Fisher 344 and Lewis rats.

| | Corticosterone (ng/ml) | |
	CTRL	Stress
Wistar	26.3 ± 6.7 (8)	289.3 ± 73.6[**] (8)
Fisher 344	51.2 ± 17.9 (8)	361.1 ± 34.5[**] (8)
Lewis	78.0 ± 4.7 (6)	213.2 ± 34.6[*] (7)

Results are expressed as means ± SEM; numbers of animals are given in parentheses. Statistically significant differences are indicated: [*]P<0.05 and [**]P<0.001 compared with the values of control unstressed animals (CTRL).

To determine whether stress might be associated with changes in hippocampal sensitivity to corticosterone, we measured the expression of 11HSD1, GR and MR in the ventral and dorsal CA1 hippocampus. For all measurements, a 3 x 2 ANOVA was examined using genotype (strains WIS, LEW, F344) and stress conditions (control vs. stress) or hippocampal regions (CA1 ventral vs. CA1 dorsal) as variables. For all measurements, stress conditions or hippocampal regions did not significantly interact with rat strain, so the following discussion will focus on the main effects of strain, stress conditions and hippocampal regions. Exposure to a three-day stress resulted in a significant effect on the expression of hippocampal 11HSD1 in the ventral (F[1,32]=6.47; p<0.05) but not dorsal hippocampus (F[1,36]=0.88; n.s.). In addition, there was a significant effect of genotype for the ventral (F[2,32]=6.02; p<0.05) but not dorsal hippocampus (F[2,36]=0.13; n.s.). A *post-hoc* comparison (Fig. 1) showed that the ventral CA1 region of control unstressed animals of F344 and LEW strains had a significantly lower expression of 11HSD1 than WIS rats (F344: p<0.01; LEW: p<0.05) and that stress significantly increased 11HSD1 expression in F344 rats (p<0.05), whereas the effect in LEW rats was just shy of statistical significance (p=0.07); no changes were observed in the WIS strain. The expression of GR in the dorsal hippocampus was altered by stress (F[1,36]=35.78; p<0.001) but did not depend on genotype (F[2,36]=2.55; n.s.). In contrast, genotype (F[2,35]=11.61; p<0.001) but

not stress (F[1,35]=1.34; n.s.) significantly affected MR expression in the dorsal hippocampus. As depicted in Figure 2, a marked decrease in GR expression was observed in the dorsal CA1 hippocampus of all three rat strains exposed to stress (WIS, LEW: p<0.01; F344: p<0.05). The *post-hoc* analysis also revealed that the CA1 region of the dorsal hippocampus exhibited a significantly greater expression of MR in control WIS rats than in F344 and LEW (both p<0.01). In contrast, neither stress nor genotype influenced the expression of GR (stress: F[1,34]=1.12; n.s.; genotype: F[2,34]=1.75; n.s.) and MR (stress: F[1,33]=0.01; n.s.; genotype: F[2,33]=2.89; n.s.) in the ventral hippocampus (Fig. 2).

Moreover, the analysis performed to compare differences between the dorsal and ventral CA1 hippocampus found significantly different expression of all studied transcripts in control animals, i.e. 11HSD1 (F[1,32]=30.93; p<0.001), GR (F[1,33]=80.20; p<0.001) and MR (F[1,32]=69.47; p<0.001). A *post-hoc* analysis revealed that the dorsal hippocampus had a higher expression of 11HSD1, GR and MR than the ventral hippocampus of all three rat strains (p<0.05 or p<0.001). Differences in the expression of 11HSD1, MR and GR expression in the dorsal and ventral CA1 hippocampus were also significantly pronounced in rats that had been exposed to stress (11HSD1: F[1,34]=16.55; p<0.001; MR: F[1,36]=68.99; p<0.001; GR: F[1,36]=53.41; p<0.01). *Post-hoc* testing indicated that the 11HSD1 expression levels of the dorsal hippocampus were significantly higher than that of the ventral region in F344 (p<0.01) and LEW (p<0.05) but not in WIS rats (n.s.). Similarly, a *post-hoc* analysis for MR and GR showed

a significantly higher expression of both receptors in the dorsal compared to the ventral hippocampus of all three rat strains (MR: p<0.01 or p<0.001; GR p<0.001).
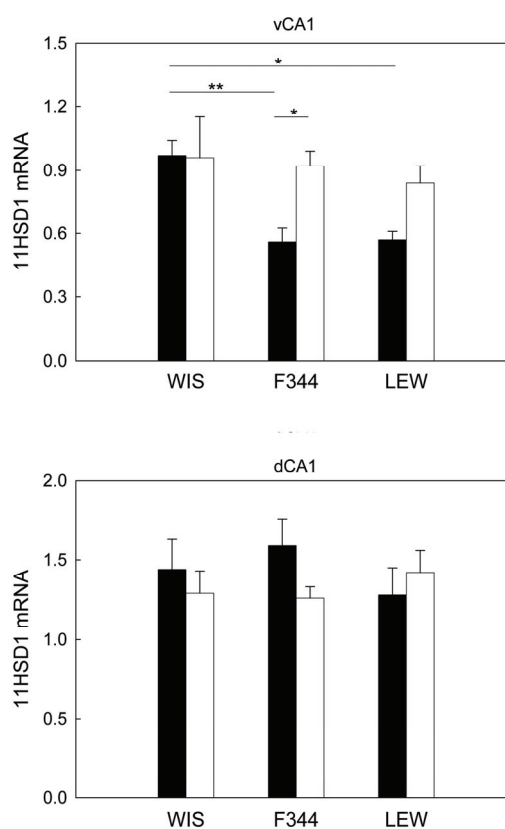

Fig. 1. Effect of stress on expression of 11HSD1 in CA1 region of ventral (vCA1) and dorsal (dCA1) hippocampus of stressed (open bars) and unstressed (filled bars) Wistar (WIS), Fisher 344 (F344) and Lewis (LEW) rats. Data are expressed as means ± SEM (6-8 animals per group). Significant differences are indicated: *P<0.05 and **P<0.01.
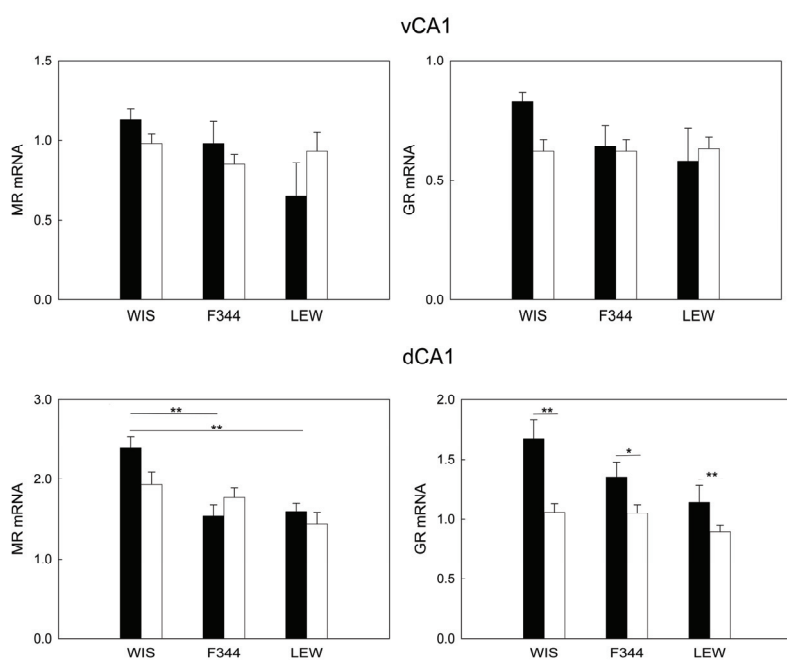

Fig. 2. Effect of stress on expression of mineralocorticoid (MR) and glucocorticoid (GR) receptors in CA1 region of ventral (vCA1) and dorsal (dCA1) hippocampus of stressed (open bars) and unstressed (filled bars) Wistar (WIS), Fisher 344 (F344) and Lewis (LEW) rats. Data are expressed as means ± SEM (6-8 animals per group). Significant differences are indicated: *P<0.05 and **P<0.01.

Discussion

In this study we have presented novel data indicating regional differences in the regulation of 11HSD1, GR and MR in the dorsal and ventral CA1 hippocampus, and have characterized the response of these areas to stress in three rat strains that differ in their HPA reactivity and stress response. The hippocampus richly expresses both corticosteroid receptors and 11HSD1 (Herbert *et al.* 2006) and thus changes in the expression of GR/MR or 11HSD1 during stress might modulate the sensitivity of the hippocampus to corticosteroids. Indeed, two lines of evidence suggest that this may be sufficient to influence hippocampal functions. First, the increased GR gene dosage is associated with an enhanced resistance to stress (Reichardt *et al.* 2000) whereas a reduction in GR gene dosage leads to a prolonged activity of the HPA axis during stress (Ridder *et al.* 2005). Second, the deletion of 11HSD1, a glucocorticoid-producing enzyme, modulates the sensitivity of the HPA axis (Harris *et al.* 2001) and hippocampal overexpression of 11HSD2, a glucocorticoid-degrading enzyme, attenuates the negative impact of an excessive elevation of glucocorticoids on synaptic transmission and spatial memory (Dumas *et al.* 2010).

The key finding in this study is that 11HSD1 expression is increased by stress in the hippocampus, i.e. in the limbic structure that is activated by stress (Ulrich-Lai and Herman 2009), and that this increase depends on the hippocampal region and genotype of the rat. The finding of increased 11HSD1 in the ventral but not dorsal CA1 region is of particular interest. Emerging evidence indicates that the ventral hippocampus is connected to stress and emotion whereas the dorsal hippocampus performs primarily cognitive functions (Fanselow and Dong 2010). The increase in 11HSD1 in our study is consistent with a previous study of the effect of arthritic stress on the undivided rat hippocampus (Low *et al.* 1994) but not with the effect of psychosocial stress on the hippocampus of tree shrews (Jamieson *et al.* 1997). The reason for this discrepancy is unknown, but might reflect a species-specific control of 11HSD1 or the type of stress. The putative role of corticosteroid status in the regulation of 11HSD1 is also supported by the finding of reduced hippocampal 11HSD1 mRNA and enzyme activity in adrenalectomized rats that can be reversed by substitution therapy of the glucocorticoid agonist (Low *et al.* 1994). As hippocampal cells reactivate inactive

11-dehydrocorticosterone to active corticosterone (Rajan *et al.* 1996), it can be hypothesized that the increase in 11HSD1 during stress might intensify the glucocorticoid signaling in the hippocampus. The increased transcript level of 11HSD1 in stressed F344 but not WIS and LEW rats suggests that F344 may amplify the hippocampal glucocorticoid signal more effectively than the stressed WIS and LEW rats, or that the positive effect of stressful stimuli on 11HSD1 requires a longer stress exposure in the WIS and LEW strain. This is in accordance with the well known hyperresposiveness of F344 rats to stressful stimuli.

As described in other studies, corticosteroids have been shown to act as structural and functional modulators of the hippocampus, a structure that modifies the negative feedback effect of glucocorticoids following stressful stimuli *via* the neural inhibition of stress responses (Feldman and Weidenfeld 1999, de Kloet *et al.* 2005, Herbert *et al.* 2006). Our results showed that the dorsal hippocampus had a higher expression of GR and that stressed rats had consistently suppressed GR mRNA in the dorsal but not ventral CA1 hippocampus of all three strains. This implies a potential attenuation of glucocorticoid signaling in the dorsal CA1 hippocampus, i.e. in the area that is involved in cognitive functions but not in regulation of the HPA axis (Fanselow and Dong 2010). In addition, the decreased expression of GR in the dorsal CA1 hippocampus is in agreement with the findings of previous studies in which chronic stress decreased hippocampal GR mRNA (Paskitti *et al.* 2000) and this effect did not depend on the responsiveness of the strain to stress (Gomez *et al.* 1996). In contrast to GR, the expression of MR was neither influenced by stress in the dorsal nor ventral region of CA1. Similarly, neither acute nor chronic stress modulated the expression of hippocampal MR mRNA in an *in situ* hybridization study (Paskitti *et al.* 2000). These findings could be understood in light of the differences between GR and MR. In rats, MRs have an approximately tenfold higher affinity for corticosterone than GR (de Kloet *et al.* 1998), which means that GR and MR are occupied in different ways. MRs are extensively occupied under basal unstressed conditions, while the saturation of GRs requires higher corticosterone levels that are usually reached in stressful situations.

In summary, the results of this study suggest inhomogeneous expression of 11HSD1, GR and MR in the dorsal and ventral CA1 hippocampus and indicate that glucocorticoid signaling in the CA1 region is regulated

by stress through different mechanisms. Whereas in the dorsal area of CA1 the decreased expression of GR without any changes in 11HSD1 and MR expression indicates a stress-induced downregulation of glucocorticoid signaling, the increase in 11HSD1 expression without any changes in GR and MR in the ventral part of CA1 indicates an amplification of glucocorticoid signaling. The increased 11HSD1 in ventral CA1 might participate in the inhibitory feedback mechanisms of the HPA axis, whereas the attenuated GR expression in dorsal CA1 might play a role in ameliorating the negative effect of glucocorticoid excess on hippocampal plasticity, learning and memory. This conclusion is supported by the finding of reduced glucocorticoid negative feedback in mice lacking 11HSD1 (Harris *et al.* 2001) and by the finding of facilitated spatial learning in the moderately stressful water maze after hippocampal injection of the GR antagonist (Oitzl *et al.* 1998).

Conflict of Interest
There is no conflict of interest.

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