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# Differential impact of stress on hypothalamic—pituitary—adrenal axis: Gene expression changes in Lewis and Fisher rats

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**Summary** The aim of the present work was to study the influence of variable stress on the expression of 11β-hydroxysteroid dehydrogenase type 1 (11HSD1) and the neuropeptides corticotropin-releasing hormone (CRH), urocortins 2 and 3(UCN2, UCN3), arginine vasopressin (AVP), oxytocin (OXT) and adenylate cyclase-activating polypeptide (PACAP) in two inbred rat strains: stress hypo-responsive Lewis (LEW) and hyper-responsive Fisher 344 (F344) rats. We found site-specific and strain-dependent differences in the basal and stress-stimulated expression of 11HSD1, CRH, UCN2, UCN3 and PACAP. In LEW rats, stress upregulated 11HSD1 in the prefrontal cortex and lateral amygdala, whereas in F344 rats 11HSD1 was upregulated in the central amygdala and hippocampal CA2 and ventral but not dorsal CA1 region; no effect was observed in the paraventricular nucleus, pituitary gland and adrenal cortex of both strains. The expression of glucocorticoid receptors did not parallel the upregulation of 11HSD1. Stress also stimulated the expression of paraventricular OXT, CRH, UCN3 and PACAP in both strains but amygdalar CRH only in LEW and UCN2/UCN3 in F344 rats, respectively. The upregulation of PACAP and CRH was paralleled only by increased expression of PACAP receptor PAC1 but not CRH receptor type 1. These observations provide evidence that inbred F344 and LEW rats exhibit not

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only the well-known phenotypic differences in the activity of the HPA axis but also strain- and stress-dependent differences in the expression of genes encoding 11HSD1 and neuropeptides associated with the HPA axis activity. Moreover, the differences in 11HSD1 expression suggest different local concentration of corticosterone and access to GR in canonical and noncanonical structures of the HPA axis.


## 1. Introduction

Stress is a common aspect of modern life that produces profound physiological and behavioral disturbances and may contribute to many psychiatric disorders, including depression and post-traumatic stress disorders (de Kloet et al., 2005). Exposure to stressors triggers activation of the nervous, endocrine and behavioral systems to promote physiological adaptations and maintain homeostasis (Herman et al., 2003). The principal endocrine component of the stress response is the activation of the hypothalamic–pituitary–adrenocortical (HPA) axis, a self-regulatory pathway that utilizes its end products (cortisol and corticosterone) to control its own activation and responsiveness through a negative feedback mechanism. The HPA axis is controlled by neurons located in the paraventricular nucleus (PVN) of the hypothalamus but also by central stress excitatory and inhibitory circuits that are activated by stressors in both intrahypothalamic and extrahypothalamic structures (Ulrich-Lai and Herman, 2009). Various neurotransmitters, neuromodulators and steroid stress mediators are released to stress response and can influence distinct neuronal circuits. As summarized by Joëls and Baram (2009), a number of neuropeptides are released by stress in specific populations of neuronal cells and contribute to the activations of the stress response or counteract it. The canonical stress-activated neuropeptides include vasopressin (AVP) and corticotropin-releasing hormone (CRH), which is expressed together with its receptors CRHR1 and CRHR2 not only in the PVN but also in other brain structures. Similar to CRH, stress upregulates the expression of two other members of the CRH family, the urocortins UCN2 and UCN3, which have much higher potency to bind CRHR2 than CRHR1 (Tanaka et al., 2003; Jamieson et al., 2006), the oxytocin (OXT), a neuropeptide that reduces physiological and behavioral indices of stress (Lee et al., 2009), and the adenylate cyclase-activating polypeptide (PACAP), a pleiotropic neuropeptide that is an important regulator of neuroendocrine stress response pathways (Lezak et al., 2014) and has a less-appreciated modulatory role in biosynthesis and secretion of some pituitary hormones (Halvorson, 2014). In addition, the effects of individual mediators on the HPA axis are modulated by glucocorticoids whose release is triggered by stress and receptors are ubiquitously expressed in brain (Joëls and Baram, 2009).

The response of the target cells to glucocorticoids is not dependent merely on the level of free hormones in blood or the activity of multidrug resistance efflux pumps and receptor density in target cells but also on the prereceptor metabolism of glucocorticoids that determines the intracellular concentration of the hormone. In the majority of cells and tissues, this metabolism depends on 11β-hydroxysteroid dehydrogenase type 1 (11HSD1), an enzyme, which converts in vivo biologically inactive 11-oxo-steroids (cortisone, 11-dehydrocorticosterone) to cortisol and corticosterone and thus amplifies cellular glucocorticoid action (Tomlinson et al., 2004; Wyrwoll et al., 2011). This enzyme is expressed in the brain (Wyrwoll et al., 2011; Bisschop et al., 2013; Vodička et al., 2014), pituitary gland (Hanafusa et al., 2002), adrenal gland (Shimojo et al., 1996) and many other peripheral organs (Tomlinson et al., 2004). Another enzyme in the 11HSD family is 11HSD type 2 (11HSD2), which catalyzes the oxidation of cortisol and corticosterone to the inactive cortisone and 11-dehydrocorticosterone, thereby reducing the local glucocorticoid signals (Wyrwoll et al., 2011). It is expressed predominantly in mineralocorticoid target tissues but also in the adrenal gland (Shimojo et al., 1996), where it plays a role in regulation of basal and stimulated adrenal steroid secretion and modulates the expression of phenylethanolamine-N-methyltransferase, a glucocorticoid-dependent enzyme, which catalyzes the conversion of norepinephrine to epinephrine (Musajo et al., 1996; Shimojo et al., 1996). Moderate levels of 11HSD2 expression were also found in some loci of brain (Wyrwoll et al., 2011).

The expression of 11HSD1 in the principal components of the HPA axis and in brain areas that are responsible for the positive and negative regulation of this axis suggests that 11HSD1 might modulate the activity of the HPA axis. Two findings support this hypothesis. First, targeted inactivation of enzyme hexose-6-phosphate dehydrogenase, which regenerates NADPH required for 11HSD1 catalyzed reduction of 11-dehydrocorticosterone to corticosterone, is associated with decreased negative feedback of the HPA axis in spite of elevated circulating levels of corticosterone (Rogoff et al., 2007). Second, 11HSD1 knock-out mice have elevated corticosterone levels and exaggerated ACTH and corticosterone responses to stress (Harris et al., 2001), but the HPA axis phenotype is dependent on the background strain of the mice (Carter et al., 2009). These findings suggest that the regeneration of glucocorticoids by 11HSD1 may be an important regulator of glucocorticoid feedback of HPA axis in vivo and that the genetic background may influence the interaction between 11HSD1 and HPA axis.

Some data indicate that stressful situations modulate the expression of 11HSD1 in the brain and some peripheral organs, but the results are contradictory (Low et al., 1994; Monder et al., 1994; Jamieson et al., 1997; Sesti-Costa et al., 2012; Vodička et al., 2014). In addition, little is known regarding whether genetic background determines the effect of stress on 11HSD1 in specific brain areas associated with the regulation of the HPA axis and in pituitary and adrenal glands. One approach used to investigate these questions is testing animals with different genotypically determined HPA axes. For such study, can be used the histocompatibly similar Lewis (LEW) and Fisher 344 (F344) inbred rat strains, which differ in their responses of both the HPA axis and the immune system to stressogenic stimuli (Sternberg et al., 1989). Generally, LEW rats display a markedly smaller reaction to a wide range of stressors compared with F344 rats, even if there is no difference in the GR level between both strains in the hippocampus and HPA axis (Dhabhar et al., 1993; Grota et al., 1997).