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Research Report

CRH mRNA expression in the hypothalamic paraventricular nucleus is inhibited despite the activation of the hypothalamo-pituitary-adrenal axis during starvation

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ABSTRACT

Corticotropin-releasing hormone (CRH) is one of the anorexigenic neuropeptides, and indeed the expression of hypothalamic CRH is known to be inhibited by starvation. To clarify whether elevated plasma glucocorticoid during starvation is responsible for the CRH suppression, we examined the expression level of hypothalamic CRH mRNA after food deprivation in adrenalectomized, plasma corticosterone (B)-clamped animals. Male Wistar rats were divided into 2 groups: one group had adrenalectomy (ADX) and B pellet implantation (ADX+B, $n=42$), and the other group had only sham operation (sham, $n=42$). Rats were then treated with either ad libitum food supply or food deprivation for up to 96 h. The expression of CRH mRNA in the paraventricular nucleus (PVN) was estimated by in situ hybridization. After food deprivation, mean plasma B level was markedly elevated in sham group, but almost clamped in the ADX+B group. In this experimental condition, CRH mRNA in the PVN was significantly decreased in the sham group, whereas no change was obtained in the ADX+B group. Our data suggest the decrease in CRH mRNA seems to be related to the elevated glucocorticoid level during starvation. The status of hyperadrenocorticism without activation of CRH led us to speculate that adrenocortical function is predominant in the hypothalamic-pituitary-adrenal (HPA) axis during starvation.

1. Introduction

Corticotropin-releasing hormone (CRH) is a key neuropeptide implicated in the neuroendocrine, autonomic and behavioral responses to stress (Bale et al., 2004; Charmandari et al., 2005; Makino et al., 2002; Venihaki et al., 2002). CRH neuron is located in the hypothalamic paraventricular nucleus (PVN), which plays a pivotal role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, a major stress-coping system in higher organisms. During stress, glucocorticoid, an end-product of HPA axis, is released from the adrenal gland and suppresses CRH expression in the PVN, thus operating a negative feedback regulation (Beyer et al., 1988; Jingami et al., 1985a,b; Makino et al., 1994). CRH is also known to be involved in the regulation of food intake and energy homeostasis (Heinriches et al., 1999; Richard et al., 1993). Indeed, animal experiments show that acute intracerebroventricular (icv) administration of CRH significantly reduces food intake and body weight (Arase et al., 1988; Glowa et al., 1991; Krahn et al., 1988).

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Starvation and/or food restriction causes robust changes in circulating hormones and neuropeptide expression in the brain, reflecting the adaptive changes in endocrine and neuroendocrine systems related to appetite regulation and energy expenditure. During starvation, circulating satiety signals, such as glucose, insulin and leptin, are decreased, whereas corticosterone (B) is markedly elevated (Ahima et al., 1996; Dallman et al., 1993; Schwartz et al., 1995). In the brain, starvation causes increase in the expression of orexigenic peptides such as neuropeptide Y (NPY), Agouti-related protein (AgRP), and melanin-concentrating hormone (MCH), whereas it causes decrease in the expression of anorexigenic peptides like proopiomelanocortin (POMC) and CRH (Brady et al., 1990; Mizuno et al., 1999; Qu et al., 1996). These changes are considered to be important in maintaining energy homeostasis. However, the regulatory mechanism(s) responsible for the altered expression of these peptides is not completely understood.

Regarding CRH, it has been reported that CRH mRNA expression in the PVN and CRH peptide in the hypothalamus are decreased after starvation or food restriction (Brady et al., 1990; Dallman et al., 1999; Hwang et al., 1997; Suemaru et al., 1986). One remaining question is whether the decrease is primarily caused by adaptive response to starvation per se, or a secondary response to the negative feedback inhibition by elevated circulating B. To address this issue, we explored the effect of starvation on the mRNA expression levels of CRH under circulating B-clamped condition, i.e., adrenalectomized and B-replaced (ADX+B) rats. We found that, when plasma B levels were clamped, starvation-induced decrease in CRH mRNA was abolished. Thus, the decrease in CRH mRNA level during starvation seems to be caused by a concomitant elevation of plasma glucocorticoid level due to activated HPA axis.

2. Results

2.1. *Effects of starvation on plasma corticosterone and ACTH levels*

In sham-operated group, plasma B levels were significantly elevated at 48 or 96 h after the start of food deprivation (both in the morning and afternoon), indicating that food deprivation potently activated HPA axis throughout the day (Fig. 1). In contrast, no significant difference in plasma B obtained in the afternoon was observed in the ADX+B group, indicating that plasma B was successfully clamped during the starvation. Mild but significant difference in plasma B obtained in the morning in the ADX+B group was observed at 96 h, but the difference was minimal (≈2.5-fold) compared with that in the sham group (>100-fold).

In the sham-operated group, plasma ACTH levels were not significantly increased following starvation at both the morning and afternoon measurements. In the ADX+B group, plasma ACTH in the afternoon was consistently elevated regardless of the food supply, which was also seen in the previous reports (Cascio et al., 1987). Interestingly, plasma ACTH in the morning significantly increased after starvation, possibly due to the lack of plasma B elevation following food deprivation.

We also measured blood glucose and plasma insulin concentration to verify the effect of starvation. Blood glucose and plasma insulin were significantly decreased in the

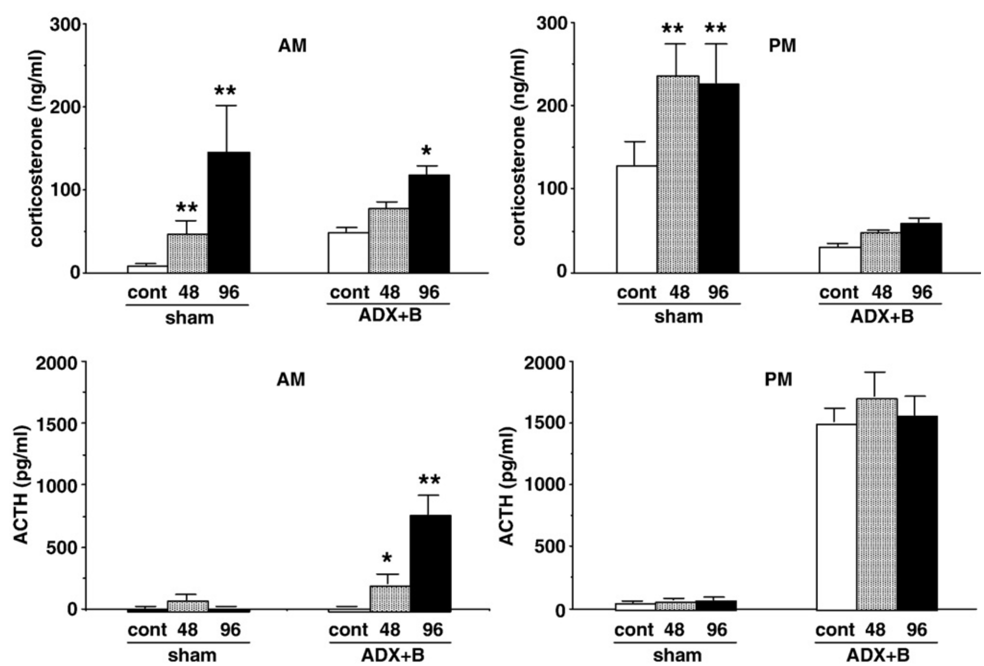

Fig. 1 – Plasma concentrations of B and ACTH in free-feeding rats (control; white bar), or starved rats for 48 h (gray bar) or 96 h (black bar) in sham and ADX+B groups. Samples were obtained in the morning (AM) or afternoon (PM). *P<0.05, **P<0.01 vs. corresponding control.