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Research Report

Regulation of forebrain GABAergic stress circuits following lesion of the ventral subiculum

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ABSTRACT

Ventral subiculum (vSUB) lesions enhance corticosterone responses to psychogenic stressors via trans-synaptic influences on paraventricular nucleus (PVN) neurons. Synaptic relays likely occur in GABA-rich regions interconnecting the vSUB and PVN. The current study examines whether vSUB lesions compromise stress-induced c-*fos* induction and GABA biosynthetic capacity in putative limbic–hypothalamic stress relays. Male Sprague–Dawley rats received bilateral ibotenate or sham lesions of the vSUB. Animals were divided into two groups, with one group receiving exposure to novelty stress and the other left unstressed. Exposure to novelty stress increased c-*fos* mRNA expression in the PVN to a greater degree in vSUB lesion relative to shams, consistent with an inhibitory role for the vSUB in the HPA stress response. However, c-*fos* induction was not affected in other forebrain GABAergic stress pathways, such as the lateral septum, medial preoptic area or dorsomedial hypothalamus. vSUB lesions increased GAD65 or GAD67 mRNA levels in several efferent targets, including anterior and posterior subnuclei of the bed nucleus of the stria terminalis and lateral septum. Lesions did not effect stress-induced increases in GAD65 expression in principal output nuclei of the amygdala. The current data suggest that loss of vSUB innervations produces a compensatory increase in GAD expression in subcortical targets; however, this up-regulation is insufficient to block lesion-induced stress hyperresponsiveness, perhaps driven by amygdalar disinhibition of the PVN.

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Abbreviations:
ACTH, adrenocorticotropic hormone
ANOVA, analysis of variance
BST, bed nucleus of the stria terminalis
CeA, central amygdaloid nucleus
CORT, corticosterone
CRH, corticotropin releasing hormone
DG, dentate gyrus
GAD, glutamic acid decarboxylase
HPA, hypothalamo–pituitary–adrenal
KPBS, potassium phosphate-buffered saline
MeA, medial amygdaloid nucleus
PVN, paraventricular nucleus
vSUB, ventral subiculum

1. Introduction

The ventral subiculum (vSUB) plays a prominent role in neuronal regulation of the hypothalamo–pituitary–adrenocortical (HPA) axis. Lesions of the vSUB enhance glucocorticoid responses to restraint stress and novelty (Herman et al., 1995, 1998; Mueller et al., 2004) and enhance corticotropin releasing hormone (CRH) expression in paraventricular hypothalamic nucleus (PVN) neurons controlling HPA axis responses to stress (Herman et al., 1995, 1998). Anatomical studies indicate that the influence of the vSUB on the PVN is trans-synaptic, involving intervening neurons in the regions such as the bed nucleus of the stria terminalis (BST), medial preoptic area, dorsomedial hypothalamus, subparaventricular zone and the peri-PVN region (Cullinan et al., 1993). Combined anterograde–retrograde tract-tracing studies indicate that the vSUB likely relays with the PVN via synaptic contacts with GABAergic neurons in these regions (Cullinan et al., 1993).

Previous studies indicate that GABA plays a prominent role in inhibition of the HPA axis. Electron microscopic studies suggest that up to 50% of synapses in the PVN are GABAergic (Decavel and Van Den Pol, 1990). In addition, PVN CRH neurons express GABA-A receptor subunits (Cullinan, 2000), suggesting direct actions of GABA on hypophysiotrophic neurons. Accordingly, corticosterone responses to restraint can be attenuated by injection of the GABA-A receptor agonist muscimol into the PVN (Cullinan, 1998), whereas PVN neurons can be directly activated by microinjections of the GABA-A receptor antagonist bicuculline (Cole and Sawchenko, 2002). These data verify that GABA, acting through GABA-A receptors, is prominently involved in inhibition of the PVN.

Notably, GABA-rich PVN-projecting regions are activated by stressful stimuli, supporting a putative role in HPA integration. For example, the medial preoptic area, dorsomedial hypothalamus and peri-PVN region show marked c-*fos* induction in response to a variety of stressors (Cole and Sawchenko, 2002; Cullinan et al., 1995; Emmert and Herman, 1999; Martinez et al., 1998; Sawchenko et al., 2000). In the case of the dorsomedial hypothalamus and medial preoptic area, c-*fos* immunoreactivity is localized in neurons expressing the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) (Cullinan et al., 1996), suggesting that stress activates GABAergic neurons in these regions. In addition, both GAD65 and GAD67 isoforms show marked induction by acute or chronic stress. In the case of acute restraint, GAD67 is up-regulated in the dorsomedial nucleus, medial preoptic area and anterior subnuclei of BST; the same regions showed up-regulation of GAD65 following chronic intermittent stress exposure (Bowers et al., 1998). Up-regulation of GAD isoforms suggests increased synthesis of GABA, perhaps as compensation for prior stress exposure. Thus, changes in both c-*fos* and GAD65/67 expression support the hypothesis that PVN circuits in regions targeted by the vSUB are engaged by stressful stimuli.

Affective disease states are associated with hippocampal dysfunction and HPA axis abnormalities (Bremner et al., 1995; Carroll et al., 1976; Sheline et al., 1996; Yehuda et al., 1991). As the ventral subiculum–PVN circuitry is a primary inhibitory pathway for HPA responses to psychogenic stimuli (Herman and Cullinan, 1997; Herman et al., 2003), it is highly likely that glucocorticoid hypersecretion consequent to depression is associated with altered transmission along this disynaptic pathway. Therefore, understanding the mechanism whereby the hippocampus modulates HPA tone may be of substantial importance in guiding new therapeutic and ameliorative approaches. The data summarized above suggest that the disinhibitory effects of vSUB lesion on the HPA axis may be associated with altered activation of PVN-projecting GABAergic cell populations. To test this hypothesis, the current study assesses the effects of vSUB lesion on induction of c-*fos* mRNA and expression of GAD65/67 mRNA isoforms following acute novelty stress exposure. The data suggest that selective regulatory changes in key GABAergic relays accompany vSUB lesion in these rats and may be involved in mediating hippocampal influences on HPA axis function.

2. Results

Histological assessment revealed that injections of ibotenate caused extensive damage to pyramidal cells of the vSUB,