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Research report

Postnatal handling does not normalize hypothalamic corticotropin-releasing factor mRNA levels in animals prenatally exposed to ethanol

Kara I. Gabriel[b,*], Maria M. Glavas[a], Linda Ellis[a], Joanne Weinberg[a,b]

[a]Department of Cellular and Physiological Sciences, Canada
[b]Department of Psychology, The University of British Columbia, Vancouver, British Columbia, Canada

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Abstract

Postnatal handling has been shown to attenuate some of the deficits in developmental outcome observed following prenatal ethanol exposure (E) although it appears to be ineffective at ameliorating the hypothalamic–pituitary–adrenal (HPA) hyperresponsiveness to stressors that has been observed in adult E animals. However, the effects of postnatal handling on central regulation of HPA activity in E animals, particularly with regard to alterations in steady-state hypothalamic corticotropin-releasing factor (CRF) activity, have not been examined. In the present study, offspring from E, pair-fed (PF), and ad-libitum-fed control (C) groups were exposed to daily handling during the first 2 weeks of life (H) or were left entirely undisturbed until weaning (NH). Basal CRF and arginine vasopressin (AVP) mRNA in the parvocellular portion of the paraventricular nucleus (pPVN) of the hypothalamus were assessed at 90–110 days of age. Prenatal ethanol exposure resulted in elevated basal pPVN CRF mRNA levels compared to those in ad-libitum-fed controls. Handling altered CRF mRNA levels in a sex-specific and prenatal treatment-specific manner. Females showed no significant effects of handling. In contrast, handling decreased CRF mRNA levels in PF and C but not E males compared to their NH counterparts. There were no effects of prenatal ethanol or postnatal handling on AVP mRNA levels. These findings indicate that prenatal ethanol exposure results in elevated basal CRF mRNA levels in adulthood and that handling appears to be ineffective in normalizing those elevations, supporting the suggestion that altered basal HPA regulation in E animals may, at least in part, underlie their HPA hyperresponsiveness to stressors.

Theme: Neural basis of behavior
Topic: Drugs of abuse: alcohol, barbiturates, and benzodiazepines

Keywords: Prenatal ethanol exposure; Postnatal handling; Corticotropin-releasing factor; Arginine vasopressin; mRNA

1. Introduction

The ability to respond to stress is an important basic adaptive mechanism, and hypothalamic–pituitary–adrenal (HPA) activation is a central feature of this response. Environmental stimuli and the endogenous circadian rhythm stimulate secretion of corticotropin-releasing hormone (CRF) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus. CRF and AVP act synergistically to regulate the release of adrenocorticotropin (ACTH) from the anterior pituitary, which, in turn, activates the release of glucocorticoids, namely

Abbreviations: E, Prenatal ethanol exposure; PF, Pair-fed; HPA, Hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropin; CORT, Corticosterone; CRF, Corticotropin-releasing factor; AVP, Arginine vasopressin; mRNA, Messenger ribonucleic acid; H, Postnatally handled; NH, Nonhandled; pPVN, Parvocellular paraventricular nucleus

* Corresponding author. Current address: Department of Psychology, California State University, East Bay, 25800 Carlos Bee Boulevard, Hayward, CA 94542-3091, USA. Fax: +1 510 885 2553.
E-mail address: kgabriel@csuhayward.edu (K.I. Gabriel).

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corticosterone (CORT) in the rat, from the adrenals. Elevations in glucocorticoids are normally self-limiting because glucocorticoids act on higher levels of the axis such as the hippocampus and prefrontal cortex to inhibit further hypothalamic and pituitary activity through negative feedback. Dysregulation of the stress system may lead to disturbances in growth and in many aspects of physiological and behavioral development. As well, it has been implicated in a variety of psychiatric disorders and vulnerability to autoimmune diseases [6,42].

In rats, prenatal and postnatal manipulations have been shown to influence the development of the HPA axis, resulting in long-term alterations in endocrine function and responsiveness. Of interest to the current work, animals prenatally exposed to ethanol (E) display HPA hyperresponsiveness (i.e., increased and/or prolonged elevations of ACTH and/or CORT) to a variety of stressors [36,43,46,47,53]. Elevated steady-state [2,25,39] and stress-induced [26,27] levels of hypothalamic CRF and/or CRF mRNA have also been reported in E animals, although, depending on the parameters of the test situation or the age of testing, this has not always been observed [2,15,24,26].

The mechanisms underlying HPA hyperresponsiveness in E rats are unclear at present but may result, at least in part, from both deficits in feedback inhibition of hypothalamic or pituitary activity [12,14,37,40,59] and increased HPA drive [13,40,59]. Dysregulation of HPA function in E animals is indicated by findings from our laboratory and others showing that, although basal pituitary–adrenal hormone levels are typically normal in E animals [7,36,37,47], under conditions of challenge or manipulation of the HPA axis (e.g., adrenalectomy or glucocorticoid receptor blockade), E animals show increased basal hormone levels compared to those in controls [13,14]. These findings provide support for previous data, as cited above, suggesting that prenatal ethanol exposure may not only alter stress responsiveness but may also alter central regulation of basal HPA activity [2,25,39].

Several environmental interventions, including postnatal handling and environmental enrichment, have been examined for their ability to mitigate the behavioral and hormonal effects of prenatal ethanol exposure [7,8,36,44,52]. Postnatal handling, in which animals are removed daily from the home cage for a brief period (3–15 min) during the first weeks of life, typically results in attenuated or better modulated HPA responsiveness to stressors and/or a faster recovery to basal activity levels compared to that in NH animals [29,34,50,51]. Handling has been shown to lower basal hypothalamic CRF mRNA levels and median eminence CRF content [38], as well as to upregulate hippocampal glucocorticoid receptor expression [45], both of which may play a role in mediating the altered HPA responsiveness observed in handled animals.

Previous studies have shown that, although postnatal handling is effective at modulating pituitary–adrenal responses to stressors in normal or control animals and may alter some aspects of development in E offspring, e.g., preweaning weight gain, hypothermic responses to challenge [52], it appears to be ineffective in attenuating pituitary–adrenal hyperresponsiveness in E animals [7,36]. However, dissociations between hormone responses have been noted before in investigations of HPA function in E animals, in which the response of one hormone may be altered independently of another [53], perhaps due to differences in peptide degradation or because of the specific stressor or time point chosen for sampling. This, in combination with evidence that alterations in central HPA regulation early in development are crucial for the expression of differences in HPA responsiveness in adulthood [3], underscores the importance of assessing the effects of postnatal handling on central regulation of the HPA axis in E animals. Therefore, the present study investigated the effects of postnatal handling on basal hypothalamic CRF and AVP mRNA levels in E animals. We hypothesized that basal CRF mRNA levels would be elevated in E animals and that postnatal handling would normalize elevations in steady-state CRF that may drive the stress-induced HPA hyperresponsiveness observed in E animals.

2. General methods

2.1. Breeding and subjects

Details of the breeding and handling procedures have been previously published [7,8]. In brief, adult Sprague–Dawley males (350–375 g) were co-housed with females (250–275 g) until the presence of vaginal plugs indicated day 1 of gestation (G1). Females were then singly housed and assigned to one of three groups:

1. Ethanol (E): liquid ethanol diet (36% ethanol-derived calories), ad libitum.
2. Pair-fed (PF): liquid control diet, with maltose-dextrin isocalorically substituted for ethanol, in the amount consumed by an E dam (g/kg body weight/day of gestation).
3. Control (C): laboratory chow and water, ad libitum.

Diets were prepared by Bio-Serv, Inc (Frenchtown, NJ) and formulated to provide adequate nutrition to pregnant females regardless of ethanol intake [48]. Fresh diet was placed on the cages daily in the late afternoon, just prior to lights off, in order to avoid a shift in the CORT circadian rhythm typically observed in animals on a restricted feeding schedule such as that of the PF group [9]. Bottles were removed and weighed at this time to determine amount consumed. Diets were administered from G1 to G22 and, thereafter, animals received lab chow and water. Pregnant females were weighed on G1, G7, G14, and G21. At birth,