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Endogenous opioids as substrates for ethanol intake in the neonatal rat: The impact of prenatal ethanol exposure on the opioid family in the early postnatal period

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HIGHLIGHTS

- Pregnant rats were exposed to moderate amounts of ethanol during late gestation.
- Offspring were examined, as infants, for changes in opioid gene expression and protein.
- Assessed factors in the nucleus accumbens, ventral tegmental area, and hypothalamus
- Results suggest relatively long-term changes stemming from prenatal exposure.
- The nucleus accumbens emerged as a primary target for ethanol's effects.

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ABSTRACT

Background: Despite considerable knowledge that prenatal ethanol exposure can lead to devastating effects on the developing fetus, alcohol consumption by pregnant women remains strikingly prevalent. Both clinical and basic research has suggested that, in addition to possible physical, behavioral, and cognitive deficits, gestational exposure to alcohol may lead to an increased risk for the development of later alcohol-related use and abuse disorders. The current work sought to characterize alterations in endogenous opioid signaling peptides and gene expression produced by ethanol exposure during the last days of gestation.
Methods: Experimental subjects were 4-, 8-, and 12-day old infant rats obtained from pregnant females that were given daily intubations of 0, 1, or 2 g/kg ethanol during the last few days of gestation (GDs 17–20). Using real-time RT-PCR, western blotting analysis, and enzyme immunoassays, we examined mRNA and protein for three opioid receptors and ligands in the nucleus accumbens, ventral tegmental area, and hypothalamus.
Results: Three main trends emerged — (1) mRNA for the majority of factors was found to upregulate across each of the three postnatal ages assessed, indicative of escalating ontogenetic expression of opioid-related genes; (2) prenatal ethanol significantly reduced many opioid peptides, suggesting a possible mechanism by which prenatal exposure can affect future responsiveness towards ethanol; and (3) the nucleus accumbens emerged as a key site for ethanol-dependent effects, suggesting a potential target for additional assessment and intervention towards understanding the ethanol's ability to program the developing brain.
Conclusion: We provide a global assessment of relatively long-term changes in both opioid gene expression and protein following exposure to only moderate amounts of ethanol during a relatively short window in the prenatal period. These results suggest that, while continuing to undergo ontogenetic changes, the infant brain is sensitive to prenatal ethanol exposure and that such exposure may lead to relatively long-lasting changes in the endogenous opioid system within the reward circuitry. These data indicate a potential mechanism and target for additional assessments of ethanol's ability to program the brain, affecting later responsiveness towards the drug.

1. Introduction

Despite considerable knowledge that drinking ethanol during gestation leads to devastating effects to the developing fetus, alcohol

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consumption by pregnant women remains strikingly prevalent. Consequences of ethanol consumption during gestation depend, in part, on the overall amount of alcohol consumed per intake session and the gestational period in which the drug was administered (e.g., [1–3]). For example, the intake of large amounts of alcohol throughout gestation often leads to severe developmental consequences, sometimes including mental retardation and the hallmark cranial–facial malformations associated with Fetal Alcohol Syndrome [1,4]. Consumption of smaller amounts of ethanol during this critical period of development, however, may result in less obvious, yet equally devastating consequences. Recent research suggests that even moderate exposure to ethanol during the last portion of gestation or early postnatal life may enhance ethanol intake [5–11], preference [6,12,13], and reinforcement [14–17] throughout life. The neurochemical and neuroanatomical mechanisms mediating these effects, however, are currently unknown.

Studies examining alcohol intake and reinforcement have indicated that the endogenous opioid system plays an important role in many prenatal ethanol effects. Known to be involved in ethanol intake and reinforcement during adulthood, the endogenous opioid system has also been implicated in ethanol's appetitive effects during early postnatal life (e.g., [18,9,19,20,14]). Administration of a mu or kappa opioid receptor antagonist, for example, was sufficient to eliminate ethanol reinforcement normally observed in the infant rat [19,20]. Additionally, naloxone, a nonselective opioid antagonist, administered in combination with ethanol to pregnant females eliminated evidence of augmented alcohol consumption typically found following prenatal exposure to ethanol alone [5,7]. Taken together, these results imply the activation of the endogenous opioid system following ethanol administration and, perhaps, an obligatory role of opioid activity in order for ethanol to function as an effective positive reinforcer during early infancy.

Multiple groups have already reported a relationship between ethanol-induced opioid activity and increased ethanol preference and/or intake [21–26]. It appears that, in alcohol preferring strains of rodents and individuals considered to be at high risk of developing alcoholism, the endogenous opioid system is particularly sensitive to ethanol-induced activation. This is in contrast to low-risk individuals and animals genetically selected to avoid alcohol, for whom ethanol does not seem to be particularly effective at stimulating opioid peptide release [27,22]. Ethanol-induced enhanced sensitivity seems to be particular to the mu-opioid system in which beta-endorphin release, the endogenous ligand for the mu-opioid receptor, is especially responsive to ethanol administration [22]. Augmented opioid signaling contributes to enhanced ethanol reinforcement and/or intake through the release of dopaminergic cell bodies from GABAergic inhibition [22,23]. Similar work in animal models has implicated not only mu, but the delta-opioid system as well [22]. In contrast, the kappa-opioid receptor system is typically thought to convey aversive aspects of ethanol administration. While this generally seems to be the case for older infants [28] and adult animals, KOR activation is thought to convey appetitive and not aversive information during early postnatal life [20,52,53]. What remains to be determined, however, is the consequence of prenatal ethanol administration on the basal development or activity of the endogenous opioid system (i.e., in the absence of a subsequent postnatal ethanol challenge).

Given that increases in alcohol intake are reported following prenatal exposure to the drug, it would be especially useful to examine the molecular and neurochemical mechanisms associated with prenatal exposure to ethanol during early infancy. Certainly, prenatal ethanol-induced changes in the neural mechanisms thought to mediate alcohol addiction may serve as one possible means by which fetal exposure to ethanol might increase the likelihood of future alcohol-related use and abuse disorders.

The current body of work sought to do just this by examining the expression of some of the key members of the opioid signaling family (including both ligands and receptors) in infant rats born to females intubated with ethanol during the last days of gestation. Specifically, we used real time RT-PCR to examine mRNA for the opioid receptors, mu, kappa, and delta, along with precursors for their endogenous ligands — proopiomelanocortin (POMC), preprodynorphin (PPD), and preproenkephalin (PPE), and western blotting analysis or enzyme immunoassay to assess protein for these same receptors and endogenous ligands (i.e., endorphin, dynorphin, enkephalin). All factors were assessed in the ventral tegmental area and nucleus accumbens, key mesolimbic structures known to be involved in ethanol intake and reinforcement. Additionally, we chose to assess these same factors in a third, offsite structure — the hypothalamus, due to its rich opioid activity, involvement in consummatory behavior, and connection to ethanol intake [30] and reinforcement [22]. Brain tissue was collected across several days during early postnatal life (PDs 4, 8 and 12), focusing on ages at which ethanol intake is known to be relatively low, moderate, and high, respectively [29,30]. To the extent that ethanol intake is mediated by the endogenous opioid system (e.g., [31], but see [32]) and differs across early ontogeny, and prenatal exposure to the drug alters subsequent consumption (e.g., [5–11,33]) and reinforcement [14–17] of the drug, we expected to see differences in basal levels of opioid mRNA and/or protein as a function of both ontogeny and prenatal alcohol exposure (PAE). More specifically, we expected to see ontogenetic-dependent upregulation of opioid systems, indicative of ongoing developmental changes within this system. Additionally, based on previous work [34] and others [33], we expected to see ethanol-induced changes in mu-, kappa, and possibly delta- opioid receptors that reflect site-specific responsiveness to PAE. We anticipated that KOR expression would be reduced, while MOR may, depending upon the structure, be found in greater concentration following PAE. Lastly, we predicted that the majority of ethanol-dependent effects would be observed within the nucleus accumbens, a structure that seems especially responsive to ethanol-dependent effects.

2. Materials and methods

2.1. Breeding

Rat pups derived from experimentally naïve Sprague–Dawley rats (Taconic, Germantown, NY) were used as experimental subjects. For breeding, a single male and female were housed in a wire-hanging cage and the droppings below each cage were checked daily for the appearance of a waxy sperm-plug. On the day detected, deemed as gestational day (GD) 0, females were removed and re-housed in standard maternity tubs with at least one other female impregnated on the same day. On GD 20, females were singly housed and observed daily for parturition; the day of delivery was deemed as postnatal day (PD) 0. On PD 1, litters were culled to a total of 10 animals, maintaining equal sex ratios whenever possible [35]. All animals were maintained in a temperature controlled environment (22 ° C), on a 14:10 light–dark cycle with lights on at 0700 h and both food and water available ad libitum (Purina "Formulab Diet", 5008, breeding formula, Ralston-Purina, St. Louis, MO). All animals were treated in accordance with the guidelines set forth by the National Institutes of Health (1986) and the protocols approved by the IACUC of Binghamton University.

2.2. Subjects and procedures

A total of 45 pregnant females, giving rise to 72 experimental subjects, were assigned to 1 of 3 prenatal treatment groups and given daily intubations (i.g.; GDs 17–20) of 0, 8.4, or 16.8% ethanol (v/v; total volume of 1.5% of body weight) to obtain a final dose of 0, 1, or 2 g/kg ethanol, respectively. Briefly, females were gently restrained in a soft towel and a stainless steel feeding tube, roughly 7.6 cm in length, was inserted through the intraoral cavity and into the stomach where fluid was infused. On PDs 4, 8, or 12, a total of 2 pups from each litter (1 male and 1 female) were removed from the dam and immediately