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Research report

Prenatal ethanol exposure alters met-enkephalin expression in brain regions related with reinforcement: Possible mechanism for ethanol consumption in offspring

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HIGHLIGHTS

- Prenatal ethanol exposure induces drug intake in offspring.
- Ethanol and opioids during early ontogeny.
- Prenatal ethanol induces changes in Met-enkephalin content in offspring.

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ABSTRACT

The endogenous opioid system is involved in ethanol reinforcement. Ethanol-induced changes in opioidergic transmission have been extensively studied in adult organisms. However, the impact of ethanol exposure at low or moderate doses during early ontogeny has been barely explored. We investigated the effect of prenatal ethanol exposure on alcohol intake and Methionine-enkephalin (Met-enk) content in rat offspring. Met-enk content was assessed in the ventral tegmental area [VTA], nucleus accumbens [NAcc], prefrontal cortex [PFC], substantia nigra [SN], caudate-putamen [CP], amygdala, hypothalamus and hippocampus. Pregnant rats were treated with ethanol (2 g/kg) or water during GDs 17–20. At PDs 14 and 15, preweanlings were evaluated in an intake test (5% and 10% ethanol, or water). Met-enk content in brain regions of infants prenatally exposed to ethanol was quantitated by radioimmunoassay. Ethanol consumption was facilitated by prenatal experience with the drug, particularly in females. Met-enk content in mesocorticolimbic regions – PFC and NAcc – was increased as a consequence of prenatal exposure to ethanol. Conversely, Met-enk levels in the VTA were reduced by prenatal ethanol manipulation. Prenatal ethanol also increased peptide levels in the medial-posterior zone of the CP, and strongly augmented Met-enk content in the hippocampus and hypothalamus. These findings show that prenatal ethanol exposure stimulates consumption of the drug in infant rats, and induces selective changes in Met-enk levels in regions of the mesocorticolimbic and nigrostriatal systems, the hypothalamus and hippocampus. Our results support the role of mesocorticolimbic enkephalins in ethanol reinforcement in offspring, as has been reported in adults.

1. Introduction

Ethanol experiences during early ontogeny – even during prenatal periods – facilitate posterior acceptance and consumption of the drug in these organisms [1–3]. The predisposition to recognize and prefer ethanol as a function of pre- or postnatal exposure has not only been observed in animal models. Human studies suggest

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that maternal intake of ethanol during pregnancy results in neonatal detection of ethanol odor [4]. In addition, alcohol consumption in children has been recently proposed as a serious risk factor that further increases the probability of drinking among these subjects [5].

In animal models, several authors have established that ethanol seeking and intake are modulated by the appetitive and aversive properties of the drug [2]. Preweanling rats have proven valuable for assessing these phenomena. A pattern of high acceptance of ethanol early in ontogeny seems to be associated with the pharmacological effects of the drug, rather than with its orosensory properties [1,2,6–9]. In addition, preweanling rats are sensitive to the locomotor activating effects of ethanol [10,11], suggesting that infants are prone to process the stimulating effects of ethanol rather than its sedative consequences.

The motivational aspects of alcohol in neonates and infant rats have been analyzed using operant approaches [12–17]. These studies strengthen the notion that the developing rat is highly sensitive to the appetitive motivational effects of ethanol, as demonstrated through rapid and robust instrumental learning.

The endogenous opioid system (enkephalins, endorphins and dynorphins) has been shown to play a major role in ethanol reinforcement and drinking behavior. Opioid peptides and ethanol exhibit similar pharmacological properties and behavioral effects. For instance, low doses of ethanol or opioids stimulate locomotor activity through dopaminergic (DAergic) activation in the ventral tegmental area (VTA), while high doses activate DAergic terminals in the nucleus accumbens (NAcc) [18,19]. Activation of the DAergic mesocorticolimbic system by mu (μ) and delta (δ) opioid agonists induces reinforcement, while kappa (κ) opioid receptor activation is associated to dysphoria [20]. These actions are mediated by increases or decreases in DA release from the NAcc, respectively, suggesting that opioid and alcohol reinforcement underlies a common neurobiological mechanism that involves activation of DAergic reward circuits [21].

In adult rats, ethanol increases the release of β-endorphin and Met-enkephalin (Met-enk), particularly during the ascending limb of the blood ethanol curve [22–24]. Ethanol intake is reduced by κ opioid receptor agonists and selective δ and μ receptor antagonists [25–27]. Lower ethanol preference and self-administration have been reported in μ knockout mice [28], in contrast to the greater preference and drug consumption observed in δ knockout animals [29]. The opioid system is also involved in ethanol-induced appetitive conditioning [2,30,31]. Recently, μ but not δ receptors, have been shown to be involved in the psychomotor stimulant effects of ethanol [32–34].

Mu, δ and κ receptors follow different patterns of development, but all are functional by the second postnatal week of life [35]. In this sense, similar to adult rodents [36], ethanol reinforcement and acceptance in preweanling rats seem to be regulated, at least partly, by the opioid system. For example, non-selective opioid antagonists (such as naloxone or naltrexone) co-administered with ethanol during gestation disrupt future increases in appetitive responding towards ethanol [12,37–39]. Furthermore, opioid antagonist administration, prior to conditioning with ethanol, disrupts appetitive reinforcement towards the drug [13]. In newborn and infant rats, μ and κ opioid systems modulate ethanol-mediated appetitive reinforcement [40] through inhibition of positive behaviors, such as the attachment to an artificial nipple [41]. Ethanol intake can also be reduced by non-selective or selective (μ or δ) opioid antagonists during the preweanling period [34,42,43].

Recent research conducted in our laboratory indicates that early in life the opioid system is involved not only in ethanol consumption, but also in operant behavior mediated by the drug (i.e., ethanol seeking and drug consumption). Overall, these results show that a fully functional opioid system is needed to promote ethanol reinforcement during the second postnatal week. Disruption by either a non-selective (i.e., naloxone) or selective opioid antagonist (μ, δ, κ) or agonist (κ) is sufficient for substantial reduction in consummatory and seeking behaviors associated with ethanol reinforcement [13,14].

Changes in opioid neurotransmission are relevant during ethanol intoxication, as well as in the adaptive neural responses induced by the drug. Ethanol-induced changes in opioidergic transmission occur at different levels, such as the expression and release of opioid peptides, as well as ligand binding to opioid receptors (for reviews, [44–46]). We have previously shown that ethanol induces selective changes in enkephalinergic and β-endorphinergic systems in adult rats, particularly in mesocorticolimbic regions involved in the reinforcing aspects of the drug. Acute ethanol administration dose-dependently increases Met-enk release from the NAcc and decreases peptide content in both the NAcc and caudate-putamen (CP) [23]. In contrast, acute ethanol does not modify β-endorphin content in these regions, but decreases peptide levels in the hypothalamus [47]. In addition, ethanol administration differentially alters the binding of selective ligands to μ and δ opioid receptors with different kinetic patterns. Ethanol (2.5 g/kg) significantly reduces ([^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin ([^{3}H]-DAMGO) binding to μ receptors in the VTA and the shell region of the NAcc, but increases binding in the prefrontal cortex (PFC) [48]. The same ethanol dose increases ([^{3}H]-[D-Pen^2,D-Pen^5]-enkephalin) ([^{3}H]-DPDPE) binding to δ receptors in the PFC, NAcc, CP and substantia nigra (SN) [49]. These results indicate that ethanol selectively alters neurotransmission of both opioidergic systems, but has more pronounced effects on δ than on μ receptors. These findings also suggest that opioid receptor down- and up-regulation mechanisms may be involved in these actions. Differential opioid receptor sensitivity in specific brain areas may account for the impact of acute ethanol intoxication [45].

Chronic ethanol exposure selectively affects Met-enk- and β-endorphinergic systems as well. Ethanol (10% v/v, 4 weeks) increases Met-enk content in the adult rat VTA and PFC, but does not change β-endorphin levels in these brain areas [47,50,51]. However, this treatment does not alter ligand binding to μ or δ opioid receptors, suggesting that neuroadaptive changes in enkephalin- and β-endorphin-containing neurons in the mesocorticolimbic system may have occurred along prolonged ethanol exposure.

Even when ethanol-induced molecular changes in opioid systems have been extensively studied in adults, knowledge about the impact of exposure to low or moderate ethanol doses during early ontogeny is scarce in the literature. Therefore, the aim of this work was to investigate the effect of prenatal ethanol exposure on alcohol intake and Met-enk content in offspring. We studied ethanol effects on peptide levels in brain areas involved in reward mechanisms (i.e., mesocorticolimbic system), as well as in other regions in which Met-enk content is commonly high and are sensitive to ethanol actions (i.e., CP, SN, amygdala, hypothalamus and hippocampus) (for reviews, [44,45]). We hypothesized that selective molecular changes occur at the level of the enkephalinergic system when animals are exposed to ethanol during early ontogeny, since previous pharmacological studies from our group show that ethanol rewarding effects, mediated by prenatal ethanol exposure and infantile experiences with the drug, is disrupted when either non-selective or selective opioid antagonists are administered [13,14]. These findings allow us to predict that, as in adult animals, ethanol reinforcement is mediated, at least partially, by the enkephalinergic opioid system.