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Alcohol alters hypothalamic glial-neuronal communications involved in the neuroendocrine control of puberty: In vivo and in vitro assessments

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ABSTRACT

The onset of puberty is the result of the increased secretion of hypothalamic luteinizing hormone-releasing hormone (LHRH). The pubertal process can be altered by substances that can affect the prepubertal secretion of this peptide. Alcohol is one such substance known to diminish LHRH secretion and delay the initiation of puberty. The increased secretion of LHRH that normally occurs at the time of puberty is due to a decrease of inhibitory tone that prevails prior to the onset of puberty, as well as an enhanced development of excitatory inputs to the LHRH secretory system. Additionally, it has become increasingly clear that glial-neuronal communications are important for pubertal development because they play an integral role in facilitating the pubertal rise in LHRH secretion. Thus, in recent years attempts have been made to identify specific glial-derived components that contribute to the development of coordinated communication networks between glia and LHRH cell bodies, as well as their nerve terminals. Transforming growth factor-α and transforming growth factor-β1 are two such glial substances that have received attention in this regard. This review summarizes the use of multiple neuroendocrine research techniques employed to assess these glial-neuronal communication pathways involved in regulating prepubertal LHRH secretion and the effects that alcohol can have on their respective functions.

Introduction

Over the years it has been well documented that alcohol suppresses hypothalamic luteinizing hormone-releasing hormone (LHRH) secretion (Dees, Rettori, Kozlowski, & McCann, 1985; Dees, Srivastava, & Hiney, 2009; Dissen, Dearth, Scott, Ojeda, & Dees, 2004) and causes a delay in puberty-related events in rats (Dees & Skelley, 1990), rhesus monkeys (Dees, Dissen, Hiney, Lara, & Ojeda, 2000) and humans (Peck, Peck, Skaggs, Fukushima, & Kaplan, 2011; Richards & Oinonen, 2011). The hypothalamus plays the critical role in synchronizing events leading to the activation of mammalian puberty. This process requires the interaction of both glial and neuronal regulatory circuitries that serve to control the secretion of LHRH (Brann & Mahesh, 1994; Ojeda & Urbanski, 1994). Understanding mechanisms by which glial cells contribute to LHRH secretion and how alcohol can affect those actions is important for discerning the mechanism by which alcohol suppresses the pubertal process.

In mammals, the LHRH peptide is synthesized mainly in neurons within the preoptic area (POA), with the vast majority of the nerve processes coursing caudally into the medial basal hypothalamus (MBH) and ending near capillaries within the median eminence (ME). The difference between rats and primates is that the latter, including humans, also have LHRH cell bodies in the arcuate nucleus (ARC) of the MBH, whereas rats do not (Kozlowski & Dees, 1984). However, it is well accepted that the mechanisms governing the release of the peptide are very similar. The enhanced secretion of LHRH leads to increased pituitary gonadotropin secretion followed by an elevation in production of ovarian estradiol and subsequently, reproductive maturity. The secretory activity of LHRH neurons is triggered by several trans-synaptic inputs of both inhibitory and excitatory nature (Brann & Mahesh, 1994; Crowley, Parker, Sahu, & Kalra, 1995). Decreased secretion of inhibitory neurotransmitters (Terasawa, 1999; Terasawa & Fernandez, 2001), and the increased secretion of numerous excitatory neurotransmitters (Claypool, Kasuya, Saitoh, Marzban, & Terasawa, 2000; Hiney, Ojeda, & Dees, 1991; Hiney, Srivastava, Nyberg, Ojeda, & Dees, 1996; Hiney, Srivastava, Pine, & Dees, 2009; Lee, Hiney, Pine, Srivastava, & Dees, 2007; Navarro et al.,

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2004; Ojeda, Urbanski, Costa, Hill, & Moholt-Siebert, 1990) initiate the cascade of events that ultimately lead to the rise in pubertal LHRH release. Some of these transmitters are growth factors of glial origin and are important at the time of puberty because of their involvement in glial-neuronal signaling processes by which the glial cells, through their intimate association with the LHRH nerve terminals in the MBH, regulate LHRH secretion during mammalian puberty (Ma, Berg-von der Emde, Moholt-Siebert, Hill, & Ojeda, 1994; Ma, Costa, & Ojeda, 1994; Ojeda, Lomniczi, & Sandau, 2008). Two glial-derived members of the epidermal growth factor (EGF) family, transforming growth factor-α (TGFα) and transforming growth factor-β1 (TGFβ1), have been shown during the past decade to be significantly involved in the release of LHRH at puberty. Furthermore, their functions have been shown to be altered by alcohol. In this review, we will first describe the importance of their respective roles and physiological mechanisms of action pertaining to the control of LHRH secretion at puberty, and then detail the means by which alcohol alters their actions and disrupts glial-neuronal communications, hence, resulting in suppressed LHRH release. The material presented not only shows how TGFα and TGFβ1 contribute to normal puberty, but also provides insight as to how alcohol can detrimentally affect pubertal maturation.

ErbB receptor activation and prepubertal LHRH release

While EGF and TGFα can both stimulate LHRH release (Ojeda et al., 1990), TGFα plays the more pivotal role in regulation of LHRH neuronal function during puberty (Ma, Berg-von der Emde et al., 1994; Ojeda et al., 2008). TGFα is highly expressed in both astrocytes and tanycytes of the MBH and increases markedly around the time of puberty (Ma, Junier, Costa, & Ojeda, 1992). TGFα binds to and activates the erbB1/erbB2 receptor complex on adjacent glial cells in MBH. Activation of these receptors results in the production of a glial substance, prostaglandin-E2 (PGE_2), which then induces the release of prepubertal LHRH secretion upon binding to specific receptors on nearby LHRH neuron terminals in the ME region of the MBH (Ma, Berg-von der Emde, Rage, Wetsel, & Ojeda, 1997). Studies have shown that TGFα stimulates LHRH release via an indirect mechanism that involves a paracrine effect of this growth factor on glial cells. In this regard, the receptors for TGFα have been shown only in glial cells (Ma, Berg-von der Emde et al., 1994). *In vitro* studies have shown that exposure to TGFα stimulates secretion of PGE_2 from hypothalamic glial cells into the medium, and that placing this conditioned medium on immortalized LHRH-secreting neurons referred to as GT1 cells causes LHRH release (Ma et al., 1997). Additionally, in hypothalamic glial cells, TGFα induced PGE_2 formation, and the stimulatory effect of the TGFα-conditioned medium on LHRH release is prevented by erbB receptor inhibition or blockade of prostaglandin synthesis (Ma et al., 1997; Ojeda & Ma, 1999). Taken together, these studies demonstrate that TGFα acts by indirectly influencing hypothalamic glial-neuronal communication networks contributing to mammalian puberty.

In vitro and in vivo effects of alcohol on erbB1 receptor activation and LHRH release

Understanding the mechanism of alcohol-induced suppression of LHRH release is important for determining how this drug disrupts pubertal development. Critical to this issue is the role of PGE_2, which plays a major role in the LHRH secretory process in prepubertal animals (Ojeda, Urbanski, Katz, & Costa, 1988; Ojeda, Urbanski, Katz, Costa, & Conn, 1986). Furthermore, it is a critical component for the glial-dependent regulation of LHRH release (Ma et al., 1997; Prevot, Cornea, Mungenast, Smiley, & Ojeda, 2003). An earlier report (Hiney, Dearth, Srivastava, Rettori, & Dees, 2003) showed that acute *in vitro* exposure to alcohol blocks PGE_2 and LHRH secretion from the same ME tissue fragments containing the LHRH nerve terminals (Fig. 1). Only recently, however, have the mechanisms of alcohol actions on the TGFα-PGE_2 pathway been critically assessed with regard to prepubertal hypothalamic glial-neuronal communications (Srivastava, Hiney, & Dees, 2011). Specifically, it was shown in immature female rats that short-term alcohol exposure via a liquid diet feeding regimen for 4 and 6 days caused an increase in hypothalamic TGFα gene and protein expressions. TGFα gene expression was increased markedly at 4 days and was still elevated after 6 days (see Srivastava et al., 2011). This effect paralleled the increased TGFα protein expressions on both days (Fig. 2A–D). To determine whether the increased levels of TGFα protein were due to diminished release, basal TGFα secretion was assessed from MBHs incubated *in vitro* after 6 days of alcohol exposure *in vivo*. Results indicated that alcohol exposure suppressed TGFα release into the medium (Fig. 3). Taken together, these findings demonstrate that the alcohol suppressed the release of this glial peptide, resulting in an accumulation of hypothalamic TGFα mRNA and protein.

With regard to erbB1 receptors, alcohol exposure for 4 and 6 days did not elicit changes in erbB1 gene expression or the synthesis of total, non-phosphorylated erbB1 protein, but caused a marked decrease in the synthesis of the phosphorylated form of the receptor at 4 days (see Srivastava et al., 2011), as well as at 6 days

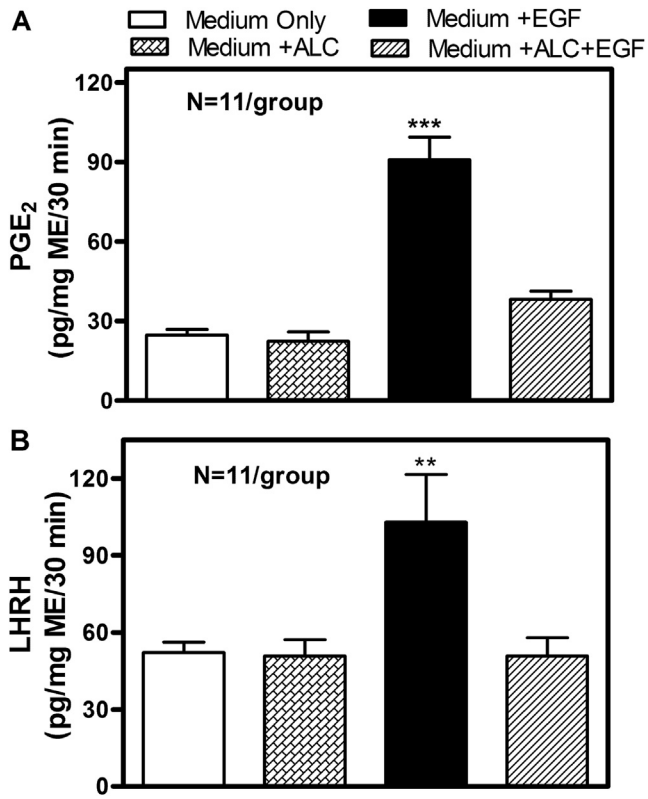

Fig. 1. Effect of alcohol on EGF-induced PGE_2 (Panel A) and LHRH (Panel B) release *in vitro* from the median eminence of prepubertal female rats. Open bars represent basal release of PGE_2 and LHRH. Hatched bars represent basal release of PGE_2 and LHRH in the presence of 50 mM alcohol, which would be a blood alcohol level of approximately 230 mg/dL *in vivo*. Solid bars represent EGF-induced PGE_2 and LHRH release, and lined bars represent EGF-induced release of PGE_2 and LHRH release in the presence of 50 mM alcohol. Note that EGF significantly stimulated both PGE_2 and LHRH release, which was blocked by alcohol. Bars represent the mean ± SEM. $^{**}p < 0.01$ vs. medium only, medium + alcohol, and medium + alcohol + EGF; $^{***}p < 0.001$ vs. medium only, medium + alcohol, and medium + alcohol + EGF. Modified from Hiney et al., 2003.