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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.

Keywords

alcohol; puberty; transforming growth factor-α; transforming growth factor-β1; glia

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

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(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). A 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., 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, 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. 4). Apparently, the 4–6-day duration of alcohol exposure used was not long enough to down-regulate erbB1 gene expression; however, the translation of phosphorylated erbB1 protein was diminished. Interestingly, alcohol did not affect the synthesis of total and phosphorylated erbB2 (not shown), which further demonstrates a specific effect of alcohol on the erbB1 receptor. We suggest that because TGFα binding initiates autophosphorylation of the erbB1 receptor (Ebner & Derynck, 1991), it is plausible that the action of alcohol to suppress TGFα release (Fig. 3), at least in part, is a contributing factor to the alcohol-related decrease in erbB1 phosphorylation. However, we cannot rule out the possibility of a direct action of alcohol on the erbB1 autophosphorylation process that is independent of suppressed TGFα secretion. Several studies supporting this have demonstrated that chronic alcohol exposure disrupts phosphorylation of erbB1 by altering receptor affinity and/or tyrosine kinase activity (Tuma, Todero, Barak-Bernihagen, Casey, & Sorrell, 1998; Wang, Feng, & Wu-Wang, 1997).

Because suppressed erbB1 phosphorylation was expected to result in decreased PGE_2 release, rats were exposed to alcohol *in vivo* for 6 days. Their hypothalami were then removed and subsequently incubated *in vitro* for assessing the release of PGE_2. In this regard, the *in vivo* alcohol exposure caused the suppression of PGE_2 released *in vitro* (Fig. 5). This action was associated with the suppressed phosphorylation of the erbB1 receptor shown in Fig. 4.

Collectively, these results demonstrate the inhibitory effects of alcohol on the glial TGFα/erbB1 pathway contributing to the production and secretion of PGE_2 within the MBH, hence, indicating that this is one of the mechanisms by which alcohol suppresses PGE_2 and subsequently, LHRH secretion (Hiney & Dees, 1991; Lomniczi et al., 2000; Srivastava et al., 2011). The fact that alcohol can negatively affect the erbB1 receptor is important since it is known that an alteration in the function of this receptor is associated with delayed puberty (Apostolakis, Garai, Lohmann, Clark, & O'Malley, 2000).

TGFβ1 and prepubertal LHRH synthesis and release

In addition to TGFα regulation of LHRH secretion, another growth factor that is produced and secreted by hypothalamic astrocytes, TGFβ1, is now being shown to also influence LHRH synthesis and release by several methodologies, including astrocyte and neuronal cell line cultures, *in vitro* incubations of hypothalamic tissue, and *in vivo* studies.

Hypothalamic glia are not only associated with LHRH nerve terminals in the MBH/ME, but also have been demonstrated in the rat to have a connection with LHRH neuronal parakarya in the POA (Witkin, Ferin, Popilskis, & Silverman, 1991; Witkin & Silverman, 1985). TGFβ1 gene expression has been observed in hypothalamic astrocytes in culture (Buchanan, Mahesh, & Brann, 2000; Galbiati et al., 1996) and in both the POA and MBH *in vivo* (Bouret, De Seranno, Beauvillain, & Prevot, 2004). Furthermore, LHRH neurons in the POA express both TGFβ-receptor-1 (Prevot et al., 2000) and TGFβ-receptor-2 (Bouret et al.,

2004). Taken together, these results suggest that this peptide may regulate LHRH synthesis and possibly release through direct activation of the LHRH neurons in the POA and may also contribute to release of the peptide, possibly through glial-nerve terminal interactions in the MBH/ME. Initial studies conducted *in vitro* demonstrated that TGFβ1 secreted from astrocytes grown in culture can act directly on GT1 neurons, a LHRH-secreting cell line, to stimulate LHRH gene expression and release of the peptide (Buchanan et al., 2000; Galbiati et al., 1996; Melcangi et al., 1995). GT1 cells and their processes/terminals are both present in the culture dish. Therefore, this does not take into account that, in the rat, for example, the LHRH is synthesized in neurons located in the POA, yet the area with the greatest concentration of its nerve terminals is located in the MBH. Thus, while the results using the GT1 cultures are interesting, they do not rule out the possibility that LHRH release in the live animal may occur not only following direct activation at the level of the nerve cell body, but also through actions, either direct or indirect, at the level of the nerve terminals. The nature of this question as to what are the sites and mechanisms of TGFβ1 actions on the control of LHRH has required the use of several experimental methods. The sections below will describe how the combination of cell culture, *in vivo*, and *in vitro* techniques, as well as assessing alcohol interactions, have contributed to our understanding of glial-neuronal communications regarding TGFβ1 control of LHRH synthesis and release.

Actions and interactions of TGFβ1 and alcohol on LHRH neurons

The TGFβ1 peptide has been shown (Srivastava, Hiney, & Dees, 2014) to induce LHRH gene expression in the POA within 6 h after a third ventricular injection, an action that was blocked by alcohol (Table 1). Hence, these data support the previous *in vitro*/cell-culture studies using GT1 cells, showing that LHRH gene expression is up-regulated by astrocyte-derived TGFβ1 (Galbiati et al., 1996). This information, along with results derived from rat POA tissues that show TGFβ1-expressing astrocytes are closely associated with LHRH neurons that are immunoreactive for both TGFβ receptors (Bouret et al., 2004; Prevot et al., 2000), provides *in vivo* evidence supporting the concept that TGFβ1 can up-regulate the LHRH gene.

To further investigate glial-neuronal communications, we recently used a cell culture approach to assess the effects of alcohol and another glial-derived peptide, insulin-like growth factor-1 (IGF-1), on TGFβ1 and its potential action on LHRH neurons. In this regard, hypothalamic astrocytes were grown in culture and exposed to medium only, medium containing either alcohol or IGF-1, or medium with both alcohol and IGF-1. In Fig. 6A, astrocytes exposed to alcohol showed suppressed basal secretion of TGFβ1, while exposure to IGF-1 markedly stimulated the secretion of the peptide, and this action was blocked when alcohol was present in the medium. After this 18-h incubation, the medium from each of the four above groups was removed and used to replace the medium in which GN11 cells, another LHRH-secreting cell line, were growing in culture. In this regard, the medium that was originally exposed to the alcohol only, and then contained suppressed TGFβ1, caused a decrease in LHRH released from the GN11 cells (Fig. 6B). This figure also shows that the medium that was originally exposed to IGF-1 only, and then contained increased TGFβ1, caused a marked increase in LHRH released from the GN11 cells, whereas the medium that was originally exposed to both IGF-1 and alcohol and did not

result in elevated TGFβ1, was unable to stimulate LHRH secretion from these cells. Collectively, the above studies using *in vivo* tissue assessments, as well as astrocyte cultures, along with two different LHRH cell lines, have provided convincing evidence for actions of glial-derived TGFβ1 on LHRH neurons.

Effects of TGFβ1 and alcohol on LHRH release from nerve terminals within the MBH

While the TGFβ1 action on the LHRH neuron is convincing, its ability to stimulate release of the peptide from nerve terminals when LHRH cell bodies are not present is less clear. An earlier report using the rat model indicated that TGFβ1 was ineffective in causing the release of the LHRH peptide from nerve terminals when only the ME was incubated *in vitro* (Ojeda et al., 1990). In a more recent study (Srivastava et al., 2014), we assessed the effect of TGFβ1 and alcohol on LHRH release *in vitro* from a tissue block that contained the entire MBH from prepubertal female rats, which includes both the ARC nucleus and the ME. In this regard, the TGFβ1 induced a marked increase in LHRH released from the MBH into the medium (Fig. 7). This figure also demonstrates that alcohol was capable of blocking the stimulatory effect of the TGFβ1 peptide on LHRH release. The difference between the results of this study compared to the previous one, indicating the lack of LHRH stimulation with the ME only, is the presence of the ARC nucleus in the tissue block along with the ME. Although more research is needed, it is likely that the TGFβ1 is acting indirectly by stimulating a neurotransmitter produced by neurons located in the ARC nucleus that, once secreted, is capable of inducing LHRH release from the nerve terminals in the ME. The potential for this action is supported by the fact that both TGFβ receptors are present in the ARC nucleus (Bouret et al., 2004; Prevot et al., 2000).

It is worth noting that TGFβ1 can facilitate LHRH release from the terminals within the MBH/ME via other mechanisms. For example, IGF-1 plays an important role at puberty (Hiney et al., 1996). Glial synthesis of this peptide increases in the hypothalamus as puberty approaches and furthermore, it is known that peripherally derived IGF-1 crosses the blood-brain barrier to enter this region, which contains the greatest number of IGF-1 receptors (Lesniak et al., 1988). This peptide can stimulate PGE_2 release and subsequently, LHRH release *in vitro* (Hiney, Srivastava, Lara, & Dees, 1998) and LH *in vivo* (Hiney et al., 1996). Since alcohol inhibited IGF-induced TGFβ1 from cultured hypothalamic astrocytes, the question arose as to the ability of IGF-1 to stimulate TGFβ1 protein synthesis in MBH tissue from prepubertal female rats *in vivo*. Thus, we recently showed (Hiney, Srivastava, Volz, & Dees, 2014) that the central administration of IGF-1 stimulated an increase in TGFβ1 at 6 h post-injection, and that this action was blocked by alcohol (Fig. 8). This IGF-1 action was subsequently shown to be mediated by the transduction signal Akt, which was also suppressed by alcohol. In order to determine the mechanism of alcohol's action on the IGF-1 signaling pathway in this brain region, we demonstrated that the IGF-1-stimulated phosphorylation of the IGF-1 receptor was inhibited by alcohol, thus resulting in the downstream alterations in Akt and TGFβ1 protein levels (Hiney et al., 2014).

The ability of IGF-1 to activate the glial production of prepubertal TGFβ1 is important with regard to the regulation of LHRH secretion at the time of puberty. Because IGF-1 and

TGFβ1 are produced in glia, their glial to glial interrelationship is relevant. IGF-1 can affect TGFβ1 through activation of the above-mentioned TGFα-PGE_2 pathway from adjacent glial cells. Oct 2 POU homeodomain genes are expressed in hypothalamic glia and increase during pubertal development (Ojeda et al., 1999). This gene is an upstream modulator of the TGFα, which, as stated earlier, is involved in LHRH release. We have shown that Oct 2 genes are a link between IGF-1 and TGFα (Dees, Srivastava, & Hiney, 2005). Specifically, centrally administered IGF-1 stimulated Oct 2c in the MBH and furthermore, this action was blocked by alcohol. Importantly, TGFα acts within the MBH through erbB1 receptors to stimulate glial-derived PGE_2 release (Ma et al., 1997). Once released, PGE_2 not only can induce LHRH release directly from its neuron terminals in the ME (Hiney & Dees, 1991; Ojeda, Negro-Vilar, & McCann, 1979), but it can also act on specialized glial cells called tanycytes that line the third ventricle of the hypothalamus (Prevot et al., 2003). With regard to the latter, PGE_2 stimulates the release of TGFβ1, which then causes retraction of tanycyte processes within the ME to better allow for entry of LHRH into the hypophyseal portal blood (Prevot et al., 2003). Collectively, this information clearly depicts the importance of glial to glial communications in regulating prepubertal LHRH secretion. Also, an alcohol-induced suppression of TGFβ1 synthesis, either alone or coupled with the alcohol inhibition of IGF-1-induced PGE_2 release (Hiney et al., 1998), would markedly affect tanycyte functions related to the LHRH secretory process.

Conclusions

A recent survey indicates that there has been a significant increase in alcohol use and abuse between adolescent and early teenage development (Miech, Johnston, O'Malley, Bachman, & Schulenberg, 2015). It is now well accepted that binge-type drinking is frequent and can easily produce BACs similar to those we have shown above. Thus, increased alcohol use poses a health concern for the young since they are vulnerable to its detrimental effects. As stated above, an indication of this is that girls who drink have four times the chance for showing delayed signs of pubertal maturation. Thus, identifying the underlying mechanisms by which alcohol can alter adolescent development is important and will help define treatment methods for aiding in recovery and lessening the impact of the adverse effects of alcohol during this critical stage of development.

In this review, we have focused on the contributions of two glial-neuronal communication pathways with regard to pubertal LHRH secretion and revealed how these interactions are influenced by alcohol. One of the pathways addressed is the TGFα-erbB receptor signaling system. Glial TGFα activates the erbB1/erbB2 receptor complex on adjacent glia in the MBH. This activation causes a cascade of events leading to the increased synthesis and release of PGE_2, which in turn binds to its receptor on nearby LHRH nerve terminals, thus inducing release of the peptide. Additionally, evidence was presented using a combination of *in vivo* and *in vitro* methods showing that alcohol is capable of interfering with hypothalamic glial to glial signaling involved with prepubertal PGE_2 synthesis/release, actions that ultimately contribute to the alcohol-induced decrease in LHRH secretion. The other communication network discussed is that of the influence of TGFβ1 on the LHRH system. With regard to the POA, we showed the potential for TGFβ1 to regulate LHRH synthesis and possibly release through a direct activation of LHRH neurons. We also

discussed how TGFβ1 may contribute indirectly within the MBH to release LHRH peptide through potential glial-neuronal interactions in the ARC nucleus and furthermore, addressed the interactions between IGF-1, TGFα, PGE_2, and TGFβ1 regarding their coordinated ability to facilitate LHRH release through their glial-glial and glial-nerve terminal interactions within the ME. Overall, this review has described the use of multiple research techniques to further our knowledge as to the importance of glial-LHRH neuronal communications at puberty, and how a toxic substance, such as alcohol, is capable of altering these important cell-cell interactions during an important time of development.

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Highlights

- Increased hypothalamic TGFα following ALC exposure was due to decreased release of peptide.
- ALC-induced decrease in TGFα secretion resulted in suppressed erbB1 activity, followed by suppression in prepubertal PGE_2 release.
- In POA, TGFβ1 stimulates LHRH synthesis and possibly release through a direct activation of LHRH neurons.
- TGFβ1 may contribute indirectly within the MBH to release LHRH peptide through potential glial-neuronal interactions in the ARC nucleus.
- IGF-1 induces TGFβ1 protein synthesis in the MBH area and this action is blocked by ALC.

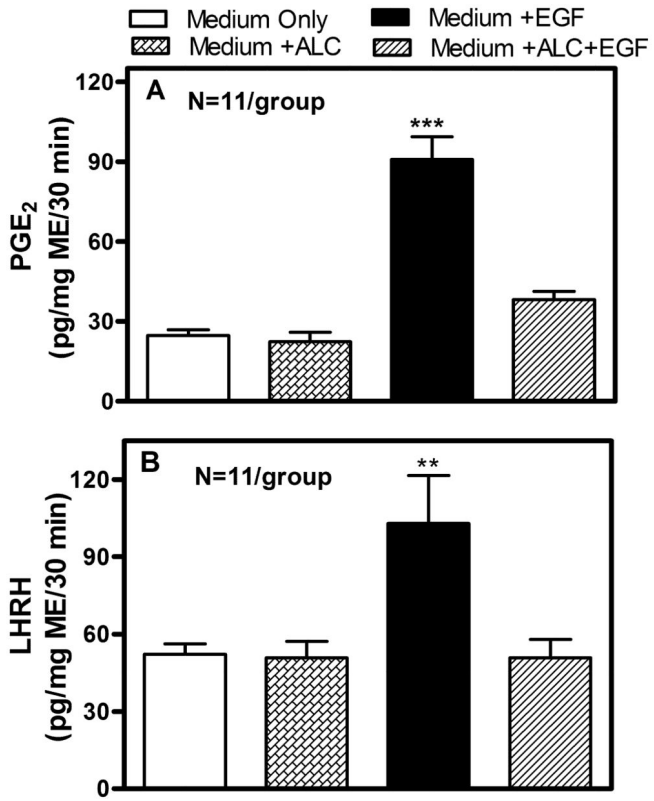

Fig. 1.

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 approximately 230 mg/dL of serum *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.

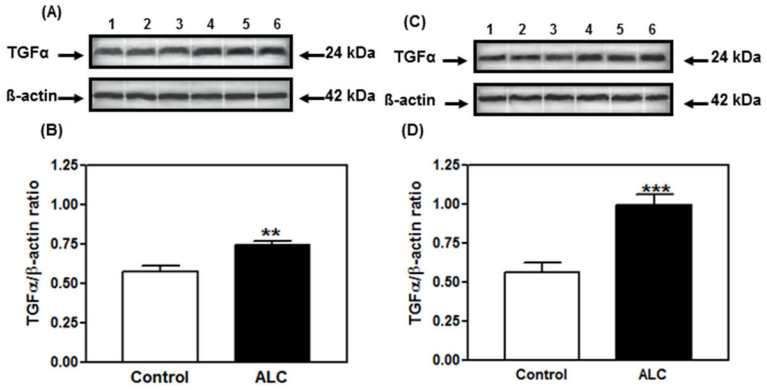

Fig. 2.

Effect of short-term alcohol exposure for 4 (panels A & B) and 6 days (panels C & D) on TGFα protein expression in the MBH of prepubertal female rats. (A & C) Representative Western immunoblot of TGFα and β-actin proteins in the MBH isolated from control (lanes 1–3) and alcohol-treated (lanes 4–6) animals. (B & D) Densitometric quantitation of all the bands from 2 blots assessing TGFα protein expression in the MBH. These data were normalized to the internal control β-actin protein, and the densitometric units represent the TGFα/β-actin ratio. Note that alcohol-treated animals showed increased TGFα protein expression on day 4 (panel B) and day 6 (panel D) compared with control animals. The respective bars illustrate the mean (± SEM) of an N of 7–8 per group. The mean blood alcohol levels after 4 and 6 days of treatment with the alcohol diet were 188 mg/dL and 210 mg/dL, respectively. $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control. Modified from Srivastava et al., 2011.

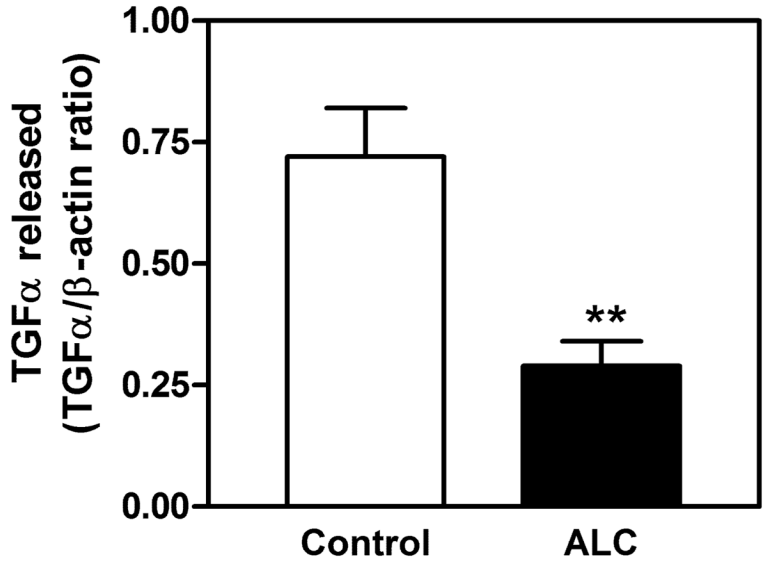

Fig. 3.

Effect of short-term alcohol exposure for 6 days on TGFα protein released *in vitro* from the MBH of prepubertal female rats. The content of TGFα released into the medium was determined by Western immunoblotting. Note that alcohol-treated animals showed a marked decrease in basal TGFα release compared with control animals. These data were normalized to the internal control β-actin protein, and the densitometric units represent the TGFα/β-actin ratio. The respective bars illustrate the mean (± SEM) of an N of 14 for control and an N of 9 for alcohol. $^{**}p < 0.01$ vs. control. Modified from Srivastava et al., 2011.

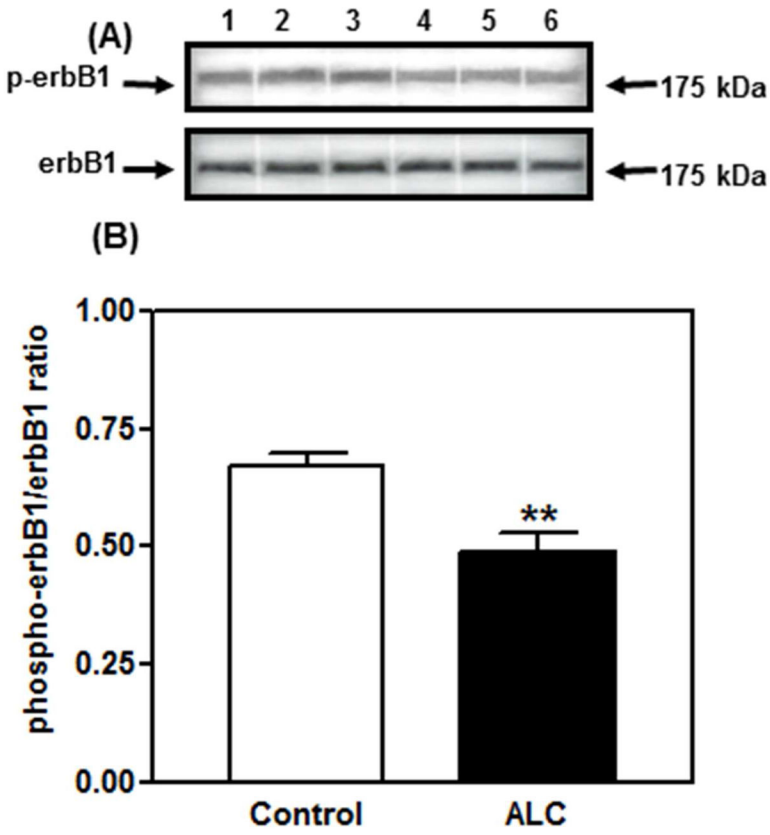

Fig. 4.

Effect of short-term alcohol exposure for 6 days on phosphorylated erbB1 protein expressions in the MBH of prepubertal female rats. (A) Representative Western blot of phosphorylated erbB1 and total, nonphosphorylated erbB1 proteins in the MBH from controls (lanes 1–3) and animals exposed to alcohol for 6 days (lanes 4–6). (B) Densitometric quantitation of all of the bands from 2 blots assessing phosphorylated erbB1 protein in the MBH. Note that phosphorylated erbB1 protein expression decreased markedly in the animals treated 6 days with alcohol diet when compared to controls. These data were normalized to the total, nonphosphorylated erbB1 protein, and the densitometric units represent the phosphorylated erbB1/total, non-phosphorylated erbB1 ratio. The respective bars illustrate the mean (± SEM) of an N of 8 per group at 6 days. $^{**}p < 0.01$ vs. control. Modified from Srivastava et al., 2011.

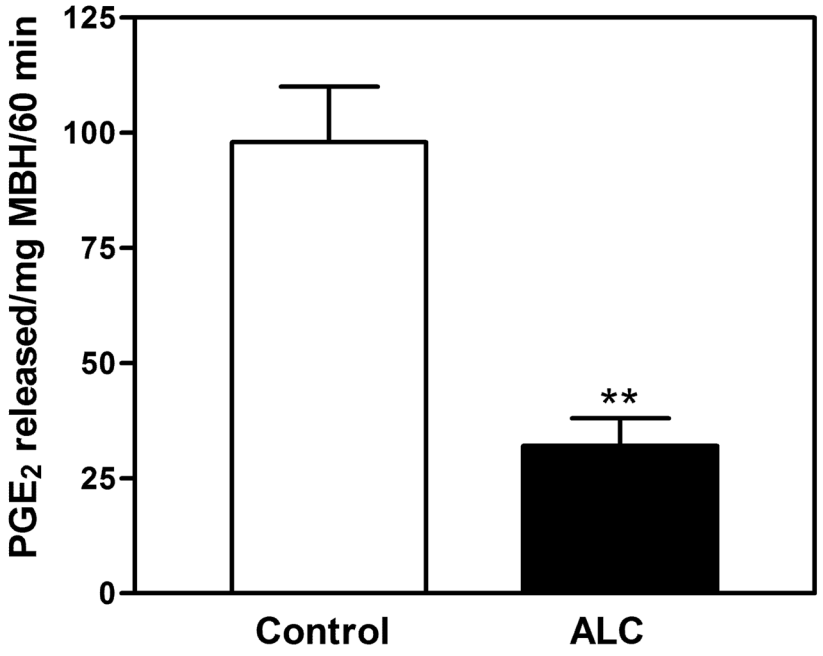

Fig. 5.

Effect of short-term alcohol exposure for 6 days on prostaglandin-E_2 (PGE_2) release *in vitro* from the MBH of prepubertal rats. The content of PGE_2 released into the medium was determined by ELISA. Note that alcohol-treated animals showed a marked decrease in the basal PGE_2 release compared with control animals. The respective bars illustrate the mean (± SEM) of an N of 14 for control and an N of 9 for alcohol. $^{**}p < 0.01$ vs. control. Modified from Srivastava et al., 2011.

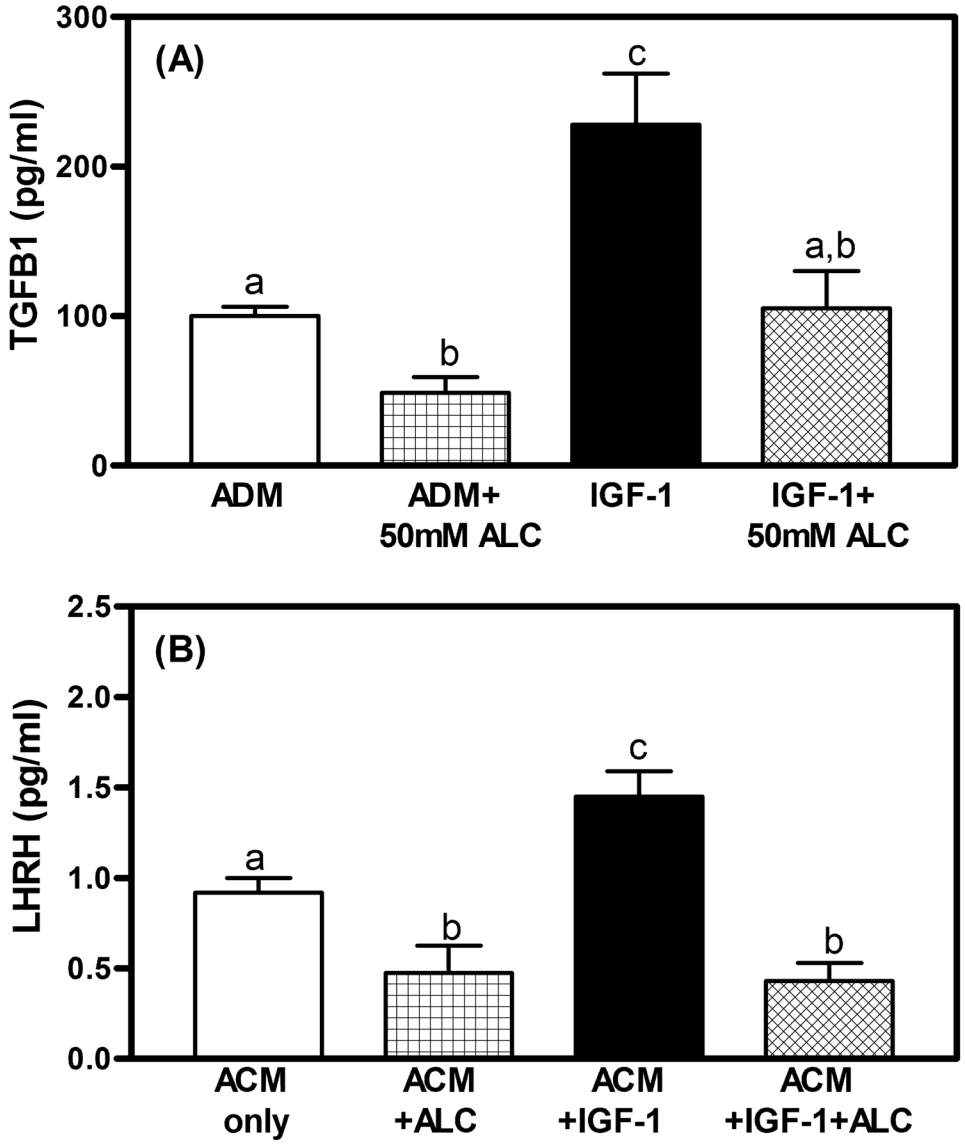

Fig. 6.

A) TGFβ1 levels in conditioned medium of hypothalamic astrocytes following 18 h of exposure to astrocyte-defined medium (ADM), ADM + alcohol (50 mM), IGF-1 (100 ng/mL), and IGF-1 + alcohol. Alcohol inhibited TGFβ1 secretion from hypothalamic astrocytes over basal release (ADM). IGF-1 increased the amount of TGFβ1 released and alcohol blocked this increase. B) LHRH release from GN-11 neurons after 60-min incubation in astrocyte-conditioned media (ACM) from the groups in 6A. ACM was added to the wells but no additional alcohol was added to the wells. LHRH release was reduced when exposed to the ACM with alcohol as compared to ACM only. GN-11 cells exposed to ACM medium with IGF-1 have augmented LHRH release over basal release and this increase did not occur when neurons were exposed to ACM with IGF-1 + alcohol. N = 6 wells/group. a vs. c: $p < 0.01$; b vs. c: $p < 0.01$; a vs. b: $p < 0.05$.

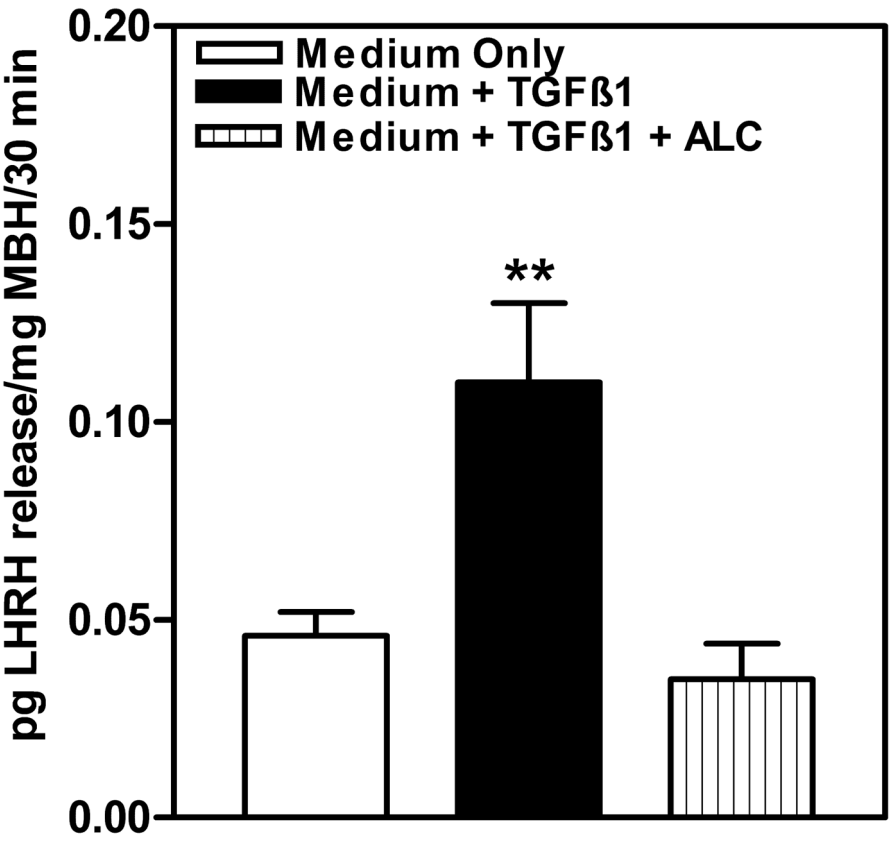

Fig. 7.

Effect of acute *in vitro* alcohol exposure on TGFβ1-induced LHRH release from the MBH of prepubertal female rats. Open bar indicates basal secretion of LHRH in medium only. Solid bar represents LHRH release in medium containing TGFβ1, and lined bar represents LHRH release in the presence of medium containing TGFβ1 plus 50 mM alcohol. Note that TGFβ1 induced the secretion of LHRH compared to the medium-only group and that the presence of alcohol in the medium blocked the TGFβ1-induced release of the peptide. The bars illustrate the mean (± SEM) of an N of 9 per group. $^{**}p < 0.01$ vs. medium only and medium + TGFβ1 + alcohol. Modified from Srivastava et al., 2014.

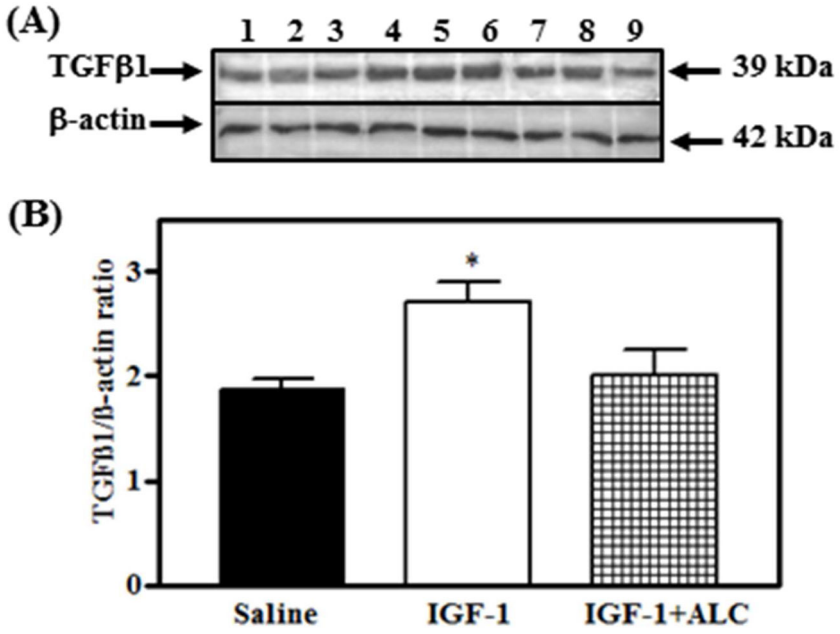

Fig. 8.

Effects of IGF-1 and acute alcohol exposure on TGFβ1 protein in the MBH of prepubertal female rats. A) Representative Western blot of TGFβ1 and β-actin proteins from saline (lanes 1–3), IGF-1 (lanes 4–6), and IGF-1 + alcohol (lanes 7–9) -treated animals. B) Densitometric quantification of all bands assessing the TGFβ1 protein. These data were normalized to the internal control β-actin protein. IGF-1 (open bar) induced an increase in TGFβ1 over saline-treated (solid bar) animals. Animals were dosed by gastric gavage with a 3 g/kg injection of alcohol, which yielded a peak serum alcohol level of 150–180 mg/dL after 90 min. At this time, IGF-1 (200 ng/mL) was injected into a third ventricular cannula. An additional 2 g/kg dose of alcohol was given 4 h after the initial dose to maintain moderately elevated serum alcohol levels. The tissues were collected 6 h after the infusion of IGF-1 and the serum alcohol levels at that time were 154 mg/dL (Hiney et al. 2014). Note that exposure to alcohol blocked the IGF-1-induced expression of TGFβ1 protein (hatched bar). Each bar represents the mean ± SEM of the TGFβ1/β-actin ratio. The number of animals represented by each bar is 6. *$p < 0.05$ vs. saline-treated and IGF-1 + alcohol-treated animals. Modifed from Hiney et al., 2014.

Table 1

The effects of transforming growth factor β1 (TGFβ1) and alcohol on LHRH gene expression in the preoptic area (POA) of prepubertal female rats. The central administration of TGFβ1 (100 ng) induced the expression of the LHRH gene at 6 h post-injection in animals that did not receive alcohol when compared to control animals. Note that this TGFβ1-induced LHRH gene expression was blocked in the alcohol-treated animals. N of 9 per group.

	Control	TGFβ1	TGFβ1+ALC
LHRH mRNA (relative expression)	1.27±0.06	1.60±0.08*	1.31±1.08

* $p < 0.05$ vs. control and TGFβ1 + alcohol.