CRITICAL REVIEW

How alcohol affects insulin-like growth factor-1's influences on the onset of puberty: A critical review

William L. Dees | **Jill K. Hiney** | **Vinod K. Srivastava**

Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA

Correspondence
William L. Dees, Department of Veterinary Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4458, USA.
Email: ldees@cvm.tamu.edu

Abstract

Alcohol (ALC) is capable of delaying signs associated with pubertal development in laboratory animals, as well as in humans. The normal onset of puberty results from a timely increase in gonadotropin-releasing hormone (GnRH) secretion, which is associated with a gradual decline in prepubertal inhibitory influences, and the establishment of excitatory inputs that increase GnRH release, which together drive pubertal development. In recent years, insulin-like growth factor-1 (IGF-1) has emerged as a pivotal contributor to prepubertal GnRH secretion and pubertal development, whose critical actions are interfered with by ALC abuse. Here we review the neuroendocrine research demonstrating the important role that IGF-1 plays in pubertal development, and describe the detrimental effects and mechanisms of action of ALC on the onset and progression of pubertal maturation.

KEYWORDS
alcohol, gonadotropin-releasing hormone, insulin-like growth factor-1, puberty

INTRODUCTION

The onset of puberty occurs following a series of glial- and neuronal-mediated events within the hypothalamus and preoptic area (POA) that promote the increased secretion of gonadotropin-releasing hormone (GnRH) to stimulate pituitary gonadotropin output and drive the pubertal process to sexual maturity. Any substance that interferes with GnRH secretion has the potential of altering the timing of puberty. Over the years, researchers questioned whether the increase in adolescent alcohol (ALC) use and abuse is associated with altered puberty-related hormones and pubertal development. In this regard, studies discovered that ALC caused diminished secretion of GnRH and delayed signs associated with the onset of female puberty. Specifically, the initial studies used prepubertal rodents and first revealed that chronic ALC exposure delayed the time of puberty in both sexes (Anderson et al., 1987; Bo et al., 1982; Dees & Skelley, 1990; Emanuele et al., 2002; Ramaley, 1982). In female

rats, the delayed puberty was associated with suppressed levels of growth hormone (GH), luteinizing hormone (LH), and estradiol (E$_2$) in blood (Dees & Skelley, 1990; Emanuele et al., 2002; Srivastava et al., 1995, 2002; Tentler et al., 1997), along with increased protein content of GH-releasing hormone and GnRH in the hypothalamus (Dees et al., 1990). In developing female rhesus monkeys, chronic ALC administration also inhibited these same reproductive hormones and delayed the development of a normal pattern of menstruation (Dees et al., 2000). Importantly, these actions were shown to be the result of a hypothalamic action of ALC to suppress GnRH release (Dissen et al., 2004; Hiney & Dees, 1991; Nyberg et al., 1993). In humans, ALC use by adolescent girls has been shown to cause delayed breast development (Peck et al., 2011) and variations in the time of first menarche (Richards & Oinonen, 2011), both associated with the age that ALC consumption began, as well as the frequency, magnitude, and duration of abuse. This is alarming since 35% of the seventh graders in the United States have reported

ALC use before the age of 13 (Swahn et al., 2008). These examples clearly depicted the general detrimental effects of ALC on the pubertal process and suggested the need to identify the central sites and mechanisms of action by which ALC causes these developmental impairments.

To discern the hypothalamic mechanisms behind the ALC actions, it became necessary to first assess upstream substances regulating GnRH secretion. Importantly, an increase in prepubertal GnRH secretion is necessary for the pubertal process to begin. This increased release is due to the gradual decline in neuronal inhibitory influences, while concomitantly developing enhanced excitatory neuronal influences. Gamma-aminobutyric acid (Terasawa & Fernandez, 2001), neuropeptide Y (Majdoubi et al., 2000), and the opioid peptides, β-endorphin and dynorphin (DYN; Faletti et al., 1999; Lehman et al., 2010; Navarro et al., 2009), are known to inhibit prepubertal GnRH release. Conversely, stimulators of prepubertal GnRH release include insulin-like growth factor-1 (IGF-1; Hiney et al., 1996; Wilson, 1998), leptin (Dearth et al., 2000; Lebrethon et al., 2000), norepinephrine (Sarkar et al., 1981), transforming growth factor α (Ojeda et al., 1990), glutamate (Claypool et al., 2000; Gay & Plant, 1987; Urbanski & Ojeda, 1990), kisspeptin (Kp; Navarro et al., 2004a, 2004b; Shahab et al., 2005; Thompson et al., 2004), and neurokinin B (NKB; Garcia et al., 2017; Navarro et al., 2009; Ramaswamy et al., 2010). Over time, research has demonstrated that of the above neuromodulators, IGF-1 has emerged as pivotal at influencing GnRH release at the time of puberty.

The IGF-1 peptide is synthesized in many tissues, including the brain, but predominantly is produced by the liver (Sara & Hall, 1990). The amplitude and frequency of GH secretion begin to increase as puberty approaches (Gabriel et al., 1992), and this induces the liver to synthesize and secrete IGF-1 into the systemic circulation (D'Ercole et al., 1984; Froesch et al., 1985). The serum levels of IGF-1 increase during pubertal development in several species, including rodents (Handelsman et al., 1987), and primates (Copeland et al., 1985), including humans (Juul et al., 1994). The peptide binds to the type 1 IGF receptor (IGF-1R) located in many organs of the body. In brain, the highest concentration of these receptors is in the median eminence (ME) of the hypothalamus (Bohannon et al., 1986; Lesniak et al., 1988; Werther et al., 1989), indicating that in this region, IGF-1 has functions in addition to cell growth. The fact that IGF-1 crosses the blood–brain barrier (Pan & Kastin, 2000) in increasing amounts during pubertal development led researchers to suggest that this peptide might be a peripheral metabolic signal to the hypothalamus for the stimulation of prepubertal GnRH release. This was shown to be the case when exposure of IGF-1 to prepubertal nerve terminals within the ME *in vitro* stimulated GnRH release (Hiney et al., 1991), initially indicating that IGF-1 may play a role in the onset of puberty. Interestingly, chronic ALC administration resulted in decreased hepatic synthesis and circulating levels of IGF-1; actions associated with suppressed puberty-related hormones and delayed female puberty (Srivastava et al., 1995). Collectively, these studies initiated the research to discern the effects of ALC on the IGF-1 system as it relates to the pubertal process.

In this review, we will first present the novel research demonstrating the importance of IGF-1 on the onset of puberty. Then, we will discuss recent information revealing mechanisms of IGF-1 and ALC actions, as well as their interactions, on puberty-related peptidergic pathways within the medial basal hypothalamus (MBH), as well as the rostral hypothalamic and preoptic areas (RHA/POA) of the brain, the principal brain regions responsible for driving the pubertal process.

OVERVIEW OF IGF-1 ACTIONS ON PUBERTY

After the initial observation that IGF-1 could stimulate the secretion of GnRH from the ME of prepubertal female rats *in vitro* (Hiney et al., 1991), a subsequent *in vivo* study confirmed the effect of IGF-1 on prepubertal GnRH secretion and its role in the onset of puberty (Hiney et al., 1996). Briefly, this study showed that the central administration of IGF-1 induced LH release in prepubertal female rats, an action blocked by the prior immunoneutralization of GnRH. Furthermore, IGF-1 gene expression did not change in the POA or MBH during late juvenile/peripubertal development; however, liver IGF-1 gene expression increased as maturation progressed. This action was accompanied by elevated serum levels of IGF-1, along with serum LH and E_2 (Figure 1). Accompanying the increase in serum IGF-1 was an increase in IGF-1R gene expression in the ME. Finally, the central administration of IGF-1 to prepubertal female rats at 1500 and 1700 hr each afternoon, to mimic the peripubertal afternoon increase in GnRH/LH secretion (Andrews & Ojeda, 1981; Urbansky & Ojeda, 1985), advanced the onset of puberty (Figure 2). Together, these results were the first to suggest that peripherally derived IGF-1 plays a role in facilitating prepubertal GnRH secretion. These puberty-related actions of IGF-1 were further investigated in both rodents and primates (Daftary & Gore, 2004; Danilovich et al., 1999; DiVall et al., 2010; Pazos et al., 1999; Wilson, 1998). Below, we will briefly discuss basic research revealing novel mechanisms of IGF-1 actions regarding the control of prepubertal GnRH release. These observations proved to be integral in subsequent studies designed to better understand the effects and mechanisms of ALC on pubertal processes.

### Puberty-related actions within the MBH

Critically important puberty-related peptides, DYN, NKB and Kp, are produced by neurons in the arcuate nucleus (ARC; Lehman et al., 2010) and contributed to the control of GnRH secretion in this brain region. Importantly, it was first observed in sheep that a subpopulation of the ARC neurons coexpressed all 3 peptides, and therefore, they were named KNDy neurons (Goodman et al., 2007). This neuronal population was confirmed in several other mammalian species (Navarro et al., 2009; Ramaswamy et al., 2010; True et al., 2011; Wakabayashi et al., 2010). The degree of colocalization varied
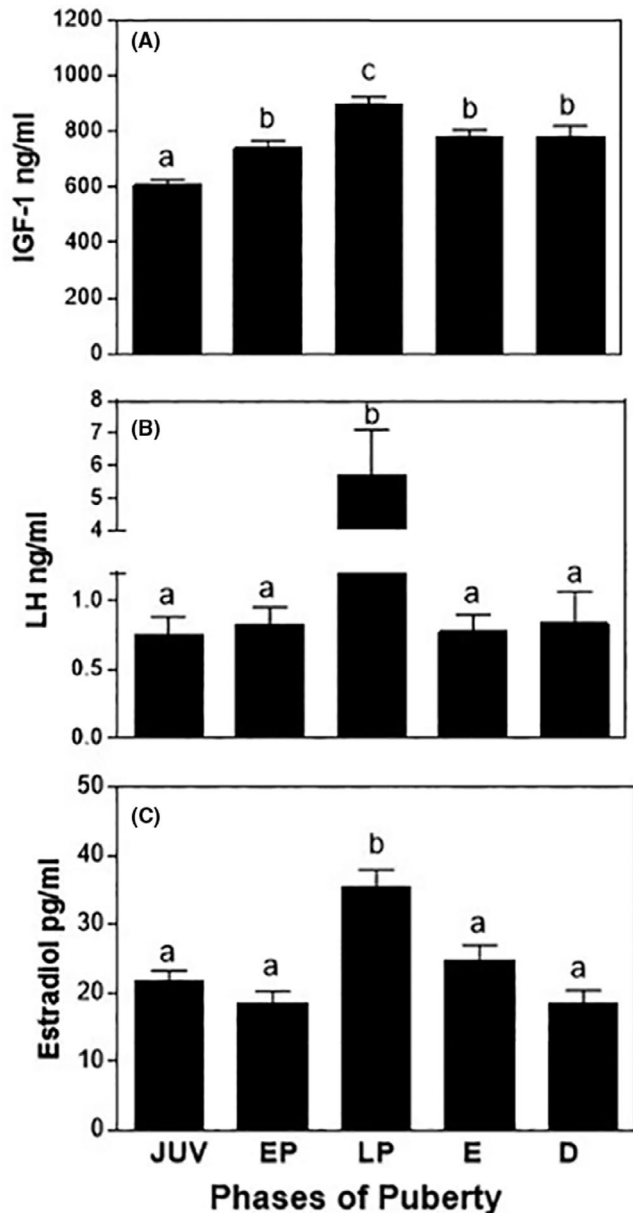
FIGURE 1   Serum insulin-like growth factor-1 (IGF-1), luteinizing hormone (LH), and estradiol (E$_2$) levels during pubertal development. (A) It depicts that serum IGF-1 levels increase significantly during early proestrus (EP), peaked during late proestrus (LP), and remained elevated over juvenile (JUV) levels through first estrus (E) and diestrus (D). (B) and (C) It demonstrates that the serum levels of LH and estradiol were also elevated significantly during LP. a vs. b and c, $p < 0.001$; b vs. c, $p < 0.05$. Differences between groups were analyzed using analysis of variance with post hoc testing using Student–Neuman–Keul's multiple range test. $n = 40$, JUV; $n = 25$, all other stages. (With permission, © 1996 by The Endocrine Society, Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL; Endocrinology published by Oxford University Press)

between species, with the human presenting the lowest degree of overlap between the 3 peptides (Hrabovsky et al., 2012).

It is important to describe the functions of these 3 peptides in the MBH before discussing the effect that IGF-1 has on their respective
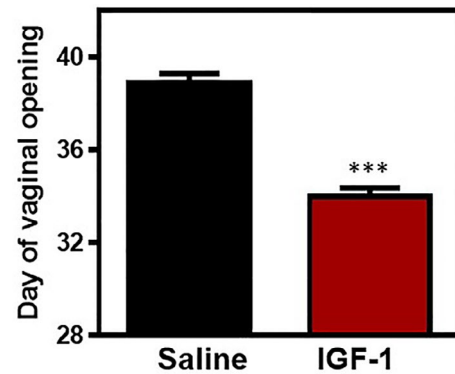
FIGURE 2   Central administration of insulin-like growth factor-1 (IGF-1) advanced the timing of puberty. Black and red bars represent the mean (±SEM) age in days at vaginal opening for saline and IGF-1-treated animals, respectively. Note that IGF-1 advanced vaginal opening by 4.9 days. $N = 6$–7/bar. ***$p < 0.001$. (With permission, © 1996 by The Endocrine Society, Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL; Endocrinology published by Oxford University Press)

actions. Prior to the onset of puberty, DYN inhibits GnRH release (Dees et al., 2019; Lehman et al., 2010; Lopez et al., 2016; Navarro et al., 2009) and thus contributes to the prepubertal restraint on the onset of puberty. Conversely, depending on physiological conditions and stage of development, NKB and Kp are both associated with the normal pubertal stimulation of GnRH release (Dees et al., 2019; Garcia et al., 2017; Gaskins et al., 2013; Grachev et al., 2012; Keen et al., 2008).

The observation that DYN neurons express the NKB receptor (NK3R; Burke et al., 2006) suggests that NKB likely contributes to the regulation of prepubertal DYN secretion. In this regard, a study revealed that the central administration of an NKB agonist for 4 days stimulated the synthesis of NK3R while causing suppressed synthesis of DYN and its receptor, kappa opioid receptor-1 (KOR-1; Dees et al., 2019). That same study showed the NKB agonist caused suppressed DYN along with increased GnRH secretion. Interestingly, the synthesis and release of Kp were not affected, hence showing an earlier action of NKB than that of Kp. Thus, these data supported previous observations that indicated NKB and Kp have independent paths in affecting prepubertal GnRH secretion (Garcia et a., 2017; Gaskins et al., 2013; Nestor et al., 2012).

Because IGF-1 levels rise at puberty, cross the blood–brain barrier (Pan & Kastin, 2000), and are associated with prepubertal GnRH release, it became necessary to determine whether a signaling pathway existed in which IGF-1 could regulate the puberty-related synthesis and secretion of NKB. Importantly, centrally administered IGF-1 for 4 days revealed increased expressions of NKB and NK3R, as well as the decreased expression of DYN and KOR-1 (Dees et al., 2019). These IGF-1 effects were associated with an increase in NKB release, an action blocked by an IGF-1R antagonist. Interestingly, the synthesis and secretion of Kp in the MBH were not altered by IGF-1, which supports a previous report (Hiney et al., 2009) and indicates

that Kp is not involved in the NKB/DYN pathway in the MBH at this early age. Collectively, this information is important because it demonstrates that IGF-1 normally stimulates NKB, which subsequently removes the prepubertal DYN inhibition on GnRH release and thus allows for the increased secretion of this peptide observed at the onset of puberty.

Puberty-related actions in rostral areas of the brain

During puberty, in rostral brain regions, only very small amounts of the DYN and NKB proteins are expressed (Burke et al., 2006), whereas the expression of Kp is markedly greater with most of the peptide in this brain area produced within the anteroventral periventricular (AVPV) nucleus (Clarkson & Herbison, 2006). Regarding GnRH, the location of neurons within the brain that synthesize this peptide can vary somewhat by species; however, in every species, the majority of GnRH neurons are located rostrally within the RHA/POA and adjacent forebrain nuclei. Although species differences exist regarding GnRH neuronal localization, it is well accepted that the mechanisms governing GnRH release are similar across species. Importantly, GnRH neurons in RHA/POA express the IGF-1R (Daftary & Gore, 2004; Danilovich et al., 1999; Miller & Gore, 2001). As pubertal development proceeds, more responsibility for driving the pubertal process is acquired by peptidergic neurons in the RHA and POA, which show an increased sensitivity to rising levels of E_2 (Clarkson et al., 2010; Hiney et al., 1996; Smith et al., 2005). The Kp gene and that of the Kp receptor (GPR-54) have been shown to increase during

pubertal development (Navarro et al., 2004a). Unlike the MBH, IGF-1 was shown to stimulate the synthesis of the Kp gene in this brain region (Hiney et al., 2009). Furthermore, as the IGF-1 levels increase, induction of the IGF-1R activates an Akt-mediated pathway that ultimately results in the increased synthesis and release of Kp from its E_2-sensitive neurons in the female AVPV nucleus (Hiney et al., 2010, 2018). This is specifically the case in females and is supported by research showing that KiSS-1 gene expression increases in the AVPV nucleus at proestrus (Smith et al., 2006), and further supported by the increase in immunoreactive Kp neurons in the female AVPV nucleus during this phase of development (Clarkson & Herbison, 2006). The Kp neurons from this region project some of their nerve fibers caudally through the MBH to the ME, whereas other fibers project rostrally into the forebrain (Decourt et al., 2008; Smith et al., 2008). Hence, once the Kp is released, it binds to its receptor, GRR-54, either on GnRH nerve terminals in the ME or on the nearby GnRH-producing neurons. Kp is a potent stimulator of GnRH synthesis and secretion and a driving force in the preovulatory GnRH/LH surge leading to puberty (Hiney et al., 2018; Navarro et al., 2004b; Shahab et al., 2005).

Effects of ALC on IGF-1 induced puberty-related events

ALC and IGF-1 both act within the MBH and RHA/POA brain regions that are responsible for controlling the pubertal process. Delayed puberty can result from any substance acting to either enhance the prepubertal inhibition of GnRH secretion or inhibit the excitatory drive for
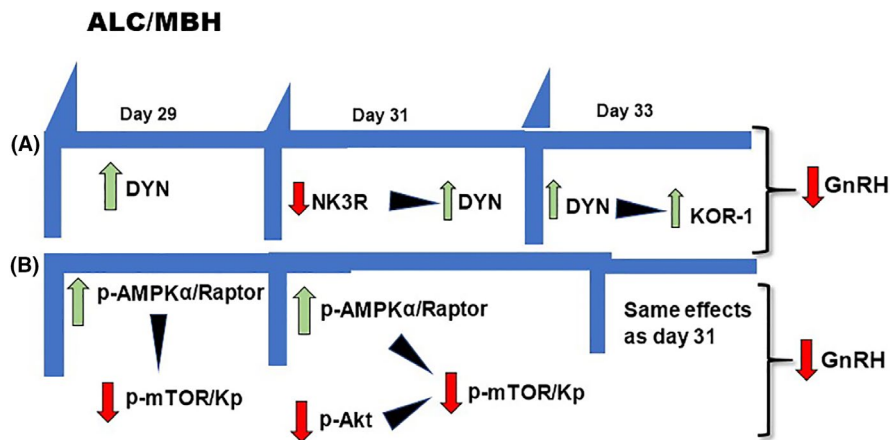
FIGURE 3 Schematic showing the timing of the effects of ALC on puberty-related pathways in the medial basal hypothalamus (MBH). Animals were administered either ALC or control liquid diets from day 27 through day 33. (A) Effects of ALC on the dynorphin (DYN) system. ALC increased the expression of DYN protein by day 29 over the control animals, an effect that continued through day 33. Furthermore, by day 31, ALC had down-regulated the expression of the neurokinin B receptor (NK3R), suggesting suppressed NKB secretion. The overall effect of this ALC-induced up-regulation in DYN protein synthesis was also associated with an increase in the expression of the dynorphin receptor (KOR-1) on day 33. B) Effects of ALC on pathways regulating the mammalian target of rapamycin (mTOR)/kisspeptin (Kp) synthesis. ALC stimulated the phosphorylation of the AMP-activated protein kinase α (AMPKα)/Raptor pathway by day 29, an action known to inhibit mTOR and Kp synthesis. This effect persisted through day 33. Additionally, on days 31 and 33, ALC caused the suppression of the stimulatory Akt pathway, further suppressing mTOR and Kp synthesis. Note that these effects are all known to cause suppressed prepubertal GnRH secretion. Green and red arrows show increases and decreases, respectively, in expression/secretion

GnRH secretion. Thus, novel research assessed the actions and interactions of ALC and IGF-1 on the control of specific puberty-related inhibitory and excitatory neuropeptides described above in the MBH and RHA/POA regions relative to the onset and progression of the pubertal process. Because these general brain areas exhibit different physiological roles during pubertal development, they will be discussed separately.

Chronic actions of ALC within the MBH

We have already established that DYN is active within the prepubertal MBH and along with other opioid peptides (Faletti et al., 1999; Lehman et al., 2010; Navarro et al., 2009; Sirinathsinghji et al., 1985) contributes to the brake on the pubertal process by inhibiting GnRH secretion. Previously, ALC was shown to increase DYN gene expression within the adult hypothalamus (Chang et al., 2007) and induce DYN secretion from the prepubertal MBH (Srivastava et al., 2015). More recently, ALC administration to prepubertal female rats from days 27 through 33 was shown to increase the expression of DYN protein by day 29 over the

control animals, an effect that was even more significant by day 33 (Srivastava et al., 2018). This ALC-induced up-regulation in DYN protein synthesis was also associated with an increase in the expression of KOR-1 on day 33. Furthermore, by day 31, ALC had down-regulated the expression of the NK3R, suggesting suppressed NKB secretion. Thus, the combined effect of an ALC-induced stimulation of DYN, along with the suppression of the NKB/NK3R pathway that normally inhibits DYN secretion at the onset of puberty, further promotes prepubertal DYN release. These actions result in the continued suppression of GnRH secretion and thus prolonging the prepubertal state (Dees et al., 2019). Additionally, the suppressed NK3R also decreases the ability of NKB to facilitate any direct stimulation of GnRH release from nerve terminals in the ME (Garcia et al., 2017; Navarro et al., 2009; Ramaswamy et al., 2010). Collectively, these ALC actions contribute to the suppression of prepubertal GnRH release and thereby delaying the onset of puberty. Figure 3A demonstrates the ontogeny of the effects of ALC on the regulation of prepubertal DYN within the MBH.

ALC exposure has also been shown to decrease Kp protein expression beginning on day 29 in the MBH (Srivastava., et al.,
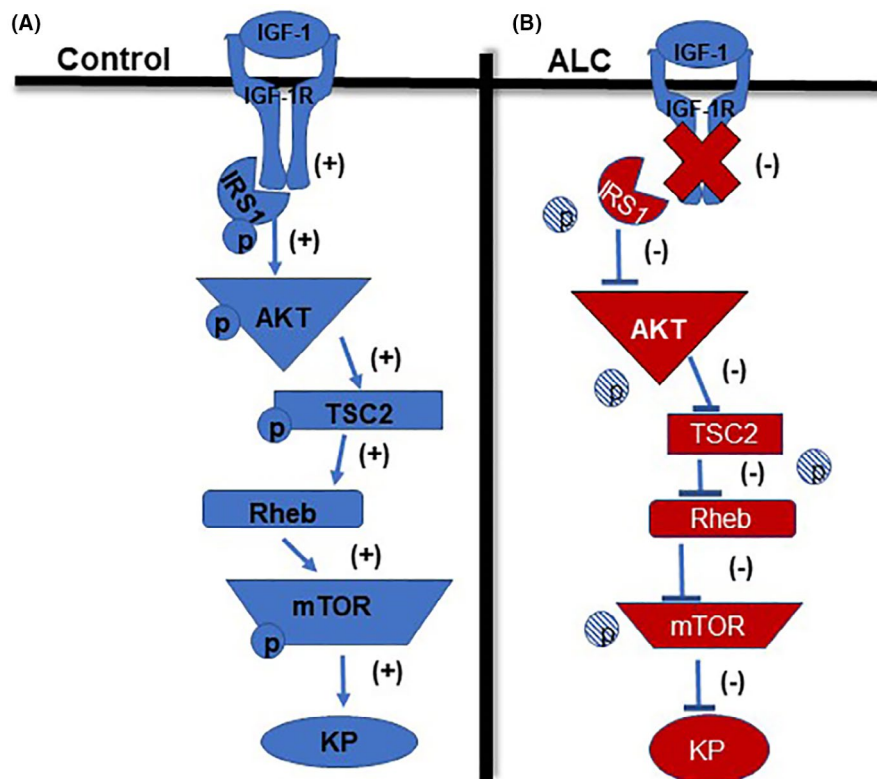
FIGURE 4 Schematic drawing showing the effects of insulin-like growth factor-1 (IGF-1) and ALC on prepubertal kisspeptin (Kp) synthesis and release. (A) IGF-1 activates the IGF-1 receptor (IGF-1R) / insulin receptor substrate-1 (IRS-1) complex, which subsequently initiates critical phosphorylation within the Akt-mediated pathway to mammalian target of rapamycin (mTOR), hence resulting in increased Kp synthesis and release. (B) ALC blocks the activation of IGF-1R/IRS-1 complex by inhibiting the phosphorylation of IRS-1 resulting in the inhibition of key downstream phosphorylation within the Akt pathway to mTOR, thus suppressing Kp synthesis and release. The detrimental effects of ALC on the IGF-1/IRS-1 complex and subsequent suppression of the Kp synthesis pathway are shown in red. Phosphorylated site, p; ras homologue enriched in brain, Rheb; tuberous sclerosis complex 2, TSC2. (With permission, © 2018 by, Hiney JK, Srivastava VK, Hartzoge NL, Vanden Anderson DN, Dees WL; Alcoholism: Clin Exp Res. published by John Wiley and Sons)

2018). That study also showed that protein expression of mammalian target of rapamycin (mTOR), an immediate upstream regulator of Kp (Roa et al., 2009), was also depressed by ALC on day 29. The depressed expressions of mTOR and Kp resulted from an ALC-induced activation of the AMP-activated protein kinase α (AMPKα)/p-Raptor pathway on day 29. This pathway is a known negative control of mTOR (Gwinn et al., 2008). Interestingly, the Akt pathway to mTOR/Kp (Inoki et al., 2002; Long et al., 2005) is not affected by ALC until day 31. This is important because it shows that mTOR activation is influenced by 2 different signaling pathways (Gwinn et al., 2008; Inoki et al., 2002) and that the ALC activates the AMPKα pathway prior to inhibiting the Akt-mediated pathway (Srivastava et al., 2018). However, clearly both pathways contribute to the ALC-induced suppression of Kp within the MBH (Figure 3B). Additionally, an ALC-induced decrease was observed in IGF-1R expression on day 31, suggesting that this down-regulation is associated with suppressed circulating levels of IGF-1 available to the MBH/ME area (Srivastava et a., 1995). While this action of ALC to alter the IGF-1 system contributes to decreased GnRH release through a direct interaction with GnRH nerve terminals, it does not contribute to the decrease in Kp expression, since the IGF-1 peptide is not an upstream regulator of Kp synthesis in the MBH (Hiney et al., 2009; Srivastava et al., 2016). Whether ALC affects the Akt/Kp pathway directly, or through some other upstream regulator of Akt within the MBH is not known. Regardless of the mechanism, the ALC-induced decrease in Kp beginning at 29 days is important because it indicates that actions of Kp to influence GnRH within the MBH is independent of IGF-1 and furthermore that the diminished excitatory influence of this peptide on GnRH occurs early in the pubertal process.

Chronic actions of ALC within rostral areas of the brain

The ontogeny of the effects of ALC has also been assessed regarding specific puberty-related peptides within the RHA (Srivastava et al., 2018). DYN and NKB protein levels are very low in this brain region, and the administration of ALC from days 27 to 33 did not alter the levels of expression. Unlike the MBH, IGF-1 does regulate Kp synthesis in the RHA. ALC did not affect the expression of the IGF-1R nor any of the downstream components of the Akt pathway to Kp within this brain region prior to 33 days of age. However, on day 33 the ALC-treated animals showed suppressed phosphorylation of the IGF-1, again due to down-regulation resulting from the drugs that continued hepatic suppression of IGF-1 synthesis (Lang et al., 2009; Srivastava et al., 1995) causing the lower circulating levels of the peptide crossing the brain (Srivastava et al., 1995). The ALC-induced decreased in IGF-1R expression in this brain area resulted in marked reductions in protein expression for each of the downstream components of the Akt/mTOR pathway to Kp, but again, not until day 33. Furthermore, the AMPK pathway to mTOR/KP was also decreased on day 33.

The inability of chronic ALC administration between days 27 and 31 to suppress neuronal Kp synthesis within the rostral brain was not unexpected. The principal action of Kp in the RHA is to stimulate synthesis and release of GnRH later in development when the peptide is needed to drive the pubertal process (Clarkson & Herbison, 2006). This increase in GnRH normally begins around prepubertal day 32 in the rat and is associated with the animal starting to enter the peripubertal phase of development. The fact that ALC suppressed Kp in the RHA at this time, along with well-known suppressions in serum levels LH and E_2, further demonstrates that the animals did not enter the peripubertal period and supports the delay in pubertal development resulting from chronic ALC administration. Thus, the information presented so far indicates that ALC plays a role in delaying the onset of puberty if consumption begins early in pubertal process.

Acute actions of ALC within the rostral areas of the brain

While progress has been made regarding the effects of ALC at the time of puberty, more attention was needed to more completely
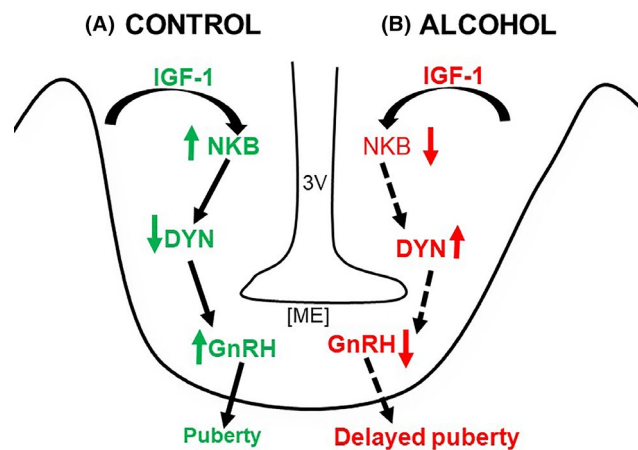
FIGURE 5 Actions of ALC within the medial basal hypothalamus (MBH) to alter the insulin-like growth factor-1 (IGF-1)/neurokinin B (NKB)/dynorphin (DYN) pathway contributing to prepubertal gonadotropin-releasing hormone (GnRH) secretion. (A) In control animals, the rising circulating levels of IGF-1 entering the medial basal hypothalamus (MBH) bind to its receptors and cause increased release of NKB. The NKB subsequently causes suppressed DYN secretion and thus allows for increased GnRH release facilitating the onset of puberty. (B) ALC causes suppressed circulating levels of IGF-1, as well as suppresses both IGF-1 and NKB receptors in the MBH resulting in decreased NKB release. This ALC-induced suppression of the IGF-1/NKB pathway results in continued prepubertal synthesis and release of DYN and therefore further promotes the inhibition of GnRH secretion and the delay in puberty. Green and red arrows indicate increased or decreased peptide responses within control or ALC-treated animals. Black arrows indicate the direction of the pathway. 3V, third ventricle; ME, median eminence
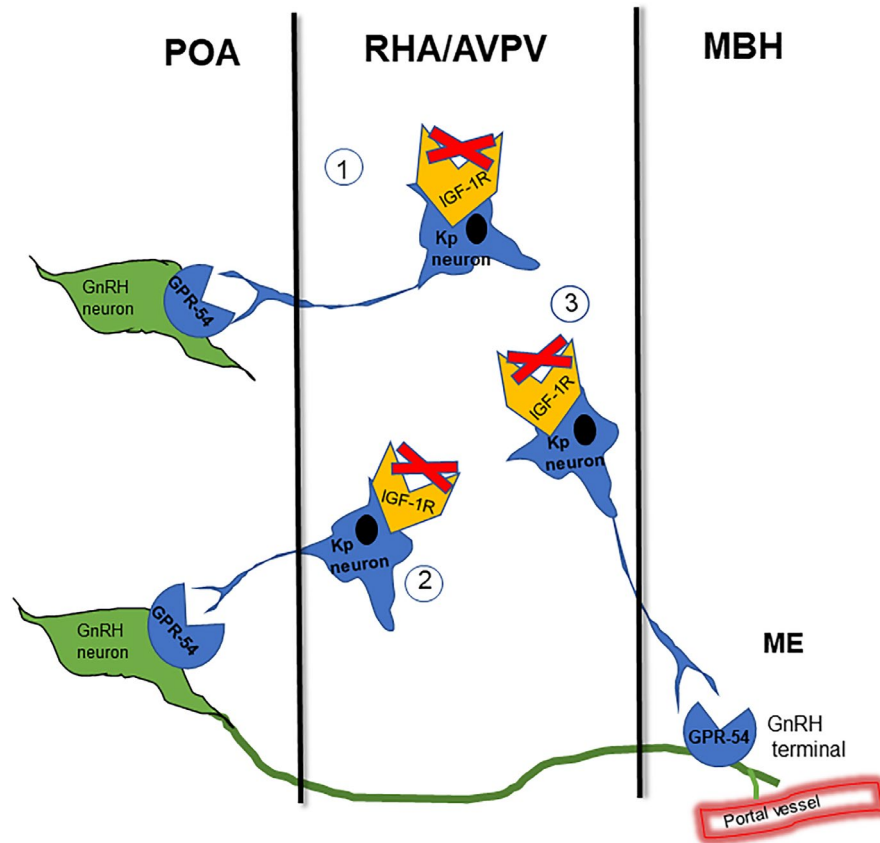
FIGURE 6 Effects of ALC on kisspeptin (Kp) neurons in the rostral hypothalamic area (RHA)/anteroventral periventricular (AVPV) brain region. ALC inhibits insulin-like growth factor-1 (IGF-1) from stimulating the synthesis and secretion of Kp by blocking (Red X) IGF-1 receptors (IGF-1R) expressed on Kp neurons. This action of ALC interferes with the downstream control of gonadotropin-releasing hormone (GnRH) in 3 ways. (1) A population of Kp neurons send their nerve processes rostrally into the preoptic area (POA) and nearby regions that bind to kisspeptin receptors (GPR-54) on GnRH neurons. These GnRH neurons then synthesize and release the GnRH peptide within that region. (2) Another population of Kp neurons also send their processes rostrally to other GnRH neurons that express GPR-54; however, these GnRH neurons send their nerve processes caudally into the medial basal hypothalamus (MBH) and release the GnRH peptide directly into the portal blood of the median eminence (ME). (3) A final population of Kp neurons send their nerve processes caudally to bind directly to GnRH nerve terminals in the ME that express GPR-54. Note the ability of ALC to block IGF-1R on Kp neurons in the RHA/AVPV is critical and prevents the potent stimulatory of actions of Kp on 3 pathways that are pivotal for increasing the levels of GnRH synthesis and secretion necessary for the final stage of pubertal development. Kp and GnRH neurons are depicted in blue and green, respectively

define the sites and mechanisms of the actions and interactions between ALC and IGF-1 regarding the physiological control of the prepubertal Kp system. Gaining a better understanding of these mechanisms was critical because Kp is the most important stimulator of prepubertal GnRH synthesis and secretion. Acute ALC studies were then used to assess specific actions within the Akt-mediated pathway used by IGF-1 to induce Kp synthesis (Hiney et al., 2018). In this regard, delivery of IGF-1 into the brain third ventricle induced the expression of Kp protein by 6 hr postinjection, an action that was blocked by acute ALC exposure. Further assessments demonstrated that ALC suppressed the phosphorylation of Akt in the RHA/AVPV region of the brain, hence preventing stimulation of the downstream pathway components tuberous sclerosis 2 (TSC2), ras homologue enriched in brain (Rheb), and mTOR (Hiney et al., 2018). Important assessments upstream from Akt indicated that centrally delivered IGF-1 to control animals

stimulated phosphorylation of the main substrate of the IGF-1R and the IRS-1 protein. Conversely, ALC prevented this stimulation, indicating the initial site of the inhibitory effect of ALC on Kp synthesis was on the IGF-1R/IRS-1 complex. A comparison of these actions is shown in Figure 4. Studies were also conducted to determine the effects of IGF-1 and ALC on the *in vitro* release of Kp from POA/RHA tissues collected from prepubertal rats (Hiney et al., 2018). In this regard, IGF-1 did not alter basal secretion of Kp from the tissue incubates collected from juvenile animals. However, IGF-1 induced the secretion of Kp protein from the tissue blocks incubated from animals in first proestrus. This indicated that Kp release occurs in the later phase of development when the neurons in this brain region are most responsive to elevated E_2. Importantly, since IGF-1Rs were blocked by ALC, no IGF-1 stimulation of Kp release was observed when tissues were incubated in the presence of ALC.

CONCLUSIONS

Chronic ALC exposure is known to delay signs of pubertal development in laboratory animals, as well as humans. The pubertal process normally begins with the timely, but gradual, decline in the inhibitory tone that has been suppressing prepubertal GnRH secretion, along with the increased responsiveness to excitatory stimuli that increase the release of the peptide. IGF-1 has emerged as a prime player in the pubertal process through its actions within the MBH, as well as in more rostral brain regions. Over the years, IGF-1 has been shown to regulate prepubertal GnRH secretion and consequently the onset and progression of pubertal development. We have shown the primary actions and interactions between ALC and IGF-1 and during pubertal development. In this regard, it is well known that IGF-1 synthesis by the liver increases at puberty, crosses the blood–brain barrier, and is not only capable of stimulating GnRH directly from nerve terminals in the ME but also activates the NKB-DYN pathway, which diminishes the prepubertal inhibition of GnRH, thus allowing the developmental process to begin. Conversely, ALC not only lowers the amount of IGF-1 entering the brain but also blocks both IGF-1 and NKB receptors, hence negatively affecting the IGF-1-NKB-DYN pathway in the MBH and thereby contributing to the ALC-induced delay in the onset of the pubertal process. The schematic shown in Figure 5 depicts both the normal puberty-related actions of IGF-1 on the NKB-DYN-GnRH pathway at the onset of puberty and the effects of ALC to alter this pathway and delay the timing of puberty.

As puberty progresses and E_2 levels continue to rise, more responsibility for the developmental process is acquired by both Kp and GnRH neurons in the RHA/POA. During normal development, IGF-R activation induces an Akt-mediated pathway in neurons within the AVPV region to synthesize and release Kp. These Kp neurons project their nerve fibers either caudally toward receptors located on GnRH terminals in the ME or rostrally toward receptors expressed on GnRH neuronal perikarya in the POA and other forebrain regions. It is now well accepted that Kp is the most potent stimulator of GnRH synthesis and release leading to the developing preovulatory surge of GnRH/LH and resulting in first ovulation. Importantly, ALC was shown to block the activation of the IGF-1R on Kp neurons by inhibiting the phosphorylation of IRS-1. This action subsequently blocks the phosphorylation of key downstream proteins in the Akt pathway and thus suppresses the synthesis and secretion of Kp. Figure 6 depicts the rostral and caudal projections of the Kp neuronal processes, as well as the sites of ALC actions on Kp neurons. Specifically, it demonstrates that ALC blocks IGF-1 receptors on Kp neurons and thus inhibits both the synthesis and release of Kp. This action of ALC to reduce the availability of Kp markedly suppresses GnRH synthesis and release at a critical time later in pubertal development when an increase in the release of the peptide is needed to drive the process to maturity.

Overall, we have clearly demonstrated that ALC affects both brain regions primarily responsible for controlling the onset and progression of pubertal processes and that it does so by detrimentally altering the key influences of IGF-1 in the regulation of GnRH synthesis and secretion. Also, it is suggested that ALC abuse can not only delay the onset of puberty by acting within the MBH before the maturation process begins but can also alter the progression of puberty-related events when exposed later in development after some maturity had already started. Clearly, ALC abuse is detrimental to adolescent health and development. While we have detailed numerous studies assessing the actions and interactions between IGF-1 and ALC in adolescent females, there is a clear need for future research to assess these actions in adolescent males, as well as in adolescent males and females following prenatal ALC exposure.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ORCID

Jill K. Hiney 🄳 https://orcid.org/0000-0001-9286-9229

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