IGF-1 Influences Gonadotropin-Releasing Hormone Regulation of Puberty

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Abstract

The pubertal process is initiated as a result of complex neuroendocrine interactions within the preoptic and hypothalamic regions of the brain. These interactions ultimately result in a timely increase in the secretion of gonadotropin-releasing hormone (GnRH). Researchers for years have believed that this increase is due to a diminished inhibitory tone which has applied a prepubertal brake on GnRH secretion, as well as to the gradual development of excitatory inputs driving the increased release of the peptide. Over the years, insulin-like growth factor-1 (IGF-1) has emerged as a prime candidate for playing an important role in the onset of puberty. This review will first present initial research demonstrating that IGF-1 increases in circulation as puberty approaches, is able to induce the release of prepubertal GnRH, and can advance the timing of puberty. More recent findings depict an early action of IGF-1 to activate a pathway that releases the inhibitory brake on prepubertal GnRH secretion provided by dynorphin, as well as demonstrating that IGF-1 can also act later in the process to regulate the synthesis and release of kisspeptin, a potent stimulator of GnRH at puberty.

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Introduction

The onset of puberty results from a series of neuronal- and glial-mediated events within the preoptic area (POA) and hypothalamus that promote the increased secretion of gonadotropin-releasing hormone (GnRH), the peptide responsible for stimulation of pituitary gonadotropin output driving the pubertal process to sexual maturity. This increased secretion of GnRH has been attributed to a gradual removal of prepubertal inhibitory influences, as well as to enhanced responsiveness to excitatory influences. With regard to inhibitory influences, gamma aminobutyric acid, as well as the opioid peptides, β -endorphin and dynorphin (DYN) [1–4], are synthesized within the medial basal hypothalamus (MBH), which includes the arcuate (ARC) nucleus. Each of these neuromodulators is capable of suppressing GnRH secretion and thus contributes to the brake on pubertal development during childhood. Regarding the excitatory influences, norepinephrine [5], excitatory amino acids [6–8], leptin [9, 10], transforming growth factor- α [11], insulin-like growth factor-1 (IGF-1) [12–14], the kisspeptins (Kp) [15–18], and neurokinin B (NKB) [3, 19, 20] have emerged as important stimulators of GnRH during pubertal development. Among these stimulatory substances, IGF-1 has emerged as playing a pivotal role in puberty-related events.

Fig. 1. The effect of third ventricular administration of IGF-1 on LH release from late juvenile female rats. Representative LH secretory profiles from rats before and after receiving either saline (**a**) or 20 ng IGF-1 (**b**). Note that the IGF-1 induced a marked postinjection increase in LH secretion. Samples were taken at 10-min intervals, and the arrows denote injections of either saline or IGF-

1 after the fourth basal sample. * $p < 0.05$ (with permission, Copyright© 1996 by The Endocrine Society [13], Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL; *Endocrinology* published by Oxford University Press). IGF-1, insulin-like growth factor-1; LH, luteinizing hormone.

IGF-1 is a 70-amino acid polypeptide mitogen that is essential for mammalian growth. Although this peptide is synthesized in many tissues including the brain, it is predominantly produced in the liver [21]. As puberty approaches, the amplitude and frequency of growth hormone secretion increases [22], which induces the liver to synthesize and then secrete IGF-1 into the systemic circulation [23, 24]. Once in circulation, IGF-1 binds to a GH-dependent, IGF-binding protein-3 and an acid labile subunit (for review, see [25]). To cross the blood-brain barrier, the peptide is released from the ternary unit via proteases and can now bind the type 1 IGF receptor (IGF-1R). This tyrosine kinase receptor is located in many organs including the brain. Although located throughout the brain, the highest concentration of these receptors is within the median eminence (ME) of the hypothalamus [26–30].

Serum IGF-1 levels increase markedly during pubertal development in rodents [31, 32] and ruminants [33, 34], as well as in primates [35, 36], including humans [37, 38]. Thus, this trophic factor has been suspected of linking somatic growth to the activation of the reproductive hypothalamus at puberty [39]; however, determining whether the peptide acts centrally to induce prepubertal GnRH secretion was needed to support this hypothesis. In 1991, research showed that IGF-1 stimulated GnRH release directly from the prepubertal ME in vitro [12] and therefore demonstrated the first indication that IGF-1 may play a pivotal role in the onset of puberty. Subsequent research efforts over the last 2 decades have utilized

numerous physiological, toxicological, and molecular approaches to discern the extent to which this peptide influences the timing of puberty. During this time, studies have shown mechanisms and pathways by which IGF-1 regulates GnRH and helps to drive the pubertal process. This review will first discuss important research using prepubertal rodents and primates demonstrating the hypothalamic site of action of IGF-1 resulting in the stimulation of prepubertal GnRH and thereby luteinizing hormone (LH) release. We will next present recently obtained evidence revealing an action of IGF-1 to stimulate a peptidergic pathway within the MBH that contributes to the removal of the prepubertal brake on GnRH secretion, and thus further allowing for the initial puberty-related increase in the release of the GnRH peptide. We will also describe the actions and interactions of estradiol (E_2) and IGF-1, as well as recently described interactions between IGF-1, Kp, and GnRH within the preoptic and rostral hypothalamic areas (POA/RHA) of the brain as puberty progresses through the peripubertal period of development to maturity.

Early Evidence for an IGF-1 Involvement in the Pubertal Process

With the knowledge that the ME is outside the blood-brain barrier, synthesizes significant concentrations of IGF-1R [26–30], and contains the GnRH nerve terminals, it was hypothesized that IGF-1 may be a peripheral meta-

Fig. 2. The central administration of IGF-1 advanced the timing of puberty. Open and solid bars represent the mean ($\pm$ 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. $N = 6-7/\text{bar}$. *** $p < 0.001$ (with permission, Copyright© 1996 by The Endocrine Society [13], Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL; *Endocrinology* published by Oxford University Press). IGF-1, insulin-like growth factor-1.

bolic signal capable of acting centrally to stimulate GnRH at the time of puberty. This hypothesis was first addressed in vitro with the results revealing a dose-dependent action of IGF-1 to stimulate GnRH secretion from isolated MEs removed from female rats during the late juvenile phase development [12]. Since IGF-1Rs were not observed directly on GnRH nerve terminals in the ME [40] and because glial cells were shown to have close associations with GnRH nerve terminals [41, 42], it appeared that the IGF-1-induced GnRH release was mediated by glial-glial and glial-neuronal communications. Several lines of evidence support that IGF-1Rs are expressed on glial cells in the ME [26, 27], and through glial-glial interactions, IGF-1 stimulates the release of glial-derived prostaglandin- E_2 (PGE_2) in vitro [43]. Importantly, once PGE_2 is secreted, it binds to its receptors on nearby GnRH nerve terminals and causes release of the GnRH peptide [44, 45].

Subsequent in vivo studies further suggested central effects of IGF-1 on GnRH secretion. In this regard, central administration of IGF-1 induced the secretion of LH in immature female rats [13], while a similar effect of IGF-1 was shown in male sheep following systemic administration of the peptide [46]. Examples of representative secretion profiles using late juvenile female rats demonstrate

the lack of an LH response to saline, compared to the increase in LH released after administration of IGF-1 (Fig. 1). Importantly, this LH response to IGF-1 was blocked by prior immunoneutralization of GnRH. Furthermore, when IGF-1 was administered centrally to immature female rats twice daily, each late in the afternoon, in order to mimic the peripubertal afternoon increase in GnRH secretion, it advanced the onset of puberty (Fig. 2). Several other studies later confirmed this action of IGF-1 on puberty. In primates, an increase in serum IGF-1 advanced first ovulation in rhesus monkeys [14]. In mice, replacement of IGF-1 advanced puberty in GH-receptor knockout mice expressing very low levels of the peptide [47]. IGF-1 administration to wild-type mice showed precocious puberty in females, but this did not occur in males, while both male and female transgenic mice lacking the IGF-1R on their GnRH neurons showed delayed puberty [48]. Furthermore, central delivery of IGF-1 antiserum to prepubertal male rats caused delayed puberty [49].

In addition to stimulating prepubertal GnRH secretion, other evidence also supports the concept that peripherally derived IGF-1 plays an early role in the pubertal process. Importantly, there are developmental differences in IGF-1 synthesis between central and peripheral sources in the days leading up to puberty in the female rat [13]. In this regard, IGF-1 gene expression did not change in the POA or MBH during late juvenile/peripubertal development (days 30–37). However, an increase in liver IGF-1 gene expression was observed on the day of first proestrus [13], a change that was accompanied by elevated serum levels of IGF-1, along with serum LH and E_2 during development. Importantly, the increase in serum IGF-1 was accompanied by an increase in IGF-1 receptor gene expression in the ME. Similar results were shown in both male and female mice indicating that hypothalamic IGF-1 gene and protein expression levels were elevated during the neonatal period, decreased through 20 days of life, and then increased by day 30 and again by day 60 [50]. Those investigators also showed that IGF-1R gene and protein expression increase developmentally in the POA and MBH/ME of male and female mice [40]. Overall, the prepubertal increase in serum IGF-1 followed by elevated hypothalamic-pituitary-ovarian activity demonstrates the general influence of IGF-1 to facilitate GnRH secretion and the pubertal process. It is important to note that more recent research, which is detailed below, has revealed specific mechanisms of IGF-1 actions within the MBH, as well as within the more rostral/anterior brain regions that occur both early and late, respectively, in the pubertal process.

Recent Evidence Revealing Puberty-Related Mechanisms of IGF-1 Actions

Novel information has emerged revealing specific IGF-1 actions and interactions that occur within the MBH, as well as the POA and RHA, the principal brain regions responsible for pubertal development. While this review deals mainly with the rodent model for puberty, there are some species differences regarding GnRH localization within these brain regions that warrant mentioning. In all species, there is an abundance of GnRH nerve fibers coursing through the MBH region in route to the ME, where the peptide is released from their nerve terminals directly into the hypophyseal portal blood. In addition, neurons that synthesize GnRH are present in large numbers within the ARC nucleus of several species such as sheep [51], guinea pigs [52, 53], hamsters [54], horses [55], cattle [56], and primates [57–59], including humans [60, 61]. Conversely, GnRH neurons are not present in the ARC nucleus of rats [62], and in mice, they are either not present [63] or have only been observed in very small numbers [64]. However, it is important to note that in all species, including rats and mice, most of the GnRH neurons are located rostrally within the POA/RHA region. The perikarya of these neurons express the IGF-1R [40, 47, 50, 65], and some of their nerve processes course caudally through the MBH into the ME. Even though differences are observed in GnRH neuronal location, it is well accepted that the mechanisms governing release of GnRH from its nerve terminals are similar across species. Importantly, IGF-1 interacts with several other neuropeptides in the MBH/ARC, as well as within the POA/RHA regions. Because each of these areas exhibits different physiological roles during pubertal development, they will be discussed separately.

Actions within the MBH

The importance of increased communication between neuronal circuitries and glial elements within the MBH that facilitates the early actions of IGF-1 to induce prepubertal GnRH release has already been discussed. In addition to the dense population of GnRH nerve fibers in this region, there are also numerous other neuropeptides present within nerves and glial elements that are capable of interacting with the GnRH-containing nerve processes to alter release of the peptide. Among these peptides are DYN, NKB, Kp, and IGF-1. The sections below will describe recent research depicting some of the specific actions and interactions of these peptides within the MBH on GnRH secretion.

Action of DYN on Prepubertal GnRH Secretion

We have already discussed the early effect of IGF-1 to cross the blood-brain barrier and stimulate GnRH release from the ME; however, for years it has been proposed that another important factor in the onset of puberty is the removal of the prepubertal brake on GnRH secretion. DYN is an endogenous opioid peptide that has emerged as a candidate for such a role since it is recognized for its ability to inhibit prepubertal GnRH/LH secretion [3, 4, 66]. Other evidence of this comes from studies showing that the administration of anti-DYN resulted in increased serum LH [67], and that a kappa opioid receptor (KOR-1) antagonist increased the pulsatile secretion of LH and advanced puberty [68, 69]. Another study demonstrated that prepubertal alcohol administration stimulated the hypothalamic synthesis and secretion of DYN [70, 71]; hence, suggesting that DYN is an important component of the prepubertal inhibition in GnRH/LH release which accompanies alcohol-induced delayed pubertal development observed following chronic exposure to the drug in rats [72–74] and monkeys [75, 76], as well as humans [77, 78]. Although these studies clearly depicted the inhibitory nature of DYN on prepubertal GnRH secretion, there was a lack of information regarding upstream substance(s) involved in controlling its synthesis and release within the MBH. Even though DYN is produced in several regions of the hypothalamus, neurons in the ARC nucleus are considered the major source of its synthesis. Importantly, a subpopulation of these DYN-producing neurons in the ARC also coexpresses NKB and Kp [79]. While these peptides are found in separate neurons within other brain nuclei, it is unique that they colocalize to these specific neurons within the ARC nucleus, although the extent of their overlap varies between species [3, 4, 19, 80, 81]. Interestingly, as opposed to DYN, stimulatory actions on prepubertal GnRH/LH release have been shown in several species for both NKB [20, 82–85] and Kp [16–18]. An important question for several years has been what may first suppress the prepubertal DYN inhibitory tone on GnRH release and thus allowing for increased secretion of the GnRH peptide at the onset of puberty. Therefore, NKB and Kp were considered prime candidates for such a role based on their presence in the cells of the ARC nucleus and ability to stimulate GnRH release.

Action of NKB on Prepubertal DYN Secretion

The fact that most DYN neurons in the MBH express the NKB receptor (NK3R) [81] suggests that NKB likely contributes to the regulation of prepubertal DYN secretion. Therefore, it was important to determine whether

the tachykinin, NKB, which increases in the MBH as puberty approaches [86], would cause the suppression of prepubertal DYN release and thus facilitate the puberty-related rise in GnRH/LH secretion [20, 82]. An initial study [87] assessed MBH explants from late juvenile female rats exposed to senktide, an NK3R agonist, and revealed a dose-dependent inhibition of DYN release, which coincided with an increase in GnRH release from the same tissue block. That same study also showed that both actions of senktide were blocked by the prior central administration of an antagonist to NK3R. This antagonist has been shown to delay the onset of puberty in female rats [88] and to block the NKB stimulation of LH release [20, 82, 83]. Importantly, the release of β -endorphin, another opioid inhibitor of GnRH secretion [63] produced in the ARC, was unaffected by senktide [87].

While the above in vitro study suggested that NKB inhibits DYN secretion, animal studies were needed to discern a better understanding of this action within the MBH. In this regard, that same study [87] showed an increase in protein expression of the NK3R (Fig. 3a), along with suppressed protein expressions of DYN (Fig. 3b) and its receptor, KOR-1 (Fig. 3c), following 4 days of senktide administration into the third ventricle of late juvenile female rats. Furthermore, in vitro incubation of MBH tissues following 4 days of senktide administration in vivo resulted in suppressed DYN and increased GnRH secretion. Previous studies have demonstrated that NKB elicits prepubertal GnRH release in several species [20, 82, 83]. Collectively, these results not only further support this action of NKB, but also suggest that the activation of the NK3R causes a suppression of the DYN brake on prepubertal GnRH secretion and, at least in part, allows for the

rise in the GnRH peptide to occur. Since NK3R are localized on GnRH nerve fibers in the MBH [85, 89, 90], it is possible that GnRH was also released by a direct action of senktide on these nerve fibers.

Fig. 3. The effects of senktide administered centrally for 4 days on NK3R, DYN, and KOR-1 protein expressions in the MBH. Western blots of all 3 proteins were assessed from animals that received either a daily 3V injection of saline or senktide (gels not shown). Each of the above proteins was normalized to β -actin protein. The bars presented represent the mean $\pm$ SEM of the densitometric quantification of all bands assessing NK3R (a), DYN (b), and KOR-1 (c) proteins. Note that senktide-induced an increase in NK3R protein expression, but it suppressed DYN and KOR-1 protein expressions over the levels of their respective saline controls. Open bars, saline controls; hatched bars, senktide treated. $N = 14$ /bar. Unpaired t test was used to compare control versus senktide-treated animal groups; ** $p < 0.01$; *** $p < 0.001$ (with permission, Copyright© 2019 [87] Dees WL, Hiney JK, Srivastava VK. *Journal of Neuroendocrinology* published by John Wiley & Sons Ltd. on behalf of the British Society for Neuroendocrinology). DYN, dynorphin; KOR, kappa opioid receptor; MBH, medial basal hypothalamus; 3V, third ventricular.

Fig. 4. The effects of IGF-1 administered centrally for 4 days on NKB and DYN protein expressions in the MBH. Western blots of NKB and DYN were assessed from animals that received either a daily 3V injection of saline or IGF-1 (gels not shown). Each of the above proteins was normalized to β -actin protein. The bars presented represent the mean $\pm$ SEM of the densitometric quantification of all bands assessing NKB (a) and DYN (b) proteins. Note that IGF-1 induced an increase in NKB protein expression, but it suppressed DYN protein expression over the levels of their respective saline controls. Closed bars, saline controls, $N = 28$; open bars, IGF-1-treated, $N = 29$. Unpaired t test was used to compare control versus IGF-1-treated animal groups. ** $p < 0.01$; *** $p < 0.001$ (with permission Copyright© 2019 [87] Dees WL, Hiney JK, Srivastava VK. *Journal of Neuroendocrinology* published by John Wiley & Sons Ltd on behalf of the British Society for Neuroendocrinology). IGF-1, insulin-like growth factor-1; NKB, neurokinin B; DYN, dynorphin; MBH, medial basal hypothalamus; 3V, third ventricular.

The Kp are peptide products of the KiSS-1 gene (for review, see [91]) and are considered potent stimulators of prepubertal GnRH and critical for driving the pubertal process [15, 17, 18, 92]. Importantly, since Kp is also synthesized by neurons in the ARC nucleus [93, 94], its secretion was also assessed from the same MBH explants described above to determine if Kp was involved in the senktide/NKB-induced suppression of DYN, along with the increased release of GnRH. In this regard, senktide did not affect Kp release; thus, indicating Kp was not involved in the senktide stimulation of GnRH release in these juvenile female rats [87]. A study using prepubertal female monkeys showed that senktide was able to stimulate Kp release following delivery into the stalk ME [20]; however, the release observed was markedly less than that produced in pubertal monkeys, an action attributed to differences in circulating E_2 levels. The differences noted between the rodent and primate studies regarding senktide stimulation of Kp could be due to a species difference or to the low levels of E_2 in the juvenile female rats. Interestingly, the study using prepubertal female monkeys revealed that NKB and Kp take independent signaling paths to affect the release of GnRH [20]. Also, a study using tissue slices from adult male transgenic mice indicated that NK3R-activated GnRH release was Kp independent [85]. Furthermore, the study detailed above showing that senktide administration caused increased GnRH release without affecting Kp release in juvenile female rats [87] also supports the concept that NK3R-activated GnRH release is independent of Kp, at least at this early stage of development. This is important because it suggests Kp is not associated with the removal of the DYN inhibitory tone on prepubertal GnRH secretion; hence, indicating an earlier role for NKB in the pubertal process than that of Kp.

Action of IGF-1 on Prepubertal NKB Secretion

The observation that senktide/NKB can suppress prepubertal DYN secretion raised the question as to what neuronal substance may regulate the puberty-related synthesis and release of NKB within the MBH. We have already discussed a direct early action of IGF-1 within the MBH to stimulate the prepubertal release of GnRH; however, it is also possible that there is another means by which this metabolic signal can facilitate GnRH secretion at the time of puberty. A recent study supports this hypothesis by showing an early action of IGF-1 to regulate NKB [87]. Specifically, central delivery of IGF-1 into the third ventricle of late juvenile female rats for 4 days caused increased NKB and decreased DYN protein ex-

pressions within the MBH (Fig. 4). IGF-1 administration, however, did not alter Kp expression in this brain region [87]. This lack of an effect of IGF-1 on Kp protein expression agrees with a previous study showing that KiSS-1 gene expression was also unaltered in the ARC nucleus [95]. Furthermore, after 4 days of IGF-1 administration, there was no change observed in the *in vitro* release of Kp; however, there was an increase noted in the release of NKB, an action that was blocked by the IGF-1R antagonist, JB-1. The exact site of this interaction between IGF-1 and NKB has yet to be determined because it is presently unknown whether NKB neurons express IGF-1Rs or whether IGF-1 is binding to its receptors expressed on an interneuron. While additional anatomical work in this regard is needed, the physiological information presented to date clearly demonstrates that IGF-1 can act as an upstream regulator of prepubertal NKB synthesis and release within the MBH, but not that of Kp. Although not related to IGF-1, another study showed that the KiSS-1 gene is expressed consistently in the ARC of prepubertal pigs and that there was no association between expression levels in this nucleus and the timing of puberty [96]. Those authors suggested that secretion levels of Kp is likely a more reliable indicator of puberty than gene expression.

Importance of the IGF-1/NKB/DYN Pathway on Prepubertal GnRH Secretion

Increasing amounts of peripherally derived IGF-1 entering the brain as puberty approaches is well known (for review, see [13]). Recently, we suggested that a novel pathway induced by IGF-1 is involved in early puberty-related events within the MBH [87]. Specifically, IGF-1 is capable of inducing NKB secretion and the subsequent NK3R-activated suppression of DYN secretion. Thus, activation of this pathway contributes to the removal of the DYN inhibitory tone on prepubertal GnRH secretion, an action that allows for the puberty-related increase in GnRH to begin. Importantly, Kp is not involved in the action of this pathway to suppress DYN, although it may participate with both NKB and DYN within the MBH in regulating pulsatile GnRH secretion later during pubertal development and during adulthood (for review, see [97]). Overall, we suggest that the newly described action of the IGF-1/NKB/DYN pathway within the MBH, along with the actions of both NKB and IGF-1 to independently stimulate GnRH release from the nerve terminals in the ME, facilitate the gradual rise in GnRH secretion at the onset of puberty.

Actions within the POA/RHA

As E₂ levels begin to increase and the pubertal process transitions from juvenile to peripubertal development, the more rostral/anterior brain regions play an increasingly important role. We have already established that this region is the major site of GnRH-producing neurons. Importantly, in addition to Kp synthesis within the MBH, the peptide is also produced by neurons in the RHA, specifically, within the anteroventral periventricular (AVPV) nucleus. KiSS-1 gene expression and that of the Kp receptor, GPR54, increases during pubertal development [16] and changes depending upon the steroid milieu [98, 99]. Furthermore, as E₂ levels rise during proestrus, the IGF-1 peptide demonstrates an increased efficiency to stimulate GnRH secretion [13]. Because of the contributions of both IGF-1 [13, 14] and Kp (for review, see [100]) to the regulation of prepubertal GnRH secretion and the timing of puberty in several mammalian species, including humans, it became important to obtain a better understanding of the potential interactions between these two puberty-related peptides.

Actions of IGF-1 in the Regulation of Kp Synthesis

As stated earlier, IGF-1 administration did not alter expression of the KiSS-1 gene in the MBH of prepubertal female rats; however, the peptide did effectively induce expressions of the KiSS-1 gene and Kp protein within the RHA/AVPV brain region, actions that were blocked by the prior administration of the IGF-1 receptor blocker, JB-1 [95]. That increase in KiSS-1 gene occurred in juvenile female rats with low levels of E₂, but later revealed that this response was E₂ dependent, since IGF-1 was ineffective in stimulating KiSS-1 gene expression in the AVPV region 20 days after ovariectomy [95]. Subsequently, it was shown that the increase in KiSS-1 gene expression induced by IGF-1 was the result of an Akt-mediated action [101]. This agrees with a previous report showing that Akt is a transduction mediator of some other effects of IGF-1 [102]. More recently, it was revealed that both the stimulation of endogenous IGF-1, as well as the direct central delivery of the peptide, caused an increase in Kp protein synthesis in this brain region, and that this stimulatory action of IGF-1 on Kp is mediated by an IGF-1R-activated Akt/mammalian target of rapamycin (mTOR) pathway [103]. Specifically, IGF-1 administered into the third ventricle induced the phosphorylation of insulin receptor substrate 1 (IRS1) by 6 h after injection, thus confirming activation of the IGF-1R receptor/IRS1 complex. This action caused the phosphory-

lation of Akt, which in turn initiates the critical downstream phosphorylation of tuberous sclerosis complex 2 (TSC2). Phosphorylation of the TSC2 complex deactivates the inhibitory tone that TSC2 has on Ras homolog enriched in brain (Rheb), and as a result Rheb levels rise. The increased levels of Rheb are associated with the phosphorylation of mTOR and finally increased Kp synthesis. Interestingly, this pathway to Kp was shown to be inhibited by alcohol blocking the IGF-1R/IRS1 complex [103].

Actions of IGF-1 in the Regulation of Kp Release

As puberty progresses through the peripubertal period, Kp synthesizing neurons in the RHA/AVPV respond to the positive feedback effect of rising serum levels of E₂ [4, 104, 105]. This response ultimately promotes an increase in the synthesis and release of GnRH, an action that drives the pubertal process to the preovulatory surge [104–106]. Because IGF-1 is also sensitive to the positive effect of E₂ regarding stimulation of GnRH/LH secretion as puberty proceeds [13, 107, 108], it was important to discern whether IGF-1 played an upstream role in stimulating release of Kp from the RHA/AVPV region. In this regard, IGF-1 did not induce the *in vitro* release of Kp from tissue incubates collected during juvenile development when E₂ levels were low, but was effective in stimulating the release of Kp from tissues collected during first proestrus when E₂ levels were higher. This action of IGF-1 to stimulate Kp secretion was shown to be inhibited by alcohol, a known blocker of the IGF-1R [103].

The observation that IGF-1 can regulate the secretion of Kp by an action within the RHA and thereby affect GnRH synthesis and secretion is further supported by anatomical and physiological information. While Kp neurons in this brain region project some of their nerve processes caudally to the MBH, there are also processes that project rostrally to adjacent GnRH synthesizing neurons in other areas of the RHA, as well as the POA [109, 110]. Importantly, most of these GnRH neurons express Kp receptors [111–113]. Thus, evidence indicates that once Kp is released from the RHA [103], it acts directly on GnRH neurons to stimulate the synthesis and release of the GnRH peptide. These interactions are important since, as stated above, Kp is a potent stimulator of GnRH/LH, especially at the time of proestrus. Interestingly, resumption of *ad libitum* feeding to previously food-restricted rats caused vaginal proestrus and a surge-like increase in LH associated with an increase in KiSS-1 gene expression in the AVPV nucleus [114]. Furthermore, the firing rates of Kp neurons increase at proestrus [115], a time when the peptide has been shown to directly enhance GnRH

neuronal firing *in vitro* [116–118]. Collectively, these facts further support the evidence that Kp neurons in the RHA region play an important role in relaying the positive feedback effects of rising E₂ in the control of GnRH neuronal function during pubertal development [119, 120]. Thus, the fact that IGF-1 stimulates Kp release within the RHA is important, since it ultimately contributes to the increased synthesis and secretion of GnRH as puberty approaches [103].

Conclusions

There are important actions and interactions of IGF-1 that occur within the MBH, as well as the POA/RHA, the principal brain regions responsible for pubertal development. This review describes research over the years supporting the hypothesis that IGF-1 is a metabolic signal capable of activating and enhancing GnRH secretion at the time of puberty. Specifically, early work showed that the hepatic synthesis of IGF-1, followed by its rising levels in peripheral circulation, markedly increases as puberty approaches. The peptide crosses into the brain at the level of the ME and acts through the IGF-1R to facilitate GnRH secretion. Furthermore, it is now well known that the peptide increasingly stimulates LH secretion throughout pubertal development via a centrally mediated effect, and that repeated exposure of the hypothalamus to increased levels of the peptide effectively accelerates the timing of puberty. We discussed that the action of IGF-1 to stimulate prepubertal GnRH secretion is coordinated by a complex series of glial- and neuronal-mediated events. Regarding glial interactions, one example that occurs within the MBH is that IGF-1 binds to its receptors located on glial cells that secrete PGE₂. Once the PGE₂ is secreted, it then binds to its receptors on nearby GnRH nerve terminals within the ME and causes release of the GnRH peptide. Regarding neuronal interactions, there are several peptides produced by neurons that are known to have either positive or negative effects on prepubertal GnRH release. It is becoming increasingly clear that IGF-1 has the capability of regulating some of these neurons in specific brain regions. In this regard, IGF-1 has been shown to act within the MBH to stimulate the synthesis and secretion of prepubertal NKB, which subsequently binds to NK3R on DYN-producing neurons and causing the suppressed release of DYN. This suggests that the NKB-induced suppression of the DYN brake on prepubertal GnRH secretion, at least in part, facilitates the puberty-related rise in GnRH/LH secre-

Fig. 5. Schematic showing early actions of IGF-1, NKB, and DYN within the MBH contributing to the prepubertal regulation of GnRH secretion. 1 Blue arrows indicate that IGF-1 directly stimulates GnRH release from the ME, while also stimulating NKB synthesis and release from neurons in the ARC nucleus. 2 Red arrows indicate that NKB likewise stimulates GnRH release from the ME, while also inhibiting DYN (orange bar) synthesis and release from neurons in the ARC nucleus. 3 The combined actions of increased IGF-1 and NKB and the removal of the inhibitory tone of DYN results in increased GnRH secretion during the initiation of puberty. 3V, third ventricle; (+) = stimulation; (–) = inhibition (with permission, Copyright © 2019 [87] Dees WL, Hiney JK, Srivastava VK. *Journal of Neuroendocrinology* published by John Wiley & Sons Ltd. on behalf of the British Society for Neuroendocrinology). IGF-1, insulin-like growth factor-1; NKB, neurokinin B; DYN, dynorphin; MBH, medial basal hypothalamus; GnRH, gonadotropin-releasing hormone; ARC, arcuate; ME, median eminence.

tion. Furthermore, a direct action of NKB to stimulate GnRH release is also likely since NK3R are localized on GnRH nerve fibers in the MBH. These actions and interactions of IGF-1, NKB, and DYN within the MBH begin early in the developmental process and are shown in the schematic revealed in Figure 5. Moreover, during the transition from juvenile into peripubertal development, neurons in more rostral regions of the brain begin to show increased sensitivity to the rising levels of E_2 . As the IGF-1 levels increase in the region, induction of the IGF-1R activates an Akt-mediated pathway to mTOR, ultimately resulting in increased synthesis and release of Kp from its E_2 -sensitive neurons in the AVPV region of the RHA. Finally, once released, Kp then binds to its receptors on GnRH-producing neurons in the RHA, as well as in the POA and other rostral nuclei, further driving the

Fig. 6. Schematic drawing showing the effects of IGF-1 in the POA/rostral hypothalamic region on prepubertal Kp and GnRH synthesis and release. IGF-1 activates the IGF-1R/IRS1 complex, causing phosphorylation of Akt. This activation of Akt initiates the downstream phosphorylation of TSC2 and thus removing the inhibitory tone on Rheb. The increase in Rheb induces the phosphorylation of mTOR leading to a stimulation in the synthesis and release of Kp, as well as GnRH. P, phosphorylated site; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor receptor 1; IRS1, insulin receptor substrate 1; TSC2, tuberous sclerosis complex 2; Rheb, Ras homolog enriched in brain; mTOR, mammalian target of rapamycin; Kp, kisspeptin; GnRH, gonadotropin-releasing hormone; POA, preoptic area.

preovulatory surge in GnRH/LH release and hence resulting in first ovulation. These peripubertal actions and interactions between IGF-1, Kp, and GnRH within the more rostral brain regions are shown in the schematic revealed in Figure 6.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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