IGF-1 in the Brain as a Regulator of Reproductive Neuroendocrine Function

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MINIREVIEW

IGF-1 in the Brain as a Regulator of Reproductive Neuroendocrine Function

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Given the close relationship among neuroendocrine systems, it is likely that there may be common signals that coordinate the acquisition of adult reproductive function with other homeostatic processes. In this review, we focus on central nervous system insulin-like growth factor–1 (IGF-1) as a signal controlling reproductive function, with possible links to somatic growth, particularly during puberty. In vertebrates, the appropriate neurosecretion of the decapeptide gonadotropin-releasing hormone (GnRH) plays a critical role in the progression of puberty. Gonadotropin-releasing hormone is released in pulses from neuroterminals in the median eminence (ME), and each GnRH pulse triggers the production of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These pituitary hormones in turn stimulate the synthesis and release of sex steroids by the gonads. Any factor that affects GnRH or gonadotropin pulsatility is important for puberty and reproductive function and, among these factors, the neurotrophic factor IGF-1 is a strong candidate. Although IGF-1 is most commonly studied as the tertiary peripheral hormone in the somatotropic axis via its synthesis in the liver, IGF-1 is also synthesized in the brain, within neurons and glia. In neuroendocrine brain regions, central IGF-1 plays roles in the regulation of neuroendocrine functions, including direct actions on GnRH neurons. Moreover, GnRH neurons themselves co-express IGF-1 and the IGF-1 receptor, and this expression is developmentally regulated. Here, we examine the role of IGF-1 acting in the hypothalamus as a critical link between reproductive and other neuroendocrine functions. Exp Biol Med 230:292–306, 2005

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Introduction

Insulin-like growth factor–1 (IGF-1) is a 70 amino acid polypeptide that exerts effects on peripheral growth, differentiation, and survival in a variety of cells and tissues. In peripheral tissues, IGF-1 is the tertiary hormone in the somatotropic axis that plays key roles in somatic growth. The growth axis, like all neuroendocrine systems, functions through hypothalamic, pituitary, and target organ interactions. At the hypothalamic level, the growth axis comprises two sets of neurons that synthesize and release growth hormone-releasing hormone (GHRH) or somatostatin, the excitatory and inhibitory regulators, respectively, of pituitary somatotropin (growth hormone, GH) release (1, 2). The secretion of GH from the anterior pituitary into the peripheral circulation stimulates the production of peripheral IGF-1 from its primary target, the liver, as well as from secondary targets such as lung, kidney, thymus, spleen, heart, muscle, and gonads (3–5). The IGF-1 receptor is localized in numerous tissues including muscle (6), ovary (7), pituitary (8), and brain (9). This wide distribution of the receptor underscores the multifaceted roles of IGF-1.

Along with its actions on somatic tissues, IGF-1 also exerts effects within the central nervous system, in which the IGF-1 receptor is widely distributed (9–14). In neuroendocrine systems in particular, IGF-1 acts as a feedback regulator of GHRH and somatotropin-secreting cells of the hypothalamus (1, 2). Insulin-like growth factor–1 exerts negative effects on GHRH, thereby diminishing its stimulatory input to the pituitary gland, while stimulating inhibitory somatotropin neurons to further inhibit pituitary GH release (2, 15). The clear and important role of IGF-1 in

Table 1. Summary of Studies on the Role of Insulin-Like Growth Factor–1 (IGF-1) in Hypothalamic-Pituitary-Gonadal (HPG) Function[a]

Experimental observations	References
IGF-1 localization in peripheral organs	Heart (3, 4); kidney (3, 4); liver (3, 4, 129); lung (3, 4, 129); mammary gland (3, 129); muscle (3, 4); ovary (5, 129); spleen (3); stomach (4); testes (3, 4, 129); thymus (3)
IGF-1 localization in central nervous system	(3, 4, 20, 33–38, 40, 41, 52–56, 129)
IGF-1R localization	Brain (8–14, 24, 60); muscle (6); ovary (7); pituitary (8, 14, 84)
IGF-1 and IGF-1R co-expression in GnRH neurons or cell lines	(10, 20, 55, 75, 77–79)
IGF-1 binding protein localization in brain	(59, 63)
IGF-1 ontogeny	(20, 35, 37, 38, 40, 52, 55–57, 59)
Effects of IGF-1 in GnRH (GT1 and NLT) cell lines	(75–79)
Effects of IGF-1 on GnRH in hypothalamic explants *(in vitro)*	(20, 80)
Effects of IGF-1 on GnRH or gonadotropins *(in vivo)*	(24, 55, 61)
Effects of IGF-1 on gonadotropes *(in vitro)*	(22, 24, 84)
Levels of IGF-1 increase during or just before puberty	Humans (90); primates (85, 89); rodents (61, 86, 87); ruminants (88)
Influence of IGF-1 on puberty	(61, 91, 96)
IGF-1 and estrogen interactions	(24, 25, 56, 98–111, 115–118)
Evidence for a role of IGF-1 in reproductive function provided by transgenic or knockout animal models	(119–122, 124–128)

[a] References are in parentheses. IGF-1R, IGF-1 receptor; GnRH, gonadotropin-releasing hormone.

the somatotropic axis does not eliminate it as a candidate for the regulation of other neuroendocrine systems, particularly the reproductive axis. This neuroendocrine system, comprising the hypothalamic gonadotropin-releasing hormone (GnRH) neurons, pituitary gonadotropes (producing luteinizing hormone, LH; and follicle-stimulating hormone, FSH), and the gonads (ovary and testis), is responsible for the regulation of reproduction in all vertebrates (16). Although changes in GnRH neurons themselves play key roles in the attainment of reproductive competence (17), it is also clear that changes in inputs to GnRH cells from neurotransmitters and neurotrophic factors in the central nervous system also play critical regulatory roles (18–21). Recent evidence from our laboratory and others, described below, implicates IGF-1 as a direct regulator of GnRH neurons. Insulin-like growth factor–1 can also regulate the hypothalamic-pituitary-gonadal (HPG) axis via actions at the pituitary (22–24) and gonadal levels (25, 26) of this axis. These findings, taken together with the role of IGF-1 in the somatotropic axis, support IGF-1 as a potential link between the reproductive and somatotropic neuroendocrine systems.

Insulin-like growth factor–1 can influence cell function via autocrine, paracrine, and/or endocrine signaling. Moreover, the role of IGF-1 in prenatal and postnatal growth is well recognized and several studies support a role for IGF-1 in the establishment and maintenance of reproductive function. Given the tight temporal coupling between growth and reproductive development, the idea that a common signal, such as IGF-1, may regulate both growth and the initiation of puberty has been the focus of recent research. In this article we will review the concept that IGF-1 regulates the release of GnRH, thereby initiating puberty, and our focus will be on IGF-1 of central origin. We will also discuss how the somatotropic axis impacts GnRH and gonadotropin secretion in post-pubertal individuals and, thus, affects the maintenance of fertility in adults. For convenience, we have provided a summary of our major points in Table 1.

Central Versus Peripheral IGF-1

The actions of IGF-1 on the brain are caused by a combination of peripheral IGF-1 that enters the central nervous system, together with local IGF-1 that is synthesized within neurons and glia. It is difficult to differentiate between actions of IGF-1 of central versus peripheral origin because its effects are all mediated at the same target receptors. The IGF-1 receptor (IGF-1R) is a member of the tyrosine kinase receptor family that signals through the phosphoinositol-3 kinase and mitogen-activated protein kinase signaling cascade (27–29). Insulin-like growth factor–1 receptors are located in numerous brain regions, discussed in detail below. The effects of IGF-1 in the brain also involve IGF-1 binding proteins (IGFBPs), which are synthesized both centrally and peripherally (30–32). In fact, these three components of the IGF-1 system (IGF-1, its receptors, and its binding proteins) are all localized to and synthesized in the central nervous system, including neuroendocrine regions (8, 9, 13, 14, 33–42). Thus, the IGF-1 system, through a combination of central and peripheral sources, and interacting with binding proteins and receptors, is poised to act as a central regulator of neuroendocrine function.

Peripheral IGF-1. The major source of peripheral IGF-1 is the liver, where its production is induced by GH (43). In addition, other organs of the body, including the

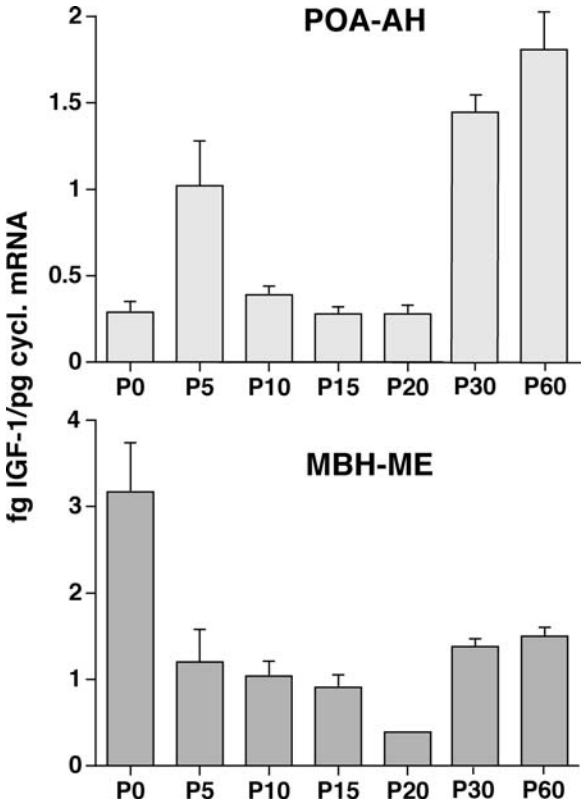

Figure 1. Insulin-like growth factor–1 (IGF-1) mRNA levels in developing mice were quantified by RNase protection assay. Insulin-like growth factor–1 mRNA levels in the preoptic area-anterior hypothalamus (POA-AH) and mediobasal hypothalamus-median eminence (MBH-ME), the site of gonadotropin-releasing hormone (GnRH) perikarya and neuroterminals, respectively, are shown for female mice at postnatal day (P) 0, 5, 10, 15, 20, 30, and 60. Similar data were obtained for males (data not shown). Mean IGF-1 mRNA levels, normalized to cyclophilin (cycl.) mRNA levels, ± SEM are indicated. Insulin-like growth factor–1 mRNA levels in the POA-AH (upper panel) show two significant developmental peaks: one at P5 and a second at P60. Insulin-like growth factor–1 mRNA levels in the MBH-ME (lower panel) also have two significant peaks: one at P0, followed by a decrease to a nadir at P20, then a second significant increase (albeit with a lower peak) at P60. (Modified from Ref. 20).

lung, kidney, thymus, spleen, heart, muscle, stomach, and gonads can produce IGF-1 (3–5). In fact, a transgenic mouse with the IGF-1 gene knocked out specifically in liver still has appreciable concentrations of circulating IGF-1 resulting from extrahepatic synthesis (44). It is notable that peripheral IGF-1 can cross the blood-brain barrier, by a mechanism that is not entirely understood (45–47). These multiple biosynthetic sources of IGF-1 synthesis complicate an understanding of how IGF-1 levels in the body are maintained and which sources may play roles in feedback on the somatotropic axis, particularly within the central nervous system. Furthermore, IGF-1 in the circulation can bind to a series of high-affinity IGFBPs, which modulate the amount of bioavailable IGF-1. Insulin-like growth factor–1 binding proteins regulate the levels of IGFs by performing several functions, such as transporting the IGFs out of the vascular compartment in the circulation; localizing the IGFs to specific cell types; and modulating both IGF binding to receptors and its growth-promoting actions (reviewed in Refs. 48, 49). Therefore, IGF-1 that is synthesized in the periphery exerts actions in the nervous system through multiple and complex mechanisms.

Central IGF-1. Insulin-like growth factor–1 is synthesized throughout the brain and spinal cord. In these regions, IGF-1 is a potent neurotrophic factor for neurons and glial cells during development (50). In the adult central nervous system, IGF-1 is a mediator of synaptic plasticity (51), regulating neuronal survival in response to injury. Many studies have demonstrated the presence of IGF-1 mRNA and immunoreactivity in neurons and glia of spinal cord, olfactory bulb, midbrain, cerebellum, cortex, striatum, amygdala, hippocampus, medulla, and pons (36, 38, 52); as well as within the key neuroendocrine centers, the hypothalamus and preoptic regions (20, 36, 38, 52–56).

The ontogeny of IGF-1 in the brain has been studied by several laboratories. Its expression exhibits a marked developmental age-dependent and region-specific variation that will be discussed here first for non-neuroendocrine brain regions. Garcia-Segura *et al.* (38) reported that IGF-1 immunoreactivity is detectable in the rat brain from as early as embryonic day (E) 15 up to adulthood, with decreased intensity of labeling and number of immunoreactive cells in cerebral cortex, hippocampus, striatum, and amygdala in adult compared with young postnatal animals (38). Further decreases are seen during aging in the hippocampus, as demonstrated by another group (57). A study using a transgenic mouse with the IGF-1 promoter linked to the luciferase reporter gene demonstrates a peak in IGF-1 gene expression at postnatal day (P) 5 in olfactory bulb, cerebral cortex, brainstem, diencephalon, cerebellum, and hippocampus (52). An analysis of IGF-1 mRNA expression in the developing rat brain from E16 to P82, using RNase protection assays, reveals region-specific developmental patterns (35). Specifically, the olfactory bulb shows a high perinatal level of IGF-1 mRNA, which declines dramatically by P8. Cerebral cortex displays maximal levels of IGF-1 mRNA at P8 and P13, which subsequently decline to adult levels (P82). Insulin-like growth factor–1 mRNA levels in brainstem and cerebellum remain unchanged throughout the time period studied (35). Notably, all of these reports indicate detectable levels of IGF-1 protein and mRNA in adult brain, and, in most regions, levels decrease following a peak early in postnatal life.

Within the hypothalamus, IGF-1 protein and mRNA expression are also developmentally regulated, although they have a unique expression pattern that differs from other brain regions. Measurements of IGF-1 mRNA in hypothalamus by RNase protection assay show an increase from E16 to P3, with levels remaining elevated through adulthood (35). Using male and female mice, our laboratory has quantified developmental changes in IGF-1 mRNA levels in the preoptic area-anterior hypothalamus (POA-AH) and the mediobasal hypothalamus-median eminence (MBH-ME),

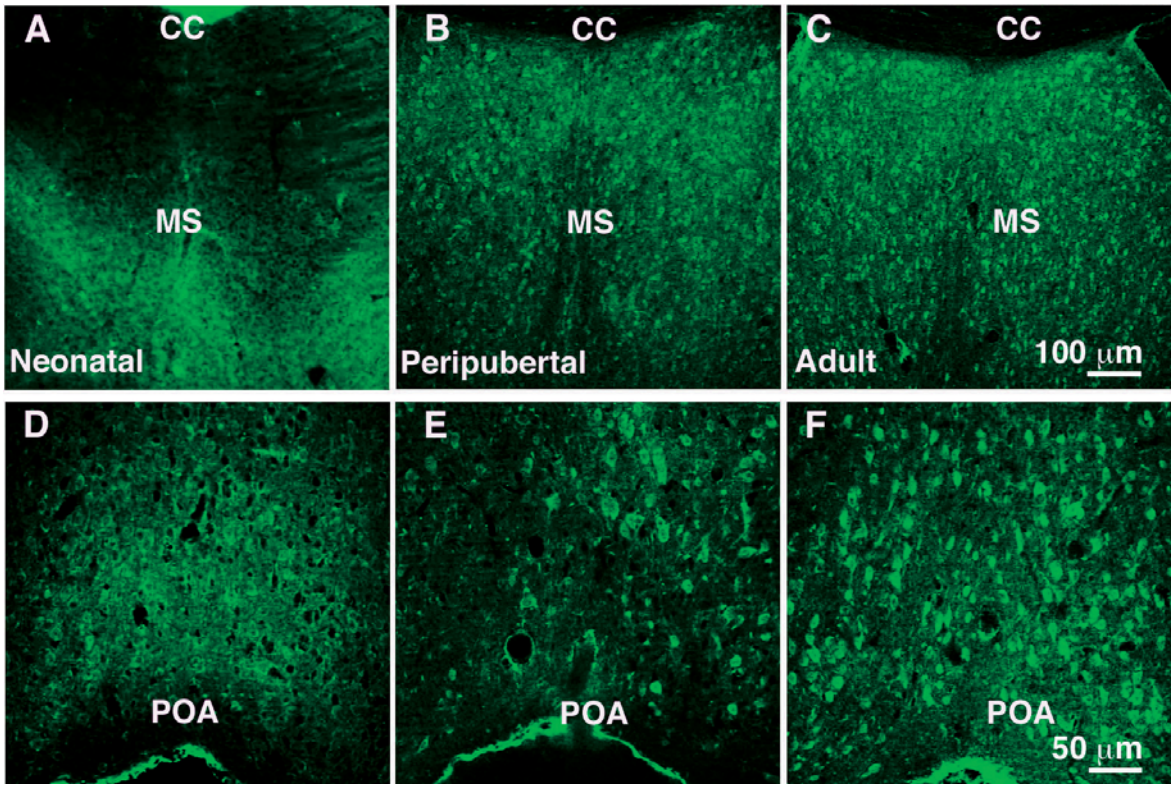

Figure 2. Insulin-like growth factor–1 (IGF-1) in preoptic brain regions. The photomicrographs show immunoreactivity for IGF-1 (shown in green with fluorescein-labeling) in septal (A–C) and preoptic (D–F) regions of representative neonatal (P5), peripubertal (P30), and adult (P60) mice. Immunoreactivity for IGF-1 in P5 mice is rather diffuse and mainly restricted to the neuropil (A and D). The intensity of immunolabeling and number of IGF-1–immunoreactive cells increase dramatically during the peripubertal period (B and E). Immunostaining for IGF-1 is abundant in the preoptic area-anterior hypothalamus (POA-AH) and MS of adult P60 mice (C and F). CC, corpus callosum; MS, medial septum; POA, preoptic area. (Modified from Ref. 20).

the major sites of neuroendocrine cells and nerve terminals, respectively. Two peaks in gene expression are detected by RNase protection assay: the first occurs at P5 and P0, in POA-AH and MBH-ME, respectively (Fig. 1; Ref. 20). These early postnatal peaks are similar to those reported in the diencephalon as a whole by Ye *et al*. using transgenic mice with the IGF-1 promoter linked to a luciferase reporter (52). Our laboratory has extended this developmental analysis further along the postnatal spectrum. We found that after reaching a nadir at P20, levels of IGF-1 mRNA then increase through adulthood (P60), the oldest age measured in our mice (Fig. 1; Ref. 20). Thus, unlike non-neuroendocrine regions, IGF-1 mRNA levels *increase* postnatally.

IGF-1 protein is localized in developing (20) as well as adult hypothalamic neurons and glia (12, 13, 54, 58). Previously, it was reported that IGF-1 immunoreactivity, assayed from hypothalamic dissections of rats from E15 through adulthood, is unchanged with age (59). However, we felt it important to perform more region-specific analyses, focusing on preoptic, hypothalamic, and septal nuclei. Using immunohistochemistry, we observed that IGF-1 immunoreactivity is robust in these brain regions in mice at ages P5, P30, and P60, and increases developmentally, with the highest immunostaining in adult (P60) mice (Fig. 2; Ref. 20). Moreover, the *pattern* of labeling of cells with IGF-1 changes developmentally, as younger mice have more diffuse immunolabeling of cells and neuropil, whereas older mice exhibit more specific and robust labeling within cells within the POA-AH and medial and lateral septum. The increase is consistent with the observation of maturational increases in IGF-1 mRNA levels described earlier, and with the suggestion that IGF-1 gene transcription and protein translation occur roughly in parallel within these brain regions (20). A similar pattern of IGF-1 immunoreactivity to that described here in mice was also observed in adult female rats, although we did not perform a developmental analysis in that latter species (55). These IGF-1 expression patterns appear to be specific to the neuroendocrine brain centers, because the non-neuroendocrine brain regions, as described above, either undergo developmental decreases or show no change in IGF-1 gene and protein expression.

Central IGF-1 Receptors. Further support for the theory of IGF-1 regulation of neuroendocrine cells is provided by the presence of the IGF-1R in the pituitary gland and rodent brain, including the hypothalamus. Binding studies (8, 14, 60), blot or *in situ* hybridization

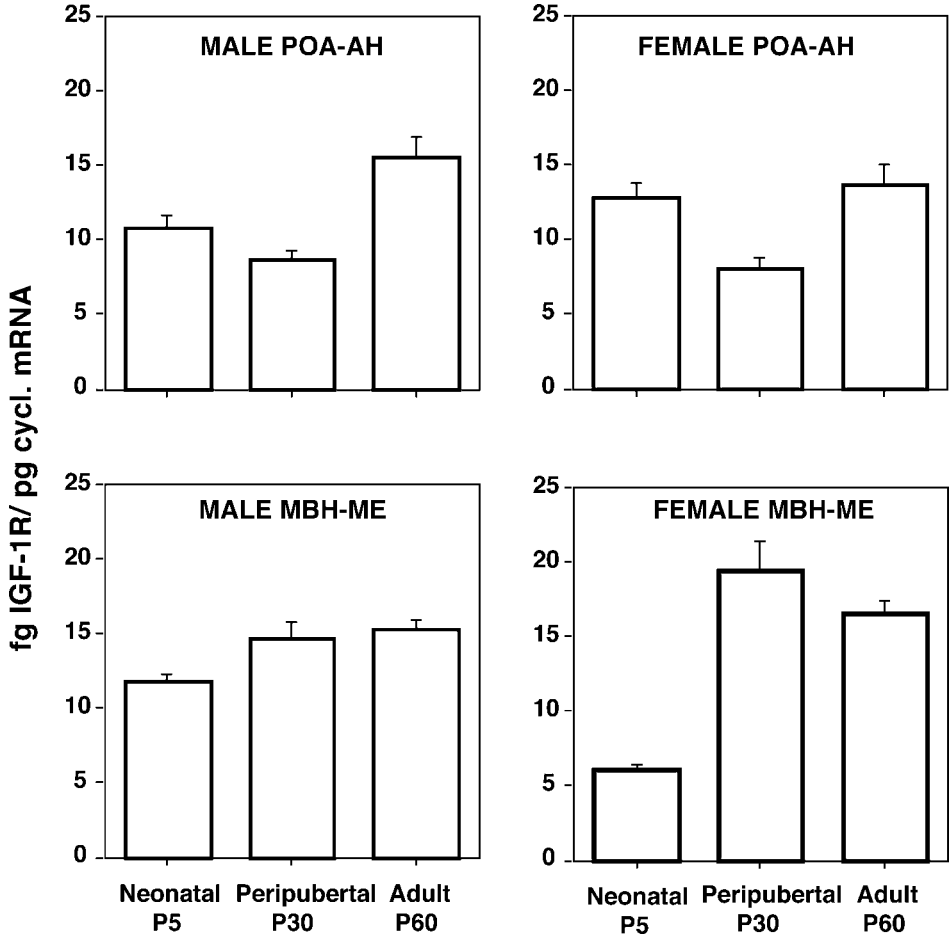

Figure 3. Quantification of insulin-like growth factor–1 receptor (IGF-1R) mRNA levels by RNase protection assay in the preoptic area-anterior hypothalamus (POA-AH; upper panel) and mediobasal hypothalamus-median eminence (MBH-ME; lower panel) of neonatal (P5), peripubertal (P30), and adult (P60); male (left) and female (right) mice. Insulin-like growth factor–1 receptor mRNA values are reported in femtograms of IGF-1R, normalized to picograms of cyclophilin (cycl.) mRNA. Levels of IGF-1R in the POA-AH of both male and female peripubertal mice decrease from P5 to P30, and increase from P30 to P60. Levels of IGF-1R in the MBH-ME of male and female mice increase from P5 to P60 in males, and from P5 to P30 in females. Each bar represents the mean ± SEM. (Modified from Ref. 10).

(9, 13), and immunohistochemical studies (11, 12, 20) demonstrate a similar developmental expression of IGF-1R mRNA and protein. Our laboratory quantified IGF-1R gene expression in the POA-AH and MBH-ME, the site of GnRH processes and terminals, respectively, in developing mice at P5, P30, and P60 (10). Insulin-like growth factor–1 receptor mRNA levels in the POA-AH are highest at P60 in male and female mice. In the MBH-ME, IGF-1R mRNA levels increase from P5 to P30 and remain at these higher levels in P60 mice (Fig. 3). Similar to our present findings in mice, Hiney *et al*. (61) also reported increases in IGF-1R mRNA levels in the median eminence (ME) of female rats undergoing pubertal maturation. We have also evaluated IGF-1R protein expression qualitatively, and immunohistochemical data support an age-related increase in the protein expression in neuroendocrine brain regions, similar to our mRNA data (10). Taken together, these studies demonstrate developmental increases in IGF-1 and the IGF-1R in neuroendocrine tissues.

Central IGF-1BPs. Insulin-like growth factors bind noncovalently to a family of six structurally and evolutionary related binding proteins, the IGFBPs (48). These IGFBPs are expressed in a tissue-specific manner, have different affinities for IGF, and modulate IGF action by regulating the bioavailability of IGFs (62). Like the IGFs, IGFBPs are synthesized mainly in the liver, exerting an endocrine action from the bloodstream. Nevertheless, IGFBPs of apparent mol wts, 24, 29, and 32 kDa are found in the hypothalamus (59). Because IGFBP-2 (mol wt, 32 kDa) and IGFBP-5 (mol wt, 21–31 kDa) are most abundantly expressed in the brain (63), these are likely candidates for those reported by Pons *et al*. (59). Each of these IGFBPs has an individual expression profile in the course of development, with the highest levels of the 29-kDa and 32-kDa IGFBPs found in fetal and early postnatal life, and levels of the 24-kDa form being highest in young adults. Hence, these IGFBPs are locally produced within the neuroendocrine hypothalamus, where their action may be autocrine and/or paracrine. Additionally, a recent study demonstrated that IGFBP-6 overexpression in the brain

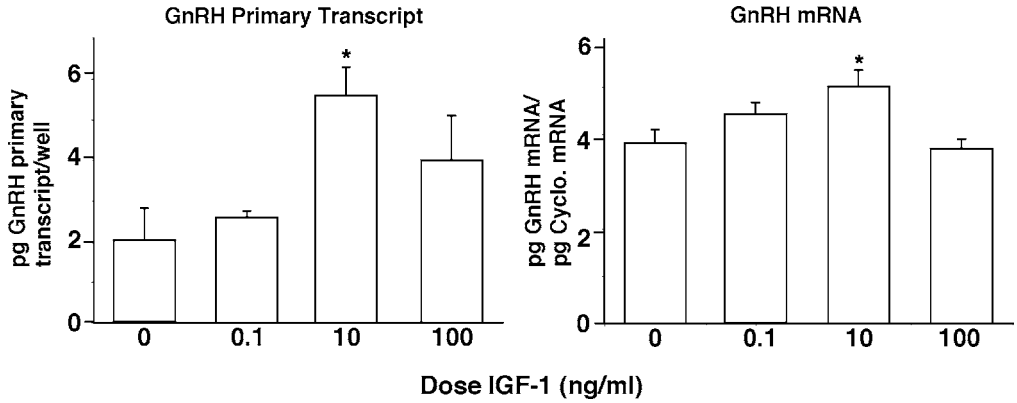

Figure 4. Dose-response analysis of effects of insulin-like growth factor–1 (IGF-1) on gonadotropin-releasing hormone (GnRH) primary transcript RNA (left panel), an index of gene transcription, and on GnRH mRNA levels (right panel) in GT1-7 cells treated for 24 hrs. Insulin-like growth factor–1 (10 ng/ml) significantly stimulated (* $P < 0.05$) both GnRH mRNA and primary transcript compared with vehicle. Data shown for IGF-1 mRNA are normalized to cyclophilin (Cyclo.) mRNA; IGF-1 had no effect on cyclophilin mRNA levels. (Modified from Ref. 77).

causes severe reproductive defects, although these may be attributable to the IGF-2 system rather than IGF-1, because IGF-2 has a higher affinity for the IGFBP-6 (42). Transgenic mice expressing human IGFBP-1 also exhibit reproductive deficiencies, such as abnormal copulatory behavior, compromised pregnancies, and spermatogenesis abnormalities, as well as significantly elevated pituitary LH concentrations (64, 65). In summary, these studies demonstrate potential roles for IGFBPs on reproductive function. Moreover, reports of developmental changes in IGFBP levels, including the brain, suggest potential roles of these molecules in mediating regulatory effects of IGF-1 on neuroendocrine functions, including growth and reproduction.

IGF-1 Regulation of Reproductive Neuroendocrine Functions

IGF-1 Regulation of GnRH Neurons. In mammals, sexual maturation and reproductive function is critically dependent on the GnRH neurons (16). In most vertebrate species, the perikarya of the hypophysiotropic GnRH neurons are located in the POA-AH, septum, diagonal band of Broca, organum vasculosum of the lamina terminalis and other related regions (66). Like other hypophysiotropic neurons, GnRH axons project to the ME, where they secrete the decapeptide into the hypophysial portal blood to trigger the production of the gonadotropins, LH and FSH, which in turn stimulate the growth and release of sex steroids by the gonads. Sex steroid hormones, produced and secreted into the circulation by the gonads, act at their respective receptors that are located remotely in many major cells and tissues. They also feed back at the level of the brain, largely, although not exclusively, at the level of the hypothalamus, as well as at the anterior pituitary, to exert profound influences on neural development and neuroendocrine function.

The biosynthesis and release of GnRH are under the complex control of a number of excitatory and inhibitory neurotransmitters and neurotrophic factors that can act at several levels of the GnRH cell, directly on the perikarya of GnRH neurons (19, 67) and at any place along the dendrite or axon, including its terminals in the ME (68–70). In addition, neuroactive factors can act indirectly on GnRH neurons via other intermediary neurons and glia (21, 71). Among the many factors implicated in GnRH regulation, IGF-1 is implicated in GnRH and gonadotropin release, the onset of reproductive ability at puberty, and the control of reproductive function through adult life. Although the specific effects of IGF-1 (to stimulate, inhibit, or have no effect) vary among species and sexes, and depend on hormonal status, our general interpretation of the literature is that IGF-1 is usually excitatory to GnRH neurons and gonadotropes or has a biphasic effect. A summary of the literature on the biological actions of IGF-1 on GnRH follows.

In Vitro *Studies in GnRH Cell Lines.* The hypothalamic GT1 and NLT cells are immortalized hypothalamic GnRH-expressing cell lines that were created by driving expression of the SV40-T antigen by the rat (GT1) or human (NLT) GnRH promoter (72–74). These constructs were used to produce transgenic mice that developed GnRH-expressing tumors that were plated into the GT1 and NLT sublines. These cells, which express the GnRH gene and synthesize and release GnRH, have provided considerable insight into understanding the mechanisms of GnRH gene regulation, including regulation by IGF-1. Olson *et al.* reported that IGF-1 treatment of GT1-7 cells is strongly mitogenic, having a strong stimulatory effect on GT1-7 cell numbers but no effect on GnRH release (75). In contrast, another group reported that IGF-1 inhibits cell proliferation but slightly elevates GnRH mRNA levels in GT1-1 cells (76). It is possible that differences between these two reports are because of the use of different GT1 sublines or other technical differences, but both reports show that GnRH cell lines are responsive to IGF-1. Our laboratory found that IGF-1 (24-hr treatment) causes significant increases in GnRH mRNA levels in GT1-7 cells (Fig. 4), accompanied

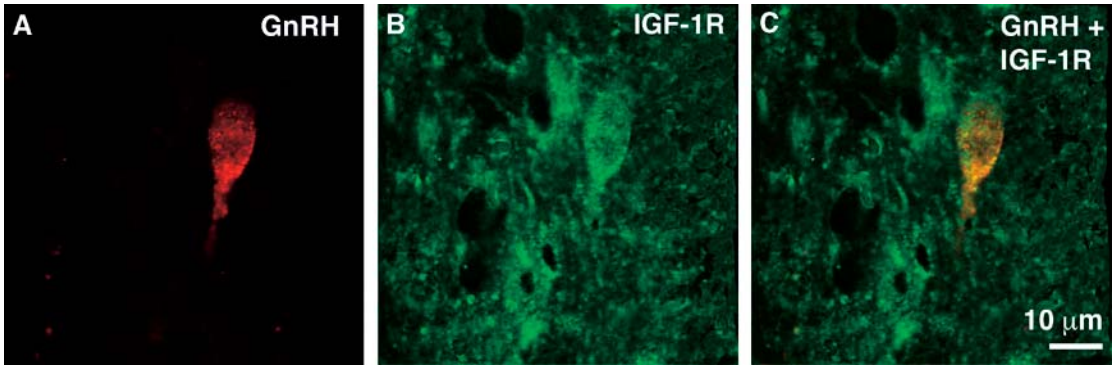

Figure 5. Confocal micrographs showing dual-label immunohistochemistry for (A) gonadotropin-releasing hormone (GnRH; shown in red, labeled with Alexa Fluor 594); (B) insulin-like growth factor–1 receptor (IGF-1R; shown in green, labeled with Alexa Fluor 488); and (C) a double exposure demonstrating that this GnRH neuron co-expresses IGF-1R. This cell was imaged from tissues of a representative P30 mouse. (Modified from Ref. 10).

by an increase in GnRH primary transcript RNA levels, an index of gene transcription (Fig. 4; Ref. 77). These results suggest that the elevation of GnRH mRNA by IGF-1 involves the stimulation of GnRH gene transcription. In our study, GnRH peptide levels in the medium were also measured, and the results showed that IGF-1 initially stimulates and then suppresses GnRH concentrations (77). This biphasic effect of IGF-1 on GnRH release was confirmed by another group (78). Together, these RNA and protein measurements indicate general stimulatory effects of IGF-1, with an uncoupling of GnRH biosynthesis (stimulatory) and secretion (biphasic).

In another GnRH cell line, NLT cells, IGF-1 signals via the IGF-1R and ras/raf/mitogen-activated protein kinase pathway to increase c-fos expression and mouse GnRH mRNA. Moreover, in NLT cells transfected with human GnRH promoters, IGF-1 significantly increases promoter activity (79). This overall stimulatory effect of IGF-1 on GnRH gene expression in NLT cells is similar to that reported for GT1-7 cells in our laboratory (77).

We have detected the presence of IGF-1 immunoreactivity in GT1-7 cells (77), and the presence of IGF-1 mRNA was reported by another group using RT-PCR in GT1-7 cells (78). In addition, binding studies show the presence of functional IGF-1 receptors in GT1-7 cells (75). Thus, the presence of both IGF-1 and the IGF-1R on GnRH cell lines suggests the potential for direct autocrine effects of IGF-1 within GnRH cell lines, and possibly on hypothalamic GnRH cells.

In Vitro *Hypothalamus.* Initial *in vitro* experiments showed that IGF-1 stimulates GnRH release from the rat ME (80), one of the brain regions with the highest concentration of IGF-1 receptors (8, 13, 41). In our laboratory, we tested effects of IGF-1 on GnRH gene expression in perifused POA-AH, a region containing GnRH cell bodies. Treatment of explants of P5, P30, and P60 mice for 1, 2, or 4 hrs with IGF-1 caused an overall stimulatory effect on GnRH mRNA levels in young immature hypothalamic explants (P5) and in explants from peripubertal mice (P30; Ref. 20). It is also notable that the rapid effects of IGF-1 on GnRH mRNA levels, occurring in 1 to 4 hrs, are coupled with significantly elevated GnRH primary transcript levels (20), suggesting that the IGF-1 stimulation of GnRH results from an increase in GnRH gene transcription (67), a result similar to that we found in the GT1-7 cell line (77). However, IGF-1 has no effect on GnRH gene expression in adult, reproductively mature mice (P60; Ref. 20). In summary, there is an age dependency of the effects of IGF-1 on GnRH neurons, with stimulatory effects on immature cells and on GnRH cell lines, but little effect on adult GnRH neurons. These results may relate to the important role of IGF-1 on puberty that is discussed below.

Effects of IGF-1 on GnRH and Gonadotropin Secretion In Vivo. Several *in vivo* studies in rats (24, 61, 80) have demonstrated the enhancement of GnRH and LH release following IGF-1 treatment in animals. For example, Hiney *et al.* (61) demonstrated that intraventricular administration of small doses of IGF-1 to immature juvenile or peripubertal rats increases plasma LH levels. This group also demonstrated that immunoneutralization of hypothalamic GnRH can inhibit the increase in LH released after the third ventricular injection of IGF-1, thus demonstrating *in vivo* that IGF-1 acts, via a centrally mediated mechanism, to stimulate LH release. Our laboratory tested effects of intracerebroventricular (icv) IGF-1 on GnRH gene expression in rats, and did not find any effect (55), suggesting that the effects of IGF-1 on GnRH release described above probably occur independently of *de novo* gene expression.

Expression of IGF-1 and IGF-1R Within GnRH Neurons. We recently demonstrated the presence of IGF-1 immunoreactivity specifically in GnRH neurons of rats and mice (20, 55). We performed dual-label immunofluorescence of IGF-1 and GnRH immunoreactivity in the hypothalamus of male and female mice (20). In P5 mice, approximately 18% of GnRH perikarya co-express IGF-1 immunoreactivity. This increases to 40% at P30 (peripubertal) and further increases to 78% co-expression in early adulthood at P60 (20). We saw similar co-expression of

IGF-1 in GnRH perikarya of adult female rats (55). To our knowledge, these reports in rodent species are the first to show colocalization of IGF-1 in GnRH cells *in vivo*, and they are consistent with reports showing IGF-1 immunoreactivity and mRNA expression in immortalized GnRH cell lines (77, 78).

Our laboratory also detected the presence of the IGF-1R on GnRH somata (Fig. 5; Ref. 10). Unlike our finding for IGF-1 within GnRH cells discussed above, the co-expression of IGF-1R specifically within GnRH neurons undergoes a significant *decrease* between the neonatal (P5) and peripubertal (P30) period. Overall, approximately 74%, 57%, and 59% of GnRH perikarya co-express the IGF-1R in P5 (neonatal), P30 (peripubertal), and P60 (young adult) male and female mice, respectively (10). The co-expression of the IGF-1R on GnRH neurons provides further evidence that IGF-1 may directly regulate GnRH neuronal function and is in agreement with observations in GnRH cell lines that express both IGF-1 and the IGF-1R (75, 77–79).

IGF-1 in Hypothalamic Neurons and Glia. Along with its expression within GnRH neurons, IGF-1 is expressed in hypothalamic neurons and glia that could regulate the GnRH system. For example, IGF-1 protein is expressed in the arcuate nucleus and ME, areas where GnRH neuroterminals traverse to regulate pituitary gonadotropin secretion (10, 55, 56). In the ME and circumventricular organs, we observed that IGF-1R immunoreactive processes and cells come in close proximity to, and appear to make contacts with, GnRH fibers and nerve terminals, although, from those analyses, we could not confirm whether the IGF-1R was localized within GnRH fibers or nerve terminals (10, 55). In these regions, IGF-1 immunoreactivity is observed in astrocytes and in specialized hypothalamic glial cells known as tanycytes. Tanycytes, whose cell bodies are located in the walls of the third ventricle and whose processes cross the arcuate nucleus and the ME, ensheath the axon terminals of GnRH neurons and modulate the secretion of GnRH into the portal capillaries (81). Interestingly, the accumulation of immunoreactive IGF-1 in the tanycytes appears to be caused by IGF-1 uptake from the blood or cerebrospinal fluid (82), and it increases during puberty in male and female rats, in contrast to an overall decrease with age in IGF-1 immunoreactivity in glial cells in other brain areas. Moreover, studies have revealed a sex difference in IGF-1 immunoreactivity, with adult females showing significantly lower numbers of labeled cells than males of the same age (56). The sex difference is abolished by early postnatal androgenization of females, suggesting that IGF-1 accumulation may be dependent on the perinatal burst of androgen production by the testis in developing male rats (56). Moreover, in females, the IGF-1 immunoreactivity fluctuates in concert with the various stages of the estrous cycle, with the highest levels observed on the afternoon of proestrus and morning of estrus compared with the morning of proestrus, diestrus, and metestrus (56). Additionally, IGF-1 peptide levels in this area decrease after ovariectomy and increase in a dose-dependent manner when the ovariectomized rats are treated with estrogen (56). Taken together, these results suggest that the glial tanycytes participate in the regulation of neuroendocrine events in adult rats by regulating the availability of IGF-1 to hypothalamic nerve terminals, and are influenced by the steroid hormonal environment.

IGF-1 Regulation of Gonadotropes. IGF-1 can also exert actions on the HPG axis via its effects on the pituitary gonadotropes (22–24). The anterior pituitary gland expresses IGF-1, the IGF-1R, and IGF-1BPs (32). Although, presumably, many actions of IGF-1 in the pituitary take place on the somatotrope (for review see Ref. 83), there is also evidence for effects on gonadotropes (for review see Ref. 15). For example, in a primary culture of anterior pituitary cells, IGF-1 significantly increases LH, FSH, and GnRH-stimulated gonadotropin secretion (22, 84). The gonadotropin-stimulating effects of IGF-1 are suppressed by administration of anti–IGF-1 and anti–IGF-1R antibodies (22), indicating that IGF-1 also acts at the level of the anterior pituitary to stimulate gonadotropin secretion. Thus, pituitary gonadotropes are also a site at which IGF-1 influences HPG function, probably via endocrine actions, either transported from the brain via the portal capillary vasculature, or from the general circulation after being released from the liver.

Influence of IGF-1 on Sexual Maturation and Puberty

In mammals, the initiation of puberty involves the complex interaction of hypothalamic, pituitary, and gonadal hormones. During this process there is an increase in the amplitude and frequency of GnRH pulses, which triggers a cascade of events including increases in the generation of FSH and LH pulses, followed by marked increases in gonadal sex steroidal output. Moreover, during puberty, serum GH release, including GH pulse amplitude, increases 2- to 3-fold from the onset of puberty, and this increase correlates with increases in IGF-1 production (85) and serum estradiol concentrations. Thus, puberty is a developmental period when IGF-1 may play a dual purpose: the regulation of somatic growth, coordinated with the attainment of reproductive maturity.

In mammals, circulating levels of IGF-1 change during key points of the reproductive life cycle. In serum, IGF-1 levels increase during puberty in rodents (86, 87); ruminants (88); and primates (85, 89), including humans (90), consistent with the possibility that the elevated levels of IGF-1 may initiate or accelerate the onset of puberty. In support of a causal role played by IGF-1 on puberty, exogenous, chronic administration of IGF-1 to prepubertal rats advances the onset of puberty (61). In primates, administration of IGF-1 prepubertally significantly advances the age at first ovulation and decreases the interval between menarche and first ovulation (91). However, it is not possible to ascertain from these studies whether the actions

of IGF-1 on puberty are caused by central or peripheral mechanisms and sites of action, or both.

With respect to peripheral IGF-1, Hiney and Dees reported that levels of two spliced forms of IGF-1 (IGF-1a and IGF-1b) increase in the liver at puberty, during the first proestrus (61). This change is accompanied by an elevation in serum levels of IGF-1 during the late proestrus phase of puberty. Our laboratory has focused on central IGF-1. As discussed earlier and therefore summarized only briefly here, we found a peak in IGF-1 mRNA levels in the POA-AH and MBH-ME of mice at P5 and P0, respectively, and thereafter IGF-1 mRNA levels undergo a second postnatal developmental increase and peak at adulthood (Fig. 1; Ref. 20). Ye *et al.* also reported a peak of gene expression in the diencephalons of reporter-transgenic mice at P5 (52). This first neonatal week of life is particularly important to reproductive neuroendocrine development. Not only is it associated with substantial amounts of neurogenesis, synaptogenesis, and gliogenesis, but it is also a critical period for sexual differentiation of the brain in rodents (92, 93). The later peak of IGF-1 mRNA in neuroendocrine regions corresponds to the pubertal period of development (17). Overall, the developmental pattern of IGF-1 gene expression in POA-AH is remarkably similar to that of GnRH release, which undergoes a transient increase neonatally, decreases during the prepubertal "hiatus", and then increases again during pubertal maturation (17, 94, 95). Although we do not know whether there is a causal relationship between the peaks of IGF-1 and GnRH, the parallel timing of these processes is certainly consistent with this possibility.

In contrast, a study by Gruaz *et al.* indicated that it is unclear whether this acceleration of IGF-1 secretion during the juvenile period affects the course of sexual maturation (96). Constant infusion of IGF-1 by Alzet minipumps was given to female rats beginning on P20 (prepubertal), to prematurely raise serum levels to normal adult levels or to supraphysiologic levels. Although IGF-1 affected somatotropic parameters, such as body weight, it did not alter the onset of sexual function. Moreover, inhibition of GH secretion by passive immunization of rat GHRH markedly reduced IGF-1 secretion, which produced a delay in growth, but no significant effects on the timing of sexual maturation and fertility (97). This lack of effect on sexual maturation is suggestive that the actions of IGF-1 are critically dependent on steroid hormone environment and developmental age, as suggested by studies in our laboratory discussed earlier in this review (20). Moreover, the pattern of IGF-1 may play a role, and constant infusion may not mimic physiologic release. Clearly, future research is necessary to better understand the inhibitory and stimulatory actions of central IGF-1 on pubertal maturation.

Interactions of IGF-1 and Estrogen

Effects of IGF-1 are modulated by levels of gonadal steroid hormones (25, 98, 99). In various reproductive tissues and cell types such as the breast, uterus, endometrium, hypothalamus, and ovarian cancer cells, estrogen can regulate the expression of IGF-1, IGFBPs, and IGF-1R proteins and genes (100–106). Conversely, IGF-1 regulates the expression and function of estrogen receptors (107, 108). Such interactions or cross-talk, either additive or synergistic, between IGF-1 and estrogen are seen at the hypothalamic level (56, 104, 109, 110). Using primary cultures from rat fetal hypothalamus, Toran-Allerand *et al.* (111) first demonstrated that IGF-1 has a synergistic action with estrogen on the induction of neuritic growth. Subsequent studies have shown that estrogen stimulates and controls IGF-1R activity and IGF-1BPs in developing hypothalamic cultures (104), and that both estrogen and IGF-1Rs are required to stimulate the differentiation of neurons (109, 112). Duenas *et al.* (112) studied the effects of IGF-1 on neuron survival and neurite outgrowth in cultures in the presence or absence of IGF-1 and found that, although both estrogen and IGF-1 increase neuron survival and neurite outgrowth, the effect of the two factors is not additive, suggesting that they both act through the same signaling mechanism/pathway in those tissues. This group also incubated the cultures with an IGF-1 antisense oligonucleotide, and found a significant decrease in survival rate and neurite outgrowth, suggesting that IGF-1 is required for the effects of the sex hormones and that the mechanism by which estrogen may induce neuronal survival and differentiation is by activating an IGF-1 signaling pathway. Although there are several explanations for this interaction, we believe this is beyond the scope of this review and recommend readers to some excellent reviews (113, 114).

Studies *in vivo* also support interactions between IGF-1 and estrogen. This is pertinent to puberty, during which circulating concentrations of these hormones increase dramatically. One study reported differences among animals in the level of IGF-1–induced LH release in pubertal rats; the authors were able to attribute these differences to the varying levels of estrogen between animals (24). In adolescent primates, chronic IGF-1 administration lowers the negative feedback effects of estrogen on LH release, thereby advancing the onset of puberty (115). All of these studies demonstrate a facilitation of the effects of IGF-1 on neuroendocrine systems by hormones, particularly estrogen. In adult rats, the IGF-1R and the estrogen receptor colocalize in neurons and astroglia in several areas of the brain, including the POA-AH, indicating that IGF-1 and estrogen may interact within the same target cells (11). Fernandez-Galez *et al.* (110) reported that IGF-1–mediated synaptic remodeling fluctuated in rats across the estrous cycle. These changes in synaptic connections and glial-neuronal contacts across the estrous cycle are regulated by estrogen (110, 116). Furthermore, *in vivo* studies using icv infusion of specific receptor antagonists indicated that both the IGF-1R and estrogen receptor are required for these synaptic and glial plastic modifications that occur during the estrous cycle (110, 116). Subsequent studies by Quesada and Etgen (117,

118) have demonstrated functional interactions between estrogen and IGF-1R in the hypothalamus of adult rodents. Estrogen treatment induces α_{1B}-adrenergic receptor expression in the hypothalamus and preoptic area, and this effect is blocked by icv infusion of the IGF-1R antagonist, JB1. Furthermore, estrogen potentiates the effects of IGF-1 on α_1-adrenergic receptor activation in the preoptic area and hypothalamus, whereas blockade of IGF-1R during estrogen priming blocks the estrogen-induced LH release and partially inhibits hormone-dependent reproductive behavior (118). The authors propose that the interaction of estrogen with IGF-1R may help coordinate the timing of ovulation with the expression of sexual receptivity.

Taken together, these studies indicate positive interactions of estrogen and IGF-1 on the regulation of reproductive function, with implications for puberty and the maintenance of adult reproductive function. All levels of the reproductive system are potential targets of this interaction, making it difficult to determine which level of interaction is primary. Nevertheless, it is becoming increasingly clear that the central nervous system, including those factors regulating GnRH neurons, is impacted by interactions between IGF-1 and estrogens.

Reproductive Neuroendocrine Functions Are Compromised in Somatotropic System Transgenic or Knockout Mice

Genetic studies have generated mice that express various foreign GH or IGF-1 genes or that are deficient in the GH, IGF-1, or IGF-1R gene. These transgenic animals have provided insight into the importance of IGF-1 on reproductive functions. Because so many IGF-1–related or GH-related knockout and transgenic mice have been produced to date, we will only provide a select and brief overview of reproductive phenotypes in this article. We recommend our readers to other recent reviews of the literature for further details (119, 120).

Ames dwarf mice, which are deficient in GH/IGF-1 secretion, are sterile (121). Growth hormone treatment during the postnatal development results in normalization of altered reproductive parameters through induction of IGF-1 secretion, which is accompanied by enhanced serum LH levels, which increase to normal levels. In female Ames dwarf mice, the plasma LH response to GnRH treatment, which is lower in the mutants, is also normalized by GH treatment. However, Ames dwarf mice are also deficient in prolactin and thyroid-stimulating hormone. Therefore, it is important to consider that some of these effects that are observed in Ames dwarf mice might be caused by the absence of prolactin and/or thyroid-stimulating hormone secretion. Nevertheless, this mouse model suggests a deficiency of HPG function in the absence of GH/IGF-1, and the ability to rescue reproductive function with somatotropin replacement. This is strong support for IGF-1 and/or GH acting as a link between growth and reproduction.

Further evidence for a role of IGF-1 in puberty and reproductive function is provided by GH receptor/binding protein knockout mice (GHR-KO; Ref. 122). Although the secretion of GH is elevated in these mice because of the lack of GH receptors, GH-dependent IGF-1 production is prevented. Danilovich *et al*. demonstrated that although fertility is reduced in GHR-KO mice, it is not totally suppressed. The female GHR-KO mouse undergoes delayed vaginal opening, a marker of puberty (123), compared with her normal siblings (122). IGF-1 treatment is able to mitigate this delay. The reduction in peripheral IGF-1 levels also causes alterations in fetal and placental growth, delay of parturition, reduced litter size, and increased time between pregnancies (122, 124). In adult male GHR-KO mice, although the basal plasma LH levels are similar relative to the normal siblings, the LH response to GnRH treatment is significantly reduced, indicating that the absence of IGF-1 alters the effect of GnRH on LH secretion (125). In addition, the percentage of GHR-KO male mice impregnating females is significantly lower than that of wild-type mice (125). Although the mechanism responsible for the maintenance of fertility in GHR-KO mice is unknown, the results indicate that reproductive neuroendocrine function in GHR-KO female mice is significantly impacted, and that this probably involves all three levels of the HPG axis.

IGF-1 knockout mice have also provided insight into a link with the reproductive axis. Whereas the heterozygous mice are healthy and fertile, the homozygous IGF-1 knockouts almost always die at birth, and the few that survive are infertile and have profound developmental and reproductive defects (126, 127). Insulin-like growth factor–1 receptor knockout mice never survive beyond birth (120). Conditional knockouts and mice that overexpress IGF-1 genes have also been produced, several of which have reproductive phenotypes. Using the Cre/loxP system, LeRoith's laboratory (44) produced conditional knockout mice that lack IGF-1 specifically in the liver. In these animals, serum levels of IGF-1 are decreased by approximately 70%. These animals exhibit no developmental defects and are fertile, suggesting that the residual IGF-1 is sufficient to maintain normal function, or that liver-derived IGF-1 may be relatively unimportant in regulating reproductive function (128). This strengthens the importance of the role of *central* IGF-1 in the development and maintenance of normal reproductive physiology.

Proposed Model of Central Regulation of the HPG Axis by IGF-1

In this review we have highlighted the significance of central IGF-1 in the control of reproductive neuroendocrine functions. Both *in vitro* and *in vivo* observations indicate that IGF-1 has a modulatory role in the reproductive axis and that it may represent one of the signals that link somatic development to the reproductive system in a development-

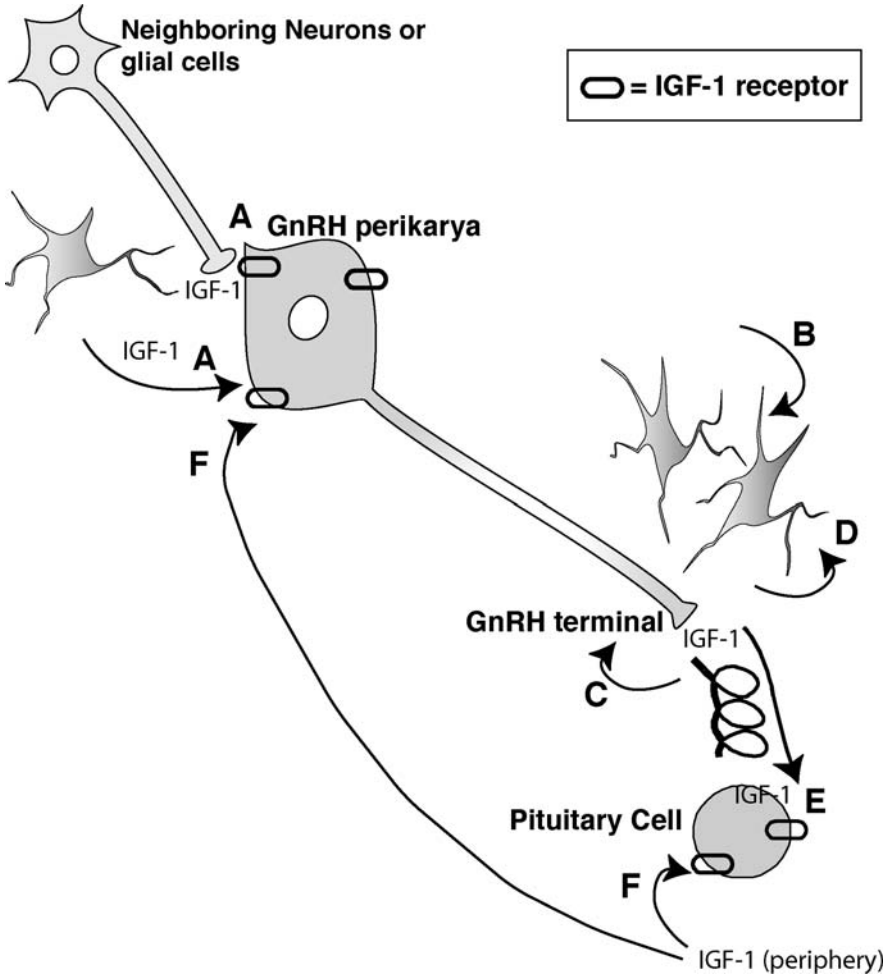

Figure 6. Model for actions of central insulin-like growth factor–1 (IGF-1) and its relationship to gonadotropin-releasing hormone (GnRH) neurons. (A) At GnRH perikarya: IGF-1 from neighboring hypothalamic neurons and glial cells can act directly via IGF-1 receptors (IGF-1Rs). (B) At GnRH neuroterminals: IGF-1 from neighboring hypothalamic neurons or glial cells can act on tanycytes, which undergo structural modifications, affecting GnRH release. (C) From GnRH neuroterminals to the median eminence (ME): IGF-1 released from GnRH terminals can exert an autocrine effect on the GnRH terminals from which it was released. (D) From GnRH neuroterminals to the ME: IGF-1 released from GnRH terminals can exert a paracrine effect on neighboring cells. (E) From GnRH neuroterminals to the pituitary gonadotrope: IGF-1 released from GnRH terminals can travel through the portal capillary vessels to exert an endocrine effect on pituitary gonadotropes or other pituitary cells. (F) IGF-1 from the periphery can act on IGF-1Rs on pituitary gonadotropes or hypothalamic loci.

and hormone-dependent manner. These effects of IGF-1 can be exerted at all three levels of the HPG axis. At the ovary, IGF-1 can exert local paracrine effects, because it is synthesized in ovarian tissues (5, 129). Circulating IGF-1 can also act on IGF-1 receptors in the ovary (7), thereby exerting endocrine actions. In the pituitary, which expresses IGF-1 receptors (8, 14, 84), IGF-1 serves the dual process of downregulating pituitary somatotrope activity and influencing gonadotrope function, probably via endocrine actions. In the hypothalamus, IGF-1 is again a regulator both of the somatotropic axis (via effects on GHRH and somatostatin neurons) and the reproductive axis (via direct and indirect effects on GnRH neurons). Because IGF-1 in the brain can arise from central or peripheral sources, these IGF-1 actions are autocrine, paracrine, or endocrine. Thus, there are multiple levels of the HPG axis at which IGF-1 can act to coordinate reproduction with growth.

That the neuroendocrine hypothalamus in general, and GnRH neurons in particular, express both IGF-1 (20, 55) and the IGF-1R (10) suggests several mechanisms by which IGF-1 could affect reproductive function at this level. In addition, the pituitary gonadotropes are responsive to IGF-1, which can originate from the brain or the periphery. We propose that, depending on the developmental and hormonal state of the animal, IGF-1 may exert the following actions that are not mutually exclusive (summarized in Fig. 6): 1). Neurons or glia in the brain that synthesize and release IGF-1 can act on GnRH perikarya and alter GnRH biosynthesis and/or release (Fig. 6A). In the brain, IGF-1 can act on tanycytes, specialized glia in circumventricular regions, including the ME, and alter the regulation of GnRH neuroterminals by tanycytes, hence affecting access of GnRH to the portal capillary vasculature (Fig. 6B). Central IGF-1 may be released by GnRH neurons, and act on these

originating cells by an autocrine mechanism, either to regulate GnRH gene expression (i.e., biosynthesis) or to cause a modification of the GnRH neuroterminal from which it was released (Fig. 6C). This model of autocrine regulation would be supported by the presence of the IGF-1R on the GnRH nerve terminal, which has not yet been confirmed but which we are actively investigating. Insulin-like growth factor-1 in the ME, released from GnRH terminals or locally synthesized in glia, may act in a paracrine manner, by exerting effects on other neuroterminals in the ME (Fig. 6D). For example, IGF-1 in the ME could act directly on GHRH and somatostatin nerve terminals by this type of paracrine mechanism. Insulin-like growth factor-1, released into the ME from neurons and/or glia, could be taken up into the portal capillaries and transported to the anterior pituitary gland, affecting gonadotropes and/or other cells such as somatotropes, thereby exerting an endocrine effect (Fig. 6E). Finally, peripheral IGF-1 can act on pituitary gonadotropes, or on cells in the hypothalamus or the ME that are particularly permeable to the blood-brain barrier, to exert effects on HPG function (Fig. 6F). By this cross-talk, IGF-1 can serve the important function of coordinating reproductive function and somatic growth.

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