

The role of insulin-like growth factor-I in neuroendocrine function and the consequent effects on sexual maturation: inferences from animal models

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SUMMARY

It is known that growth hormone (GH) plays an important role in growth and development. Additionally, emerging evidence suggest that it also influences hypothalamic-pituitary-gonadal function. We have found that GH from different species has different effects in mice. In rodents, human GH (hGH) binds to both GH and prolactin (PRL) receptors; it has both somatotrophic and lactotrophic effects. Since PRL has a profound effect on neuroendocrine function, the results obtained from hGH treatment or from transgenic animals expressing the hGH gene reflect PRL-like effects of this hormone. However, bovine GH (bGH) is purely somatogenic and therefore the effects of bGH represent the function of the natural GH produced in rodents. Furthermore, our studies in mice and rats have shown that not all effects of GH are stimulatory and the duration of exposure of

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the hypothalamo-hypophyseal-gonadal system to GH might influence the secretions of gonadotropins and gonadal steroids. In humans, excess productions of GH in acromegaly and GH resistance in Laron syndrome adversely affect reproduction. Similarly, it has been demonstrated that in transgenic mice expressing various GH genes, in insulin-like growth factor-I (IGF-I) gene-knockout mice, in GH receptor gene-disrupted (GHR-KO) mice, and in Ames dwarf mice the onset of puberty and/or fertility is altered. Therefore, excess or subnormal secretion of GH can affect reproduction. We have shown that the hypothalamic-pituitary functions are affected in transgenic mice expressing the GH genes, Ames dwarf mice and in GH receptor gene knockout mice. The majority of the GH effects are mediated via IGF-I and the aforementioned effects may be due to the GH-induced IGF-I secretion or due to the absence of this peptide production. It is important to realize that the syntheses and actions of IGF binding proteins are controlled by IGF-I. Furthermore, some IGF binding proteins can inhibit IGF-I action. Therefore, the concentrations of IGF binding proteins and the ratio of these binding proteins and IGF-I within the body might play a pivotal role in modulating IGF-I effects on the neuroendocrine-gonadal system. *Reproductive Biology* 2003 3 (1): 7-28.

Key words: transgenic mice, gene disrupted mice, growth hormone, insulin-like growth factor-I, pituitary, ovary, testis, puberty, reproduction

INTRODUCTION

It is known that gonadotropin-releasing hormone (GnRH) is the key regulator of the reproductive system, directly regulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which are essential for the normal function of the gonads. Biosynthesis and release of GnRH are under complex excitatory and inhibitory control by a number of neurotransmitters and neurotrophic factors. In addition, there are a number of paracrine factors, including GH and IGF-I, which can also modify the GnRH synthesis and action on the pituitary gonadotrophs. Here, we have attempted to review the evidence for the influence of GH/IGF-I on neuroendocrine and gonadal functions. Although the role of GH in growth and

development is well established, GH influence on pituitary and gonadal functions is poorly understood.

Growth hormone secretion is controlled by a complex system. GH secretion is under the control of two hypothalamic hormones; GH-releasing hormone (GHRH) and GH-release inhibiting hormone (somatostatin). These hypothalamic peptide hormones exert stimulatory and inhibitory influences respectively on the synthesis/release of GH by the pituitary gland. A host of neurotransmitters, other hypothalamic neuropeptides, and hormones including GH and IGF-I and their binding proteins regulate the syntheses and action of GHRH and somatostatin.

There are data to support the direct action of GH, but the majority of the physiological effects of this hormone are via IGF-I. The liver, in response to GH action synthesizes the majority of IGF-I. However, other organ systems, particularly brain and gonads, have the capacity to synthesize IGF-I. The synthesis of gonadal IGF-I might be under the control of LH. In addition to IGF-I, IGF-II is also produced in the mammalian system. IGFs are polypeptides. The human IGF-I is made up of 70 amino acids, has 50% amino acid sequence homology with insulin and 70% homology with IGF-II [69]. Both IGF-I and IGF-II have A and B chains that are linked by disulfide bonds. The IGF system includes ligands (IGF-I and IGF-II) their receptors, binding proteins (IGFBP-1 to IGFBP-6) and IGFBP proteases. It is known that IGFBPs can increase the half-lives of IGF-I and IGF-II, as well as they can potentiate or inhibit IGF-I and IGF-II actions. Thus the IGF system can influence pre- and postnatal growth and supports cell function via paracrine, autocrine and endocrine signaling. Recent experimental evidence suggests that IGF-I plays a role in the control of gonadotropin, PRL and gonadal steroid secretions. We are addressing these issues in this review article.

INDIRECT EVIDENCE FOR IGF-I EFFECTS ON PITUITARY AND GONADAL FUNCTIONS

Clinical studies have suggested a role of GH/IGF-I in human reproduction. It has been shown that in infertile women with hyposensitive ovaries, GH administration increases ovarian sensitivity to gonadotropin [41, 42]. GH

treatment increases human chorionic gonadotropin (hCG)-induced progesterone secretion by isolated human luteal cells [53] and in some cases, anovulation is associated with subnormal GH secretion [66, 76]. Excess GH secretion can also have an effect on reproduction. In acromegalic women the normal menstrual cycles are altered, possibly due to alterations in gonadotropin and PRL secretions [31].

In experimental animals, a large amount of evidence related to the role of IGF-I comes from the data obtained from GH treatment and the consequent increase in IGF-I secretion. It has been suggested that there is a relationship between GH/IGF-I and the reproductive system. In female rats, suppression of GH secretion delays puberty and reduces ovarian LH receptors [3, 68] while GH treatment augments the follicle-stimulating hormone (FSH)-induced differentiation of ovarian granulosa cells [44]. In FSH-deficient mice, although the FSH β subunit is absent, ovarian follicles develop up to preantral stage [51]. Furthermore GH, in the absence of FSH, can induce folliculogenesis in isolated ovarian preantral follicles of immature mice, strongly suggesting a role of GH in the control of ovarian function [59].

Childs and co-workers have shown that there is a relationship between GH and gonadotropin-secreting cells within the pituitary gland. GH antigens are present in pituitary cells containing FSH or LH mRNAs and in cells containing GnRH receptors indicating that either GH cells are transitory gonadotrophs, or GH is present in these pituitary cells most likely assisting to control their function [26, 27]. In addition, GH-binding protein antigens were identified in pituitary cells that contained LH and FSH, indicating a possible paracrine effect of GH in the control of the gonadotrope functions [37]. Therefore, GH may function as a "co-gonadotropin" [26, 27, 43]. It has been suggested that somatotropes may be converted to transitional gonadotropes just before proestrus. The LH and FSH antigen content of the GHRH target cells from proestrus rats indicates that the LH β and FSH β mRNAs are translated [28]. Furthermore, expression of PRL and gonadotropins in these cells indicates that these convertible somatotropes may also be somatomammotropes [28]. It has been shown in rats that at diestrus, proestrus, or estrus, GH mRNA was expressed in 50-57% of cells with LH β and FSH β antigens [29], suggesting that a multihormonal cell system

may function to help support the regulatory functions of the gonadotrope during the periovulatory period. Furthermore, female GH-deficient Ames and Snell dwarf mice have delayed puberty and reduced fertility and their pituitary functions are altered [8, 19, 34]. These studies suggest that there is an important interrelationship among somatotropes, gonadotropes and lactotropes in female rodents.

As in females, alterations in GH secretion also affect male reproduction. It has been shown that the genital organs are poorly developed in GH-deficient boys and GH treatment increases the growth of the genitalia [72]. In men, congenital GH resistance due to mutated GH receptors (Laron syndrome) is associated with a delay in sexual maturation [56, 57]. Also combined treatment with GH and gonadotropin in infertile men increased serum testosterone levels and normalized sperm parameters [73]. In these men, treatment with gonadotropins alone had no effect on these parameters.

In the experimental animals, a limited number of studies have shown that GH can influence pituitary and testicular functions. Treatment of hypophysectomized rats with GH increases the LH receptor content of the testis [88] and increases the testicular responsiveness to gonadotropin treatment [77]. In adult rats, a lack of GH secretion results in a delay in testicular growth and differentiation of germinal cells [4]. Although GH-deficient dwarf rats are fertile, their testes are small and the sperm motility is impaired [36, 75]. GH or IGF-I treatment increases motility of immature spermatozoa in GH-deficient dwarf rats [12, 83], suggesting the importance of GH and IGF-I in male reproduction. Biological neutralization of the endogenously secreted GH by active immunization against GH or administration of GH, results in alterations in neuroendocrine function in rats [20]. These studies suggest that GH plays a role in the control of neuroendocrine and reproductive functions in the male. These GH effects are most likely due to its effect on liver and other tissues to stimulate the production of IGF-I and the effects observed might have been due to IGF-I action.

We hasten to acknowledge that there are controversies related to the influence of GH on pituitary gonadotropic and testicular functions. Administration of GH to GH-deficient young adult males resulted in significant increases in total and free IGF-I levels in all patients. However, in these

individuals there were no significant effects on the pituitary gonadotropin response to GnRH, as well as basal and hCG-stimulated levels of androgens [45], indicating that the effects of GH treatment do not appear to cause major alterations in the pituitary-gonadal axis. Furthermore, in baboons, the treatment with GH or IGF-I did not alter gonadotropin stimulation of testicular function [30]. In cynomolgus monkeys, a long-term treatment with GH did not alter spermatogenesis [74], and suppression of GH by active immunization against GHRH failed to affect ongoing spermatogenesis in rats [5].

Transgenic animals expressing various foreign GH genes are good experimental candidates for evaluation of the impact of this hormone on neuroendocrine and reproductive functions. In addition, mice bearing various GH genes with the mouse metallothionein-I promoter (MT) express the foreign GH in multiple organs including liver, kidney, intestine, skin, gonads and pituitary gonadotrophs with expressions starting during fetal development and continuing throughout the entire lifespan. However, GH transgenic mice with phosphoenolpyruvate carboxykinase promoter (PEPCK), the expression of the gene starts around the time of birth and is limited to kidney and liver. Furthermore, the foreign GH genes fused to either a MT or PEPCK promoter are not subjected to the control mechanisms that normally regulate the synthesis and release of GH from the pituitary gland. In the MT-GH transgenic animals, the expression of the MT-GH gene can be controlled by heavy metal ions. Providing MT-GH transgenic mice with $ZnSO_4$ in drinking water can enhance GH gene expression. MT-GH gene may also be activated by treatment with dexamethasone. In PEPCK-GH mice, a high-protein diet can stimulate the transgene expression, while its expression is reduced by a high-carbohydrate diet. We have found that mice with the MT-GH gene secrete moderate amounts of the GH, while animals with the PEPCK-GH gene produce large amounts of GH [10, 11]. Therefore, these GH transgenic mice with two different promoters are interesting animal models to assess the effects of moderate or high amounts of the foreign GH. In male transgenic mice bearing the hGH gene with the MT promoter (MT-hGH), the plasma LH levels were increased [18, 21, 22] and LH response to castration and to testosterone treatment were attenuated [21]. In these MT-hGH mice, the expression of LH β mRNA in

the pituitary gland was increased [78]. These changes are most likely due to the PRL-like activity of hGH [70] and resemble the results observed in experimental induction of hyperprolactinemia in mice [48].

In a line of MT-bGH transgenic mice with a moderate level of hGH secretion, the plasma LH levels at estrus in transgenic females were elevated, while FSH levels were decreased relative to their normal siblings [23]. The ovariectomy-induced rise in circulating gonadotropin levels was attenuated in these transgenic mice. However, plasma FSH levels in ovariectomized, estradiol-treated transgenic mice were significantly decreased. In addition, FSH and LH responses to GnRH were significantly reduced in ovariectomized-estrogen primed mice bearing the MT-hGH gene [23]. It has been shown that these female mice are sterile, possibly due to the stimulation of the hypothalamic tuberoinfundibular dopaminergic neurons resulting in suppression of mating-induced PRL release associated with luteal failure [9]. Treatment of pregnant mice secreting hGH with PRL by PRL-secreting ectopic pituitary transplants lead to normal pregnancies [9]. Furthermore, female mice expressing hGH gene with PEPCK promoter secrete large amounts of hGH and reproduce normally [62]. The absence of an effect of very high levels of hGH secretion on reproduction in these mice is possibly due to the effective replacement of the suppressed PRL secretion and the PRL-like effect of hGH in rodents [70]. These data indicate that moderate secretion of the foreign hGH alters hypothalamic-pituitary function and affects reproduction in female mice.

We have examined the effects of excess bGH (unlike hGH, bGH has only somatogenic effect) secretion on reproduction in transgenic mice expressing the bGH gene with MT or PEPCK promoter. Transgenic mice bearing the PEPCK-bGH gene secrete high levels of bGH compared to mice expressing the MT-bGH gene [11]. Most of the female mice from MT-bGH line are fertile and their reproductive parameters are nearly normal except for the absence of gestations from postpartum estrus and the significant increase in the average interval between pregnancies [65]. In mice bearing the MT-bGH gene, GnRH-induced FSH secretion was attenuated whereas the LH responses were similar to those in controls [19]. The ovariectomy-induced increases in plasma FSH and LH levels were decreased in this line

of transgenic mice. The absolute circulating FSH levels were also reduced in estrogen-treated transgenic mice, while plasma LH level were similar in normal mice and in mice bearing the MT-bGH gene. Ovariectomized transgenic mice from this line were hyperprolactinemic [19]. High levels of bGH secretion in female mice bearing the PEPCK-bGH gene was associated with high incidence of pregnancy failure [15]. This gestation failure was most likely due to luteal deficiency [16]. The luteal failure in these mice is attributed to the failure of stimuli associated with mating to induce the normal pattern of two daily PRL surges [16]. However, treatment of mated PEPCK-bGH mice with progesterone, PRL, or a dopaminergic antagonist that releases PRL was associated with maintenance of pregnancy [14]. When normal pregnancies are carried to term in small percent of PEPCK-bGH mice, litter size was significantly increased, relating to the increased ovulation rate in these animals. It has been shown that the percentage of ovarian follicles containing apoptotic cells was lower in transgenic mice bearing the PEPCK-bGH gene than in normal mice [33]. The percentage of follicles undergoing apoptosis was lower in these transgenic mice than in control animals in preovulatory and early antral follicles. The percentage of healthy preovulatory follicles was also higher in transgenic mice relative to normals. These results indicate that GH overexpression in PEPCK-bGH mice significantly decreases follicle apoptosis and atresia in the mouse ovary, leading to increased rate of ovulation in these transgenic mice [33].

Male mice expressing the MT-bGH gene are generally fertile. It has been shown that the plasma gonadotropin, PRL, and testosterone levels were similar in these transgenic mice related to their normal siblings¹. In addition, despite reductions in pituitary FSH β mRNA and LH β mRNA levels [79], PEPCK-bGH male mice produced normal spermatozoa and were fertile [10]. Therefore, excess bGH secretion seems to have little or no significant effect on male reproduction in mice.

The ability to control the expression of the GH gene would be a useful tool in evaluating the role of GH in biological systems. Such an animal

¹Chandrashekar V, Bartke A, Wagner TE 1989 Effects of gonadotropin-releasing hormone on luteinizing hormone and testosterone secretion in male transgenic mice with bovine growth hormone gene expression. The Endocrine Society 71st Annual Meeting, Seattle, WA, #234, abstract.

model is now available. In transgenic mice bearing the ovine (o) MT 1a-oGH fusion gene (oMT1a-oGH), oGH is not expressed in animals fed a standard laboratory mouse diet and tap water, but can be stimulated by providing ZnSO₄ solution for drinking [71]. Therefore, this transgenic model is valuable experimental animal to stimulate or inhibit the production of the heterologous GH by simply providing or withholding the consumption of ZnSO₄. Transgenic female mice expressing oMT1a-oGH fusion gene can cycle, mate, and support early embryonic development; but they fail to maintain pregnancy due to luteal insufficiency [71]. Treatment of these mice with progesterone resulted in maintenance of pregnancy and normal lactation [67]. Most recently it was shown that activation of oMT1a-oGH gene for 8 weeks resulted in lower pregnancy rate associated with increased corticosterone secretion [80]. It has been suggested that increased corticosterone secretion in mice with activated oMT1a-oGH gene impairs implantation and corticosterone may induce leptin resistance causing impaired fertility in these transgenic mice [80].

We have also assessed the influence of endogenously secreted, homologous mouse GH on neuroendocrine and gonadal function in mice. Transgenic mice expressing the human GH-releasing hormone (hGHRH) gene produce large amounts of GH¹. [61]. The source of GH is from the in situ pituitary gland and the pituitary responds to increased secretion of hGHRH. Because of increased synthesis and release of this hypothalamic peptide, these mice secrete high levels of IGF-I¹. The testicular weights were significantly increased in mice expressing the hGHRH gene. Although the basal LH levels were similar in transgenic mice bearing the hGHRH gene and in their normal siblings, the GnRH-induced LH response was attenuated in these transgenic mice. Excess secretion of GH/IGF-I resulted in significant increases in testosterone levels. The increased testosterone secretion might have been due to the excess GH/IGF-I secretion which might have increased the sensitivity of the Leydig cells of the testis to LH action in these transgenic mice¹. It has been demonstrated that the number of lactotropes in the male

¹Chandrashekar V, A. Bartke 1997 Pituitary and testicular function in adult transgenic mice expressing the human growth hormone-releasing hormone gene. Society for the Study of Reproduction 30th Annual Meeting, Portland, OR, #462, abstract.

hGHRH animals was increased two fold. However, their plasma PRL levels were not changed [64]. This is due to the increased hypothalamic dopamine synthesis and release, with an increase in D₂ dopamine receptor gene expression and functional sensitivity of the pituitary gland [64]. Expression of the hGHRH gene also resulted in decreased LH β mRNA levels in the pituitary glands [64]. However, the total LH β mRNA levels per pituitary gland were significantly higher in transgenic mice expressing the hGHRH gene relative to normal mice.

NEUROENDOCRINE AND GONADAL FUNCTIONS IN IGF-I-DEFICIENT MICE

In GH-deficient Snell dwarf mice, the testicular weight, seminiferous tubular diameter and germ cell number are reduced [60]. However, GH treatment during the postnatal development resulted in normalization of these reproductive parameters. Ames dwarf mice are also deficient in GH/IGF-I secretion [17]. Administration of bGH to male Ames dwarf mice induces IGF-I secretion accompanied by enhanced plasma LH levels [17]. The effect of GnRH on LH secretion was increased in bGH-treated dwarfs, but this LH response was lower than in normal siblings that previously received the vehicle [17]. Pretreatment of these mice with GH resulted in increased production of androstenedione and testosterone by the isolated testes treated with hCG. Thus, it can be concluded that due to the absence of GH/IGF-I secretion, the hypothalamic-pituitary-testicular function was altered in male Ames dwarf mice.

In female Ames dwarf mice, the plasma LH response to GnRH treatment was decreased and GH treatment normalized this response [19]. The negative feedback effect of estrogen on LH secretion was decreased in these dwarf mice. These results suggest that GH/IGF-I plays an important role in the control of neuroendocrine function. However, both male and female Ames dwarf mice are also PRL- and thyroid-stimulating hormone (TSH)-deficient. Therefore, some of the above mentioned effects observed in Ames dwarf mice might have been due to the absence of PRL and/or TSH secretion.

The GHR-KO mice are IGF-I-deficient. Therefore, these mice are good experimental model to evaluate the role of IGF-I in reproductive functions. Although these mice secrete large amounts of GH, due to the absence of GHRs, GH-dependent IGF-I production was prevented. These mice have been designated as a model for the human Laron syndrome [49].

In the female GHR-KO mice, the LH response to GnRH treatment was attenuated relative to their normal siblings¹. Plasma LH levels 10 days after ovariectomy were lower in GHR-KO mice than in normal mice. However, the suppressive effect of estrogen on LH secretion was similar in both groups of mice. Plasma PRL levels were significantly higher in oil-injected, ovariectomized GHR-KO mice relative to the similarly treated normal mice. These results indicate that the control of LH and PRL release is altered in adult female GHR-KO mice and that systemic IGF-I is required for the normal secretions of these pituitary hormones.

In adult male GHR-KO mice, the weights of testes and male sex accessory structures were decreased [24]. These animals were hyperprolactinemic [24]. Although the basal plasma LH levels were similar in GHR-KO mice relative to those in their normal siblings, the plasma LH response to GnRH treatment was significantly reduced. We have also shown that the plasma testosterone response to LH treatment was attenuated in GHR-KO mice, while circulating androstenedione levels were not different than in their normal siblings [25]. This suggests that within the testes of GHR-KO mice, the key enzyme, 17 β -hydroxysteroid dehydrogenase that converts androstenedione to testosterone is defective/or less responsive to the exogenous LH. These results indicate that absence of plasma IGF-I alters the effect of GnRH on LH secretion as well as testicular function. In addition, the numbers of testicular LH and PRL receptors were drastically reduced in adult GHR-KO mice and they are hyperprolactinemic [25]. The rate of fertility in GHR-KO male mice was also reduced [24]. In contrast, in IGF-I gene disrupted mice, the male sex accessory structures were reduced and these animals were infertile and the in vitro testosterone response to LH treat-

¹Chandrashekar V, Bostwick MG, Panici JA, Bartke A, Kopchick JJ 2002 Neuroendocrine function in adult female growth hormone receptor gene disrupted mice. The Endocrine Society 84th Annual Meeting, San Francisco, CA, #P1-166, abstract.

ment was reduced [7]. In contrast, in GHR-KO mice fertility is reduced, but not totally suppressed. The mechanism responsible for the maintenance of fertility in GHR-KO mice is unknown. In GH-deficient dwarf rats, GH treatment elevated IGF-I secretion and increased the total number of viable spermatozoa [75], and alterations in the neuroendocrine and testicular function in GHR-KO mice suggest that IGF-I plays an important role in male reproduction.

INFLUENCE OF GH/IGF-I ON INITIATION OF PUBERTY

There are evidence suggesting the GH plays a role in gonadal development and function. In mammals, GH-dependant hepatic production of IGF-I might target testis to induce spermatogenesis. It is also possible that IGF-I is formed independently of GH action by the Sertoli and Leydig cells [13] and IGF-I can initiate the process of spermatogenesis. It has been shown that IGF-I treatment increases the motility of spermatozoa [38, 83]. Furthermore, IGF-I gene deleted male mice are infertile [7]. Similarly, patients with Laron syndrome are infertile and it has been demonstrated that administration of IGF-I initiates puberty in these subjects [57], clearly suggesting that IGF-I exerts a profound influence on sexual maturation in the male. The Sertoli, Leydig and peritubular cells within the testis produce IGF-BPs [13]. Although there is a suggestion that IGF-BP-3 and IGF-BP-4 might inhibit IGF-I-induced testosterone production by the purified Leydig cells [58], the precise role of these binding proteins in testicular growth and in male puberty is unknown.

There are strong indications to support that IGF-I system plays a key role in ovarian folliculogenesis. In the mouse ovary, IGF type I receptor is expressed in granulosa cells of the healthy and atretic follicles. However, IGF-I mRNA expression is restricted to the granulosa cells of large preantral and in the cumulus of small antral follicles [2, 84]. It is interesting to note that the expression of IGF-I in the granulosa cells is not dependent on FSH because it is not affected in FSH β gene knockout mice and in hypophysectomized animals [87]. IGF-I null mutant female mice are infertile and it has been shown that the preantraal follicles have normal viable granulosa cells

indicating a permissive role of IGF-I in ovarian follicular development [7, 46, 63]. Furthermore, ovaries of IGF-I knockout mice have no antral follicles [46, 63]. Additionally, in these mice FSH receptor expression in preantral follicles is reduced, whereas treatment with IGF-I restored FSH receptors suggesting that IGF-I plays a significant role in follicular development and possibly affects female puberty. IGFBP-2 is expressed in all follicles from primary to antral follicles and IGFBP-5 mRNA is detected in primary and secondary mouse follicles. It has been shown that these binding proteins inhibit IGF-I action on rat follicular cells [1] and it is possible that IGFBPs might retard the growth rate of ovarian follicles [63].

IGF-I is expressed in growing oocytes in infants and not in human adult ovary [86]. However, high levels of IGF-II mRNA were detected in the granulosa cells of the antral follicles [86]. It is known that in women with Laron syndrome with GH resistance, the onset of puberty is delayed [54]. Very low levels of IGF-I were found in their circulations suggesting that an effect on puberty might be due to the inadequate production of IGF-I. Furthermore, it has been reported that a patient with Laron dwarfism spontaneously ovulated and became pregnant despite trace amounts of circulating IGF-I and in the follicular fluid and an increase in IGFBP-1 levels [35]. It is possible that the GH-independent trace quantity of IGF-I and large amounts of IGF-II from large follicles might induce folliculogenesis. Additionally, androgens can stimulate follicular growth, mediated by local IGF-I and IGF type I receptors [81, 82]. It is shown that treatment with androgens stimulates follicular growth and is associated with increases in IGF-I and type I receptors in granulosa and thecal cells.

In mammals, the central endocrine involvement in initiating puberty is the increase in GnRH release from the hypothalamus, which in turn increases gonadotropin release. Gonadotropins act on the gonads initiating their steroid secretion. In many species, including humans, the circulating IGF-I levels increase during puberty [52]. This increase in IGF-I secretion might influence the onset of puberty. It has been shown in prepubertal female rats that IGF-I increases GnRH release [39]. It is known that in addition to being derived from the circulation, IGF-I is synthesized within the hypothalamus and the pituitary gland, and there is evidence for the presence of its receptors in the

hypothalamus and the pituitary gland [39]. *In vitro* studies have revealed that IGF-I increases GnRH-induced gonadotropin secretion by the pituitary cells [40], strongly suggesting that IGF-I influences the neuroendocrine function and possibly sexual maturation.

It has been shown that suppression of GH secretion in female rats delays puberty and reduces ovarian LH receptors [3, 68]. Furthermore, GH treatment increases the efficacy of FSH in differentiation of ovarian granulosa cells [44]. In male rats, experimental induction of GH deficiency causes a delay in testicular growth and differentiation of the germ cells [4], indicating a role of GH in the induction of puberty. It is also known that the sexual maturation is delayed in men with Laron syndrome [55, 73, 76] due to the mutated GH receptor genes with subsequent resistance to GH and IGF-I-deficiency. IGF-I is believed to be involved in the regulation of pubertal growth spurt and sexual maturation. In female rats, peripherally produced IGF-I was proposed to act within the central nervous system to trigger sexual maturation [40]. We have shown that in GHR gene disrupted mice the LH response to GnRH treatment was attenuated, the plasma FSH levels were lower and the testosterone response to LH administration was attenuated [24, 25]. GHR-KO disrupted mice are GH resistant, growth retarded and IGF-I is undetectable in circulation [24, 25].

Preputial separation is believed to be an external sign of pubertal development in the male rodents [50]. We have recently shown that the balano-preputial separation was delayed 5 days, and significant increase in the weights of the seminal vesicles occurred later in GHR-KO mice than in normal siblings [47]. In addition, the weights of testes, and epididymii were significantly reduced in GHR-KO mice. The intratesticular testosterone levels and the testosterone response to LH treatment were attenuated in GHR-KO mice. The elongated spermatids appeared later in the testes of GHR-KO mice relative to testes of normal siblings. These results suggest that the absence of IGF-I secretion delays the normal course of sexual maturation in male GHR-KO mice indicating that IGF-I plays an important role in the initiation of puberty in male mice.

It has been recently shown that the numbers of preovulatory follicles and corpora lutea were significantly reduced in GHR-KO mice and this was

associated with reduced plasma estradiol levels [85]. The number of atretic preovulatory follicles was reduced in GHR-KO mice. In addition, there was a reduced IGF-I mRNA expression in the ovaries of GHR-KO mice [85]. The puberty onset in these GHR deficient mice as determined by the age at vaginal introitus was delayed and it can be advanced by IGF-I treatment [32]. The numbers of active corpora lutea and uterine implantation sites were reduced in GHR-KO mice. Growth hormone resistance and IGF-I insufficiency negatively impacted follicular development/ovulation rate and sexual maturation [32]. GHR-KO female mice are fertile, but they exhibited quantitative deficits in various parameters of reproductive function [32].

A most recent study has also shown that the major effect on reproductive function seen in GHR-KO mice was due to a significant decrease in litter size as a consequence of reduction of the ovulation rate [6]. The ovulatory response to exogenous gonadotropin treatment was 3-fold reduced in GHR-KO mice relative to normal siblings. The number of follicles per ovary was reduced, although all types of follicles were present. However, the number of follicles from antral and preovulatory stages was decreased in the ovaries of GHR-KO mice. Interestingly, IGF-I treatment was unable to improve fertility or ovarian responsiveness to exogenous gonadotropins, suggesting that the effect of GH is independent of IGF-I. These results suggest that the reduction of litter size in GHR-KO mice is possibly due to the change in the growth of follicles and that the effects of GH on follicular growth are independent of IGF-I action. The aforementioned studies suggest that IGF-I plays an important role in the process of gonadal development and sexual maturation in mice.

CONCLUDING REMARKS

In this review article we have highlighted the importance of GH /IGF-I in the control of neuroendocrine and reproductive functions. GH /IGF-I, has at least a modulatory role in the aforementioned functions. Furthermore, it is important to realize that not all effects of GH are stimulatory and the duration of exposure of the hypothalamo-hypophyseal-gonadal system to IGF-I might have a strong influence on the secretions of gonadotropins and gonadal steroids.

Additionally, emerging evidence suggests that the IGF binding proteins can influence the IGF-I action. Therefore, the ratio of IGF binding proteins and IGF-I within the body might play an important role in evoking the effects of IGF-I on neuroendocrine-gonadal system. Since exogenous administration of IGF-I induces syntheses of IGF binding proteins, which might inhibit IGF-I action, one should exert caution in interpreting results obtained from such a treatment. A simple alternate experimental design is needed to evaluate the effects of IGF-I treatment without encountering the other effects. It seems reasonable to suggest that treatment with specific antibodies to IGF binding proteins might yield results that would help us to clearly understand the role of IGF-I in reproduction. We are hoping that affordable, species specific IGF binding proteins will be available soon to undertake such experiments.

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