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Congenital hypogonadotropic hypogonadism and Kallmann syndrome as models for studying hormonal regulation of human testicular endocrine functions

Hypogonadisme hypogonadotrope congénital et syndrome de Kallmann comme modèles d’étude de la régulation hormonale des fonctions testiculaires endocrines humaines

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Abstract

Men with Kallmann syndrome (KS) and those with congenital isolated hypogonadotropic hypogonadism with normal olfaction share a chronic, usually profound deficit, in FSH and LH, the two pituitary gonadotropins. Many studies indicate that this gonadotropin deficiency is already present during fetal life, thus explaining the microopenis, cryptorchidism and marked testicular hypotrophy already present at birth. In addition, neonatal activation of gonadotropin secretion is compromised in boys with severe CHH/Kallmann, preventing the first phase of postnatal testicular activation. Finally, CHH is characterized by the persistence, in the vast majority of cases, of gonadotropin deficiency at the time of puberty and during adulthood. This prevents the normal pubertal testicular reactivation required for physiological sex steroid and testicular peptide production, and for spermatogenesis. CHH/KS thus represents a pathological paradigm that can help to unravel, in vivo, the role of each gonadotropin in human testicular exocrine and endocrine functions at different stages of development. Recombinant gonadotropins with pure LH or FSH activity have been used to stimulate Leydig’s cells and Sertoli’s cells, respectively, and thereby to clarify their paracrine interaction in vivo. The effects of these pharmacological probes can be assessed by measuring the changes they provoke in circulating testicular hormone concentrations. This review discusses the impact of chronic gonadotropin deficiency on the endocrine functions of the interstitial compartment, which contains testosterone-, estradiol- and INSL3-secreting Leydig’s cells. It also examines the regulation of inhibin B and anti-Mullerian hormone (AMH) secretion in the seminiferous tubules, and the insights provided by studies of human testicular stimulation with recombinant gonadotropins, used either individually or in combination.

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Keywords: Testosterone; Estradiol; INSL3; Inhibin B; AMH; Hypogonadism; Kallmann
Résumé

Les hommes avec hypogonadisme hypogonadotrophique congénital (HHC) isolé avec olfaction normale ou avec syndrome de Kallmann ont comme point commun un déficit chronique et le plus souvent profond en gonadotrophines hypophysaires FSH et LH. De nombreux travaux indiquent que le déficit en gonadotrophines chez ces malades est déjà présent pendant la vie fœtale. Il explique la présence d’un micropénis et d’une cryptorchidie ainsi que l’importante hypotrophie testiculaire qui sont observés dès la naissance. De plus, chez les nouveau-nés de sexe masculin atteints de HHC/Kallmann, l’activation néonatale de la sécrétion des gonadotrophines est aussi compromise empêchant la première phase d’activation testiculaire post-natale. Les HHC sont enfin caractérisés par la persistance, dans la majorité des cas, du déficit en gonadotrophines au moment de la puberté puis à l’âge adulte. Ceci empêche la réactivation testiculaire pubertaire normale nécessaire à la production physiologique des stéroïdes sexuels, des peptides testiculaires ainsi qu’à la spermatogenèse. Le HHC/KS est donc un paradigme pathologique permettant d’étudier, in vivo, dans l’espèce humaine le rôle de chacune des gonadotrophines sur les fonctions testiculaires exocrines et endocrines à différents stades du développement. L’utilisation des gonadotrophines recombinantes à activité LH ou FSH exclusive a permis de stimuler de façon élective respectivement les cellules de Leydig et de Sertoli et de préciser ainsi, in vivo, leur interaction paracrine. Les effets de ces sondes pharmacologiques peuvent être évalués par les modifications des concentrations des hormones testiculaires qu’elles provoquent. Dans cette revue, nous discutons les conséquences du déficit chronique en gonadotrophines sur les fonctions endocrines du compartiment interstitiel qui contient les cellules de Leydig, lieu de sécrétion de testostéron, estradiol et INSL3. De même, nous faisons le point sur les régulations de la sécrétion hormonale d’inhibine B et de l’hormone anti-mullérienne (AMH) par le tube séminifère, ainsi que sur les enseignements obtenus par l’étude de la stimulation testiculaire par les gonadotrophines recombinantes, utilisées en monothérapie ou en association, chez ces malades de sexe masculin.

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Mots clés : Testostérone ; Estradiol ; INSL3 ; Inhibine B ; AMH ; Hypogonadisme ; Kallmann

1. Introduction

Congenital hypogonadotrophic hypogonadism (CHH) is a rare disease characterized by an often profound deficiency in pituitary gonadotropins, leading to pubertal failure in males and females [1–3]. This deficiency may result from a primary pituitary abnormality or, more often, from a failure of the hypothalamus to produce GnRH, a neurohormone essential for pubertal activation of FSH- and LH-secreting pituitary cells [1,2]. Many clinical and experimental data suggest that this pituitary gonadotropin deficiency is already present during fetal and neonatal life, preventing normal activation of pituitary gonadotropin secretion [4]. Pathophysiologically, CHH can be divided into two main forms [1–4]:

- those due to anomalies of the hypothalamic-pituitary signaling cascade, in which the development of GnRH neurons is normal [1,2];
- those due to abnormal neuronal development affecting prenatal migration of GnRH neurons [1–4].

The first category includes genetic defects responsible for altered hypothalamic GnRH secretion [5–12] or for defective pituitary responsiveness to GnRH [13,14]. These defects always lead to isolated CHH, with no other detectable clinical disorders; in particular, the sense of smell is almost always normal [1,2,15]. The second category mainly includes Kallmann’s syndrome (KS), in which CHH is associated with olfactory bulb aplasia, leading to hyposmia or anosmia [15]. KS is due to abnormal migration of GnRH neurons during fetal life, itself secondary to abnormal development of the necessary olfactory nerve endings [16–19].

2. Hormone secretion by Leydig’s cells in normal human males and CHH patients

2.1. Sex steroid secretion by the human fetal testicle

Physiologically, testicular sex steroids – testosterone and estradiol (E2) – are secreted predominantly or exclusively by Leydig’s cells during the different periods of pre- and postnatal development. In humans, synthesis of testosterone and E2 requires prenatal stimulation of Leydig’s cells via the LH/hCG receptor (RLH/hCG), first by the placental gonadotropin hCG and then by the pituitary gonadotropin LH [20–22]. The key role of RLH/hCG during fetal life is witnessed by the fact that loss-of-function mutations in this receptor lead to a lack of stimulation of fetal Leydig’s cells and thus abolish prenatal Leydig’s cell steroidogenesis [22,23]. The resulting inability of the fetal testis to secrete testosterone will prevent, partially or completely, normal virilization of the urogenital sinus and generate a female external genital phenotype or sexually ambiguous external genitalia, respectively [22,23]. In contrast, the rare bi-allelic mutations of βLH described in subjects with an XY karyotype and LH deficiency are not associated with genital ambiguity [24–28]. This suggests that the initial stages of masculinization of the external genitalia are essentially dependent on hCG and that they can take place despite the absence of pituitary LH [4,20]. However, men with congenital LH deficiency usually have micropenis, suggesting that fetal testicular steroidogenesis, which is LH-dependent during the second half of fetal life, is necessary for prenatal terminal penile growth [24–28]. In these patients with βLH mutations, it is likely that the absence of LH secretion during the first months after birth accentuates the growth defect of the external genitalia.

The role of estradiol during human fetal development of the testicles and genital organs is not clear. No clear and reproducible
phenotypic consequences for the testes or external genitalia are observed in the rare males (karyotype XY) with severe loss-of-function mutations of the aromatase or estradiol receptor alpha [29–31]. On the other hand, it is very difficult to separate the roles in fetal physiology of estrogen produced abundantly by the placenta and estrogen synthesized locally by the gonads [29–33].

2.2. Postnatal testosterone in CHH males

The markedly low testosterone levels in CHH patients were first noted many decades ago [34] and has been highlighted in particular cases diagnosed during the neonatal period [4,35,36]. Later, during childhood, this testosterone deficiency cannot be reliably diagnosed because of the physiological dramatic decrease in gonadotropin and testosterone levels that characterizes the prepubertal period [36]. The testosterone deficiency can again be detected, starting in adolescence, by simple total testosterone assay (Fig. 1A) [1,37]. Given the marked testosterone deficiency reported in most men with CHH, measurement of testosterone subfractions (non-SHBG bound, i.e., “bioavailable” or free) or the use of more sensitive and specific testosterone assay techniques such as LCMS and GCMS does not seem to provide any significant additional diagnostic information regarding the magnitude of testosterone deficit [1].

The LH dependency of testicular testosterone secretion after birth is clearly established in humans. All disorders of the gonadotropic axis so far described, associated with either genetic or non-genetic LH deficiency, are also associated with defective testicular testosterone production, the degree of which correlates with the LH deficiency [37]. The collapse of testosterone levels is similar when LH secretion is inhibited pharmacologically, by using GnRH analogs or antigonadotropic progestogens [38]. Whatever the cause of the LH deficit responsible for low testosterone levels, the latter can be corrected by recombinant hCG or LH administration but not by FSH administration [37,39–44]. This lack of any significant physiological effect of FSH on testosterone production by human Leydig’s cells is also supported by the normal testosterone levels observed in male carriers of FSH receptor mutations leading to severe loss-of-function [45].

In contrast, the conclusions that can be drawn from the description of men with FSH deficiency due to mutation of the gene encoding the FSHβ-subunit are less clear-cut. Indeed, while the essential role of FSH in ovarian steroidogenesis (stimulation of estradiol secretion by granulosa cells) is clearly established in women [46,47], data on the impact of a specific deficit in this pituitary gonadotropin on testicular testosterone secretion are somewhat conflicting. Indeed, testosterone levels were maintained in a few well-documented cases of inactivating FSH mutation [48,49], while one patient had an unexpected testosterone deficiency [50]. Further case studies are needed to settle this question. Pulsatile testosterone secretion in normal males, as measured in spermatic veins, occurs at the same frequency as pulsatile LH secretion, further supporting the dependency of testicular testosterone secretion on pituitary LH [51]. Thus, the vast majority of published human data points to a major physiological role of LH and an accessory or insignificant role of FSH. One outstanding question concerns the possible synergistic, paracrine role of the

Fig. 1. Total testosterone (A), total estradiol (B), and INSL3 serum levels in normal men and untreated men with congenital hypogonadotropic hypogonadism (CHH). A. Normal men (16–33 years old; n = 86) and patients with CHH (17–30 years old; n = 94). B. Normal men (17–46 years old; n = 60) and patients with CHH (17–45 years old; n = 76). C. Normal men (21–56 years old; n = 72) and patients with CHH (17–56 years old; n = 91); serum testosterone (T) (mean ± S.D. [range]; ng/mL) are indicated in each group. To convert the values for T to nanomoles per liter, multiply by 3.467 and to convert E2 concentration from pg/mL to picomoles per liter, multiply by 3.671.
Adapted in part from references [1,37,43].
2.3. Postnatal estradiol in CHH males

Gonadal estrogens play an important role in male skeletal maturation at puberty and in maintaining bone mineral density in adulthood [54]. Estradiol deficiency, closely associated with testosterone deficiency in males, leads to delayed fusion of epi- physeal cartilage and to poor acquisition of calcium bone mass [55–57]. If this deficiency persists into adulthood, it can cause osteopenia and, later, osteoporosis [55–58]. Estradiol secretion in adult males, like testosterone secretion, is positively regulated in adult males, like testosterone secretion, is positively regulated primarily by pituitary LH [43]. Testicular estradiol biosynthesis after birth mainly takes place in Leydig’s cells [59]. In the neonatal period, E2 secretion coincides with peak pituitary gonadotropin levels [60]. Testicular estradiol concentrations during this period [61] are also tightly correlated with testosterone levels and with histological development of Leydig’s cells [62], suggesting preferential neonatal estradiol secretion by Leydig’s cells. This is corroborated by aromatase expression in Leydig’s cells during this period [62]. Several lines of evidence suggest that Leydig’s cells are also the main source of estradiol in adult males. Indeed, aromatase is exclusively expressed in this cell type after the age of puberty [59]. These immuno- histochemical data are consistent with congenital (CHH) or acquired (AHH) gonadotropin deficiency. Indeed, in men with CHH (Fig. 2B) or AHH, we have found a large and highly significant decrease in circulating estradiol concentrations, correlating with the decrease in LH [40,43]. E2 levels in these men can be increased by LH or hCG administration, whereas FSH administration has no effect [40,43].

2.4. Insulin-like factor 3 (INSL3) in normal and CHH males

Insulin-like factor 3 is a dimeric peptide hormone encoded by the INSL3 gene [63–68]. In men, INSL3 is mainly expressed by Leydig’s cells [63–65]. INSL3 knockout leads to abdominal cryptorchidism in mice [67–69]. It is currently accepted, but not proven, that INSL3 may play a role during the initial stages of human prenatal testicular migration by acting on LGR8/RXFP2 receptors in the gubernaculum [66]. In humans, INSL3 is secreted during fetal and immediate postnatal life, then decreases during childhood [71–74]. Testicular secretion increases again at puberty, reaching its maximum in adulthood [71–74]. The abnormal INSL3 concentrations seen in some gonadal disorders [37,75,76] suggest that this hormone might, in addition to testosterone, serve as a marker of Leydig’s cell function and thus as a tool for exploring testicular endocrine function. Some small studies of patients with acquired gonadotropin deficiency suggest that testicular INSL3 secretion is dependent on the action of pituitary gonadotropins, especially LH. This is supported by the reduced levels of this hormone observed after pharmacological inhibition of pituitary LH [75,76]. More recently, we studied in a large number of men with CHH and found that the gonadotropin deficiency is associated with a consistent and highly significant decrease in circulating INSL3 levels [37]. This clearly established that testicular secretion of this peptide hormone is gonadotropin-dependent. The very marked decline in INSL3 and the lack of overlap between values in normal men and men with CHH/KS show that INSL3 can serve as a hormonal index of Leydig’s cell function in these patients, as reliably as total testosterone (Fig. 1C) [37].

Patients with CHH/KS who have not received hormone therapy exhibit a highly significant correlation between total testosterone and INSL3 levels [37], showing the close relationship in this pathological context between the capacities of Leydig’s cells to secrete each of these hormones. A highly significant correlation between INSL3 and LH levels was found [37] in the same patients, strongly suggesting a causal relationship between the severity of pituitary LH deficiency and that of testicular INSL3 secretory deficiency. This close relationship between LH and INSL3 is further supported by the fact that INSL3 deficiency can be corrected by gonadotropin therapy including a drug with LH-like activity. To formally demonstrate this LH dependency, we could compare the effects of consecutive FSH and hCG administration in the same patients. In agreement with other reports [75,76], we found that only hCG increased INSL3 levels, FSH having no effect. This provided independent confirmation that testicular INSL3 secretion is specifically upregulated by LH in patients with chronic gonadotropin deficiency. However, combined chronic FSH and hCG administration failed to normalize INSL3 concentrations, whereas total testosterone levels normalized in most cases. This dissociation suggests that, despite normalization of testicular testosterone secretion, the whole testicular Leydig’s cell number remains inadequate in these patients during prolonged...
gonadotrophin therapy. The question therefore arises as to whether pre- and neonatal testicular activation is necessary to establish a normal Leydig’s cell capital in adulthood and thus to allow normal INSL3 secretion. The precise mechanism by which LH stimulates INSL3 secretion by Leydig’s cells is controversial. Some authors consider it is due not to specific acute stimulation resembling the effect of LH on testosterone secretion [77] but rather to an indirect effect of LH on Leydig’s cell differentiation. Ivell et al. consider that INSL3 secretion by Leydig’s cells is constitutive and linked to their differentiation status [77].

3. Sertoli’s cell hormone secretion in normal men and CHH/KS males

3.1. Inhibin B

Studies of inhibin B in human physiology and pathology did not really take off until the mid-1990s, when Nigel Groome’s group developed a sensitive and specific sandwich immunoassay for this hormone [78]. Inhibin B is a heterodimeric peptide hormone composed of two subunits, α and βB [79]. The mature circulating form of 30 kDa is secreted exclusively by the testes, as shown by the consistently undetectable levels in children and men with anorchidism or castration [80]. Testicular inhibin B synthesis and secretion occur in male fetal life and in postnatal period before and after puberty in the seminiferous tubules [79,81,85,82]. Thus, when this compartment is severely damaged, as in post-pubertal males with Klinefelter syndrome, circulating inhibin B levels collapse to virtually undetectable levels [83] (Fig. 2). However, the relative contributions of the different cell types present in seminiferous tubules to the physiological synthesis and subsequent secretion of inhibin B in humans have not yet been fully established [79].

In boys, IB synthesis and secretion appear to be ensured mainly by Sertoli’s cells, which express the two subunits, α and β, at this stage, with no apparent germline contribution [84,85]. From puberty onwards, the integrity of the germline and its interaction with Sertoli’s cells seem essential for testicular IB secretion: circulating levels collapse in case of isolated germ cell (GC) damage, as in male infertility due to the Sertoli’s cell-only syndrome [85,86]. Studies of patients with Y chromosome microdeletions with maturation blockade at various levels indicate that spermatocytes play an essential role in IB synthesis by the post-pubertal seminiferous tubule [79,84–86]. The precise mechanism underlying the role of germ cells and their interaction with Sertoli’s cells in IB synthesis by the post-pubertal seminiferous tubule is not fully understood [79]. Some studies point to cooperation between certain germ cells that express the βB subunit after puberty [84]. Indeed, Marcheti et al. [86], using immunocytochemistry, studied the expression of the IB α and βB subunits in an adult human testis and demonstrated, in keeping with the results of Andersson et al. [84], that the βB subunit was expressed in germ cells but not in Sertoli’s cells, while the alpha subunit was expressed in Sertoli’s cells. These authors proposed that dimeric IB was produced jointly by the two cell types [79,84–86]. The gonadotropin dependency of testicular IB secretion was suggested very early, based on the relationship between increased secretion of pituitary gonadotropins and increased IB secretion, both in the newborn male and at the time of puberty [82,87]. This dependency was also found in primate models [88], in which gonadotropin inhibition by GnRH antagonists resulted in low circulating IB levels. It is further supported by the decline in IB levels observed during gonadotropin inhibition induced by testosterone esters, used alone or in combination with a progestagen for male contraception [89]. Studies of hypogonadotropic hypogonadism confirm that the decrease in pituitary gonadotropins is accompanied by a corresponding decrease in this testicular peptide (Fig. 2) [40,41,80,90–94]. Studies of this same model also showed that the increase in gonadotrophin levels provoked by GnRH administration to men with HH led to an increase in circulating IB levels [90,91]. To identify which pituitary gonadotropin was responsible for upregulating IB levels, men with HH were treated with recombinant FSH. Raivio et al. were the first to show an increase in IB levels in boys treated with recombinant FSH [39]. We then demonstrated that recombinant FSH was the only gonadotropin with an IB-stimulating effect in men: LH had no effect [40], while hCG had a moderate inhibitory effect [41], indicating that Leydig’s cells did not contribute to circulating inhibin B levels. Concordant results were then obtained by several independent teams in CHH patients treated during childhood [4,42,93]. The FSH dependency of testicular IB secretion is also shown by the correlation between the concentrations of these two hormones in HH [90]. These data are also consistent with a report from Lofrano-Porto et al. [94] showing that a 37-year-old man with complete FSH deficiency due to βFSH mutation had very low circulating IB levels, contrasting with high LH concentrations, and that increased after recombinant FSH administration. They are also supported by the low IB levels observed in men with FSH receptor mutations [45]. The normal IB concentrations seen in men with βLH mutations or inactivating mutations of the LH receptor further indicate that LH and Leydig’s cells do not directly contribute to IB levels in humans [25,26]. Finally, the non-contribution of Leydig’s cells is also in line with the lack of increase in IB levels in a patient with highly secretory Leydig’s cell tumor [95]. Thus, all published data indicate that human testicular inhibin B secretion is dependent on pituitary FSH secretion. The precise mechanism of FSH stimulation is not fully understood, although some evidence points to a role of the cAMP signaling pathway [79,96]. The role of germ cells remains also to be further clarified.

3.2. Anti-Mullerian hormone (AMH)

AMH is a member of the transforming growth factor-β (TGF-β) protein family. AMH is secreted by Sertoli’s cells, variably during the different phases of testicular development [97]. AMH is secreted during early fetal development, thereby repressing the development of Mullerian ducts [97]. In humans testes, AMH is exclusively expressed by Sertoli’s cells during this phase [98,99]. During pregnancy, AMH is present at high levels in cord blood from male fetus but is very much lower in female fetus serum [100]. Circulating AMH remains detectable in
newborn males and then increases during the neonatal elevation of pituitary gonadotropins [87,97,100]. This increase may be related both to Sertoli’s cell proliferation during “mini-puberty” [35,36,87,99–101] and to FSH stimulation of immature Sertoli’s cells through a mechanism that may involve the signaling pathway mediated by cyclic AMP [99,102]. Between mini-puberty and full puberty, AMH secretion continues constitutively and independently of FSH levels [36,97], which decline. During pubertal development, circulating AMH levels fall sharply in healthy males through the combined effect of the increase in intratesticular testosterone and the onset of spermatogenesis [97,103].

AMH concentrations in male patients with CHH show various abnormalities at different stages of development. A variable decline is observed in the neonatal period [4,35]. After the age of puberty, AMH levels remain at prepubertal levels in CHH males and are therefore higher than in normal post-pubertal subjects [41,92,103]. However, average values in CHH are lower than those observed in normal boys of prepubertal age [41]. These relatively low levels might reflect a decrease in production, possibly due to an inadequate testicular Sertoli’s cell number. The decrease in the number of Sertoli’s cells in these patients would result from a lack of stimulation of tubular cell proliferation that occurs during the normal fetal, neonatal and pubertal phases of testicular stimulation [4].

4. Conclusion

CHH/KS represents an ideal model for studying the integrated hormonal regulation of sex steroid and peptide secretion by the human testis, as it allows the role of each gonadotropin to be clarified. It is now clear that normal secretion of hormones produced by Leydig’s cells requires intact pituitary LH secretion. Likewise, normal inhibin B secretion requires FSH stimulation of Sertoli’s cells. Further work is needed to clarify and understand both the roles of germ cells on Sertoli’s cells functions and the roles of paracrine regulation between the interstitial compartment and the seminiferous tubules in testicular hormone secretion.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

References


