Role of FSH in male gonadal function
Le rôle de la FSH dans la régulation gonadique masculine

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RÉSUMÉ - La production des gamètes masculins dépend de l'effet concerté au niveau testiculaire des deux gonadotrophines FSH et LH. L'action de la LH permet la production de testostérone par les cellules de Leydig. Dans la mesure où les cellules germinales mâles ne possèdent de récepteurs ni pour la FSH ni pour les androgènes, l'effet de FSH et de la testostérone s'expriment par l'intermédiaire des cellules de Sertoli. Bien que l'effet précis de ces deux hormones reste mal connu, il existe aujourd'hui des preuves suggérant que la FSH et la testostérone sont toutes deux capables de stimuler toutes les étapes de la spermatogenèse. Chez l'homme, FSH est nécessaire pour la détermination du nombre de cellules de Sertoli, ainsi que pour l'induction et le maintien d'une production normale de sperme. Ce rôle crucial de FSH a été clairement illustré par la description d'un patient présentant une mutation activatrice du récepteur à FSH. Même avec une hypophysectomie pour tumeur pituitaire et sous traitement substitutif par testostérone, ce patient s'est révélé d'une manière inattendue fertile malgré des taux sériques de gonadotrophines indétectables et de testostérone. Ce patient a pu donner naissance à 3 enfants. Chez ce patient nous avons pu démontrer l'existence d'une mutation hétérozygote activatrice du récepteur à FSH, conduisant à une production d'AMPc indépendante de la stimulation par FSH. Il s'agit de la première description d'une telle mutation du récepteur à FSH et de la spermatogenèse chez l'homme.

SUMMARY - The production of male gametes depends on the concerted action of the two gonadotropins FSH and LH on the testis. The action of LH is mediated through the production of testosterone by the Leydig cells. Since male germ cells possess neither FSH nor androgen receptors, the action of FSH and testosterone occurs through the Sertoli cells. Although the precise function of these two hormones remains elusive, the existing evidence suggests that both FSH and testosterone are able to stimulate all phases of spermatogenesis. In the male FSH is required for the determination of Sertoli cell number, and for induction and maintenance of normal sperm production. The crucial role of FSH in male gonadal function has been clearly illustrated by the discovery of a patient with an activating mutation of the FSH receptor. This patient had been hypophysectomized because of a pituitary tumor and, under testosterone substitution was unexpectedly fertile in spite of undetectable serum gonadotropin levels and had fathered three children. In this patient we could demonstrate a heterozygous activating mutation of the FSH receptor which resulted in cAMP production independent of FSH stimulation. This finding represents the first description of an activating mutation of the FSH receptor and demonstrates that FSH alone maintains spermatogenesis in man.

On the other hand, the effects of the lack of FSH action are unclear. Among five men with a homozygous inactivating mutation of the FSH receptor only one was infertile and spermatogenesi-s was variably affected in the others. However, serum inhibin B values in these men were not completely suppressed and serum FSH levels were only moderately elevated, indicating the possibility that FSH receptor function was not completely abolished by the mutation. Elimination of FSH action is a prerequisite to suppress completely spermatogenesis for contraceptive purposes, while administration of both LH and FSH is necessary to induce sperm production in patients with hypogonadotro-
Spermatogenesis takes place in the testis under the control of the two gonadotropins, FSH and LH [10, 20]. The gonadotropins act indirectly on the seminiferous epithelium via the somatic testicular cells. FSH possesses specific receptors on the Sertoli cells [17]. LH induces testosterone production by the Leydig cells in the interstitium and this steroid, in turn, acts through binding to specific receptors localized in Sertoli and peritubular cells as well as in the Leydig cells. Therefore, the action of both FSH and testosterone is mediated through the Sertoli cells, although their effects at the cellular level remain fundamentally unknown.

**ROLE OF FSH IN THE INITIATION OF SPERMATOGENESIS**

Patients with hypogonadotropic hypogonadism represent a valuable experimental model to study the role of gonadotropins in spermatogenesis. Patients with secondary hypogonadism due to either Kallman syndrome or to hypopituitarism can be effectively treated with pulsatile GnRH or a combination of both gonadotropins in order to induce spermatogenesis and achieve fertility. In such patients sperm concentration increases progressively under hCG/hMG administration [4]. Sperm concentration can even reach normal values, although this is not necessary for the induction of a pregnancy and the time required to achieve a pregnancy is quite variable among the patients [4].

It is common clinical experience that both gonadotropins are required to achieve fertility in such patients, and FSH alone is not able to induce fertility [15]. However, the experimental treatment of prepubertal monkeys with FSH alone for 4 weeks was able to induce cell proliferation in the seminiferous epithelium, although only the combined administration of both hCG and FSH was capable of increasing testicular volume and number of Sertoli cells and spermatogonia [16]. Thus it is evident that FSH alone is capable of inducing proliferation of Sertoli cells and spermatogonia in the prepubertal primate, but this does not result in qualitatively and quantitatively normal spermatogenesis unless testosterone is simultaneously present.

The role of FSH for the initiation of spermatogenesis has been recently challenged by the finding that male transgenic mice in which the FSHβ subunit gene has been knocked-out are fertile [7]. In such animals spermatogenesis is only quantitatively reduced, suggesting that FSH is not absolutely necessary for initiation of spermatogenesis. However, the rodent model most probably does not reflect the situa-
tion in the human, since a man with congenital absence of FSH due to a mutation in the FSH β chain was recently reported to be infertile and hypogonadal [13]. Finally, male mice in which the FSH receptor has been knocked out are infertile [14], suggesting that the elimination of the hormone is not sufficient to exclude completely the FSH receptor activation.

**ROLE OF FSH IN THE MAINTENANCE OF SPERMATOGENESIS**

The role of FSH in the maintenance of spermatogenesis in the primate has been studied by suppressing FSH action in adult males. One possible way to reach this goal is immunization against FSH or the FSH receptor. Several experiments have shown that long-term immunization against FSH induces severe testicular regression, oligozoospermia or even azoospermia in monkeys [12]. Even if the monkeys are only oligozoospermic and not azoospermic they are infertile in mating tests, suggesting that the few sperm left are functionally impaired and that the severe oligozoospermia achieved by selective FSH suppression may be sufficient to induce infertility [12].

In a pilot study conducted recently in India human volunteers received an ovine FSH vaccine at a dose sufficient to achieve FSH deprivation throughout the period of four spermatogenic cycles. Due to the short duration of FSH deprivation, the experiment showed only marginal suppression of sperm counts (around 30-65 %) but some parameters of sperm quality such as chromatin condensation, acrosome content, and others were altered. These data would suggest that elimination of FSH action results not only in quantitative, but also qualitative damage of sperm quality such as chromatin condensation, acrosome content, and others were altered. These data would suggest that elimination of FSH action results not only in quantitative, but also qualitative damage of spermatogenesis [11].

In a classical and elegant experiment, Matsumoto et al. [9], showed that long-term administration of hCG to normal volunteers induced suppression of spermatogenesis, via suppression of endogenous FSH. This treatment resulted in oligozoospermia, which was reversed by simultaneous administration of FSH but not by testosterone, demonstrating that FSH in conjunction with testosterone is fundamental for the maintenance of quantitatively normal spermatogenesis.

In the monkey, the selective effect of FSH on the maintenance of spermatogenesis was studied by treating male adult cynomolgus monkeys with a GnRH antagonist which completely suppresses pituitary gonadotropin production, and with simultaneous administration of pure FSH. It was shown that FSH could prevent and delay the reduction of testicular volume induced by the GnRH antagonist [21]. Moreover, the combined administration of FSH and GnRH antagonist was able to maintain the number of A pale spermatogonia and, in part, of round spermatids during the 8 weeks of treatment, while in animals treated with GnRH antagonist only, the number of these germ cells decreased much faster and more drastically. These data show that FSH alone is capable of maintaining spermatogenesis in the primate at least in part [21].

This finding has very important implications for the pharmacological suppression of spermatogenesis for contraceptive purposes. In a contraceptive trial in which a long-acting testosterone ester was administered to normal volunteers in order to suppress spermatogenesis azoospermia was reached only in those subjects whose serum FSH levels were suppressed below the lower limit of the normal range, thus supporting the important role of FSH in the maintenance of spermatogenesis [3].

A particularly impressive example of the role of FSH in the maintenance of spermatogenesis in the human is the case of a hypophysectomized, fertile man with an activating mutation of the FSH receptor. The patient had been hypophysectomized because of a chromophobic adenoma and had normal fertility in spite of undetectable serum gonadotropins while he was treated with testosterone enanthate. In this patient we discovered a heterozygous mutation exchanging codon 567 of the FSH receptor from Ala to Glu. In vitro this mutation was able to induce constitutive receptor activity resulting in caMP production in the absence of FSH stimulation [5]. Serum inhibin B was at the higher limit of the normal range, corroborating the conclusion that sperm production in this man is sustained by a FSH-like activity, such as that conferred by an activating mutation of the FSH receptor. This case highlights the crucial importance of FSH in the maintenance of spermatogenesis.

**MECHANISM OF ACTION OF FSH**

In Sertoli cells FSH acts though cAMP production, but its effects at the cellular level are basically unknown. FSH stimulates Sertoli cell proliferation before puberty and perhaps Sertoli cell secretory activity in the adulthood, but the mechanism by which this is accomplished remains mysterious. Furthermore, it is not known whether FSH actions are confined
to this gonadotropin or whether they can be mimicked by testosterone. For instance spermatogenesis can be sustained in hypophysectomized monkeys by very large doses of testosterone [8], suggesting the possibility that if one hormone fails, the other can, under certain circumstances, be sufficient to sustain qualitatively normal spermatogenesis in the primate.

A n experiment in which male adult cynomolgus monkeys were treated with a GnRH antagonist for 25 days has shown that gonadotropin withdrawal induces a dramatic decrease of spermatogonia and early spermatocytes, whereas the meiotic and postmeiotic cells remain basically unaffected [21, 22]. These results demonstrate that the effect of the gonadotropins is exerted on the very early stages of the development of the seminiferous epithelium, i.e. gonadotropins, in particular FSH, seem to exert their action mainly by stimulating the proliferation of spermatogonia. Interestingly, once the spermatogonial proliferation has started the gonadotropin withdrawal does not affect their further development and eventually, in the absence of gonadotropins, spermatogenesis ceases because the spermatogonial proliferation is blocked and not due to postulated effects at the meiotic and postmeiotic level. In addition, FSH might have an effect on spermiogenesis and sperm quality [6].

DISORDERS OF FSH ACTION IN MALE INFERTILITY

M utations of the FSH molecule lead to hypogonadism and infertility in the male [13], showing clearly that FSH is fundamental for the initiation of spermatogenesis in man. Moreover, the hypogonadal testosterone levels observed in this case, in the presence of normal LH secretion, suggests that a FSH-dependent Sertoli-Leydig cell interaction might be necessary for testosterone production. The recent production of transgenic mice in which the FSH receptor has been knocked out showed that the complete abolition of FSH action in the rodent causes male infertility with disruption of spermatogenesis, progressive testicular atrophy and reduced testosterone production [14].

Naturally occurring inactivating mutations of the FSH receptor were described in Finnish families with ovarian dysgenesis [1]. The seminal parameters in 5 men carrying a homozygous inactivating mutation of the FSH receptor were also reported [18]. Of the five men analyzed, one was infertile, two had had children, while in two men paternity had not been attempted. Sperm counts were extremely reduced in most of them, and testicular volume and sperm morphology were abnormal. Serum FSH was increased, as to be expected in the presence of FSH receptor inactivation, and inhibit B levels were variably reduced. This shows that inactivation of the FSH receptor can lead to infertility and altered spermatogenesis in the human. However, paternity in two of these subjects led the authors to the conclusion that FSH is not absolutely necessary for fertility in man, although paternity was not proven in these cases [18]. More importantly, the complete inactivation of the FSH receptor due to the mutation was never really demonstrated. On the contrary, the original paper describing the mutation in the women with ovarian failure could not rule out this possibility and another study reported the histological analysis of the ovaries of the affected women and hypothesized that the mutation could permit some residual receptor activity [2]. This could explain the residual spermatogenic activity in some homozygous men.

D uring a large screening attempt of FSH receptor mutations in infertile patients we found that the receptor is polymorphic at two sites in exon 10, corresponding to the extracellular domain close to the transmembrane domain and to the intracellular domain, respectively. In particular we found two receptor isoforms carrying Ala307, Ser680 and Thr307, A n680, respectively. These two polymorphic variants show different affinity for the hormone in vitro in transiently transfected COS-7 cells. However, the two receptor variants are equally effective in mediating FSH action in terms of cAMP concentrations in response to FSH but the A la-Ser variant does so in the presence of a receptor concentration three times lower than to the Thr-A sn variant. This indicates that the A la-Ser receptor variant has a higher affinity for the hormone. This different activity in vitro, however, is not reflected in vivo by differences in parameters which might be FSH-dependent such as serum inhibin B and FSH levels and testicular volume, both in proven fathers and in infertile men subdivided according to their FSH receptor genotype. This suggests that the different receptor activity in vitro might be compensated for in vivo by a lower expression. Finally, the two receptor variants show a mendelian distribution and frequency of the two different alleles similar in proven fathers and in infertile men. This suggests that the FSH receptor genotype is not a major determinant of the fertility status in man.
CONCLUSION

Many experimental and clinical data show convincingly that FSH is necessary for quantitatively normal spermatogenesis in the primate. The recent description of infertility in men with inactive FSH due to mutations of the gonadotropin β subunit suggests that FSH might be necessary for the initiation of spermatogenesis. Both FSH and testosterone, however, are important for completely normal spermatogenesis. FSH alone is able to sustain fertility in the human, as suggested by the FSH receptor activating mutation and by the data from contraceptive trials. Finally, the FSH receptor activating mutation and by sustaining fertility in the human, as suggested by the beta-subunit of follicle-stimulating hormone.

REFERENCES