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Review Article

Genetically modified mouse models addressing gonadotropin function

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ABSTRACT

The development of genetically modified animals has been useful to understand the mechanisms involved in the regulation of the gonadotropin function. It is well known that alterations in the secretion of a single hormone is capable of producing profound reproductive abnormalities. Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally secreted by the human placenta, and structurally and functionally it is related to pituitary LH. LH and hCG bind to the same LH/hCG receptor, and hCG is often used as an analog of LH to boost gonadotropin action. There are many physiological and pathological conditions where LH/hCG levels and actions are elevated. In order to understand how elevated LH/hCG levels may impact on the hypothalamic–pituitary–gonadal axis we have developed a transgenic mouse model with chronic hCG hypersecretion. Female mice develop many gonadal and extragonadal phenotypes including obesity, infertility, hyperprolactinemia, and pituitary and mammary gland tumors. This article summarizes recent findings on the mechanisms involved in pituitary gland tumorigenesis and hyperprolactinemia in the female mice hypersecreting hCG, in particular the relationship of progesterone with the hyperprolactinemic condition of the model. In addition, we describe the role of hyperprolactinemia as the main cause of infertility and the phenotypic abnormalities in these mice, and the use of dopamine agonists bromocriptine and cabergoline to normalize these conditions.

1. Introduction

The gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are produced by the pituitary gland as heterodimers, composed of a common α-subunit and the hormone-specific β-subunit. These hormones have a key role in ovarian and testicular functions by acting through binding to their specific seven-transmembrane domain G-protein coupled receptors [1]. In the gonads, LH receptors are located

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in testicular Leydig cells and in ovarian theca, granulosa and luteal cells [2]. FSH receptors are expressed in testicular Sertoli cells and in granulosa cells of the ovary [3]. Although primarily expressed in gonads, LH receptors are also found in numerous extragonadal tissues [4,5]. However, their physiological significance is still unclear [6]. The placental analog of LH, human chorionic gonadotropin (hCG) interacts with the same LH/hCG receptor, and functions as an LH agonist with a longer half-life and higher biopotency than its pituitary counterpart [7].

There exist physiological and pathophysiological conditions where gonadotropin secretion and/or action are elevated. For instance, human pregnancy is characterized by a transient production of very high levels of hCG by the placenta during the first trimester, which is essential for the maintenance of progesterone production by the corpus luteum gravidarum, and to prepare the uterus for implantation and placental development [7]. hCG is also needed to stimulate fetal testicular testosterone production for masculinization of the male fetus [8]. There are pituitary gonadotrope adenomas, but they rarely produce high gonadotropin levels [9]. On the other hand, postmenopause is a physiological situation where women are exposed to chronically elevated levels of gonadotropins for decades, and this exposure is proposed to be a risk factor for developing ovarian [10–12] and adrenal tumor [13]. A number of articles reported the expression of hCG by a variety of cancers, such as common carcinomas, bladder, lung, pancreatic and colorectal tumors with poor prognosis [14–16]. Finally, activating mutations of gonadotropin receptors, such as those of LH, lead to chronic elevation of the gonadotropin action, with the most conspicuous phenotype of gonadotropin independent male-limited precocious puberty (testotoxicosis) [17].

Several lines of investigation in humans or different animal models have shown that changes in the action of one single hormone, either its secretion or its signaling, are able to affect the integrity of the hypothalamic–pituitary–gonadal (HPG) axis and ultimately cause infertility [17]. Increased LH/hCG action alters the endocrine balance and reproductive function in mice and humans of both sexes. Studies on genetically modified mice provide new insights into the pathophysiological role of LH/hCG in gonadal and extra-gonadal function. We have developed a transgenic mouse model overexpressing the hCGβ subunit that produces chronically elevated levels of bioactive hCG [18,19]. Multiple endocrine alterations were observed in these mice [18–25]. Profound alterations are found in function of the HPG axis, and particularly females present with increased levels of prolactin (PRL) along the lifetime and develop pituitary lactotrope adenomas in later adulthood [18,24,25]. Our recent investigations have focused on the molecular mechanisms by which elevated hCG levels affect PRL regulation in females, and the ways of reversing it by dopamine receptor agonists, such as bromocriptine and cabergoline. This review summarizes the main findings of these studies.

2. Generation of transgenic mice producing high levels of hCG

We generated a transgenic mouse model with hCGβ overexpression by using a conventional pronuclear microinjection technique [18,19]. The mice harboring the hCGβ subunit coding sequence under the human ubiquitin C promoter (hCGβ+ mice) display ubiquitous expression of the transgene both in fetal life and adulthood. hCGβ associates with the endogenously expressed glycoprotein hormone common α-subunit (*Cga*) in the pituitary gland, and produces moderate levels of the bioactive dimer. The dimerization process is expected to occur mainly in gonadotropes and thyrotropes but possibly also in some extra-pituitary tissues, such as the ovary, where *Cga* expression has been detected [26]. Circulating LH/hCG bioactivity is increased about 30-fold in females [18], but only 3- to 4-fold in males [19]. This difference is likely the result of sexually dimorphic regulation of the *Cga* gene in the pituitary gland [27]. The transgenic line was established, and transgenic mice can be produced by breeding the fertile males with wild-type (WT) FVB/N females, since transgenic females are infertile.

3. Hormone profile and reproductive aspects of female transgenic mice

As a consequence of hCG hypersecretion, hCGβ+ females show an altered serum hormone profile with increased levels of estradiol, progesterone and testosterone at peripuberty. From 2 months of age onwards, serum progesterone and testosterone levels continue increasing, but the peripubertally high estradiol rapidly reverts to normal throughout the rest of life [18]. Moreover, no significant differences were observed in FSH levels of hCGβ+ females as compared with WT females at any age studied [18,25]. These hCGβ+ females were also hyperprolactinemic, reaching up to 600-fold increases in serum PRL at the age of 10–12 months. With respect to reproduction, the hCGβ+ females exhibit precocious puberty, with vaginal opening at 21–22 days of age, which is 5–7 days earlier than in WT females. Transgenic female mice also demonstrated alterations in estrous cycles with constant diestrus-type pattern from 45 days onwards. A persistent diestrus has been observed in other animal models with hormonal alterations, such as mice with increased levels of PRL or progesterone, or pseudopregnancy [28,29]. Moreover, mice hypersecreting LH exhibit precocious puberty, and vaginal smears obtained from these females showed a persistent presence of leukocytes [27,30]. In addition, the ovarian morphology of hCGβ+ mice demonstrated the presence of occassional hemorrhagic cysts from the age of 2 months, and enlarged ovaries with massive luteinization resembling luteomas after 6 months of age [18,25]. Furthermore, luteinized unruptured follicles are a frequent histological finding on the hCGβ+ ovaries.

The luteotropic action of PRL in rodents is well known, since this hormone is responsible for progesterone production and corpus luteum maintenance in murine pregnancy [31]. The excessive luteinization observed in transgenic females may be due to the elevated levels of hCG and PRL, which caused a state of constant progesterone overproduction. Elevated LH levels are related with increased androgens concentration and this may be a cause for follicular cyst development [32,33].