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Molecular regulation of steroidogenesis in endocrine Leydig cells

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ABSTRACT

Steroid hormones regulate essential physiological processes and inadequate levels are associated with various pathological conditions. Consequently, the process of steroid hormone biosynthesis is finely regulated. In the testis, the main steroidogenic cells are the Leydig cells. There are two distinct populations of Leydig cells that arise during development: fetal and adult Leydig cells. Fetal Leydig cells are responsible for masculinizing the male urogenital tract and inducing testis descent. These cells atrophy shortly after birth and do not contribute to the adult Leydig cell population. Adult Leydig cells derive from undifferentiated precursors present after birth and become fully steroidogenic at puberty. The differentiation of both Leydig cell populations is controlled by locally produced paracrine factors and by endocrine hormones. In fully differentially and steroidogenically active Leydig cells, androgen production and hormone-responsiveness involve various signaling pathways and downstream transcription factors. This review article focuses on recent developments regarding the origin and function of Leydig cells, the regulation of their differentiation by signaling molecules, hormones, and structural changes, the signaling pathways, kinases, and transcription factors involved in their differentiation and in mediating LH-responsiveness, as well as the fine-tuning mechanisms that ensure adequate production steroid hormones.

1. Introduction

Steroid hormones are essential for life. They regulate critical phases of development and are essential for homeostasis of key physiological functions. Indeed, inadequate levels are associated with various pathological conditions including hormone-dependent cancers (prostate, breast, ovarian), PCOS, and autoimmune and inflammatory diseases (reviewed in [1–3]). Steroid hormone synthesis is finely regulated to ensure adequate amount are produced but also to avoid conditions of hormone insufficiency or excess.

The main steroid producing tissues are the adrenal glands and the gonads, although other organs, such as the brain, the skin, and the white adipose tissue can also produce steroid hormones, albeit at much lower levels (reviewed in [4]). Within the male gonad, steroid hormone production is fulfilled by Leydig cells. Since our ability to reproduce depends on successful gamete production, the need for functional Leydig cells is indispensable.

2. Origin and function of Leydig cells

While Leydig cells were described as early as 1850, their role as a hormone-producing cell essential for proper male sex differentiation was only reported by Bouin and Ancel in 1903 [5]. It is now well accepted that Leydig cells are essential for male reproductive development and health. Androgen production is mediated by two distinct Leydig cell populations: fetal Leydig cells (FLC) and adult Leydig cells (ALC).

The origin of the stem cells giving rise to FLC is still unclear but they likely originate from three possible sources: adrenal–gonadal primordium, mesonephros, or coelomic epithelium (reviewed in [6,7]). FLC stem cells start to differentiate and gradually acquire the ability to synthesize androgens required for masculinization of the foetus [6] before disappearing after birth. The ALC population is derived from undifferentiated spindle-shaped, fibroblast-like mesenchymal Leydig stem cells that are believed to be present during fetal life [8–11]. The source of these cells has been identified as peritubular, interstitial, and/or peri-vascular in mouse and human testes [10–12]. ALC differentiation occurs through well-defined cellular stages and ALCs remain active throughout life [12–14].

Leydig cells regulate male sex differentiation and fertility mainly through the production of INSL3 and testosterone. INSL3

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regulates the first phase of testis descent during fetal life; *Insl3*$^{-/-}$ mice have bilateral cryptorchid testes located high in the abdominal cavity close to the kidneys [15,16]. In adults, INSL3 regulates bone metabolism [17]. As all steroid hormones, testosterone is synthesized from cholesterol through the sequential action of transporters and enzymes. During fetal life, testosterone regulates the second phase of testis descent (reviewed in [18]), induces differentiation of the Wolffian duct, and in the urogenital sinus and genital tubercle testosterone is 5α-reduced into dihydrotestosterone (DHT) which promotes development of the male external genitalia and prostate [19]. At puberty and into adulthood, androgens are required for the development of male secondary sex characteristics and the initiation and maintenance of spermatogenesis (reviewed in [20,21]).

In humans, the importance of Leydig cells is supported by mutations that affect their number and/or function leading to INSL3 and/or testosterone insufficiency. The phenotype of affected individuals varies according to the severity of the mutation; this includes undescended testes, infertility, pseudohermaphroditism, and ambiguous genitalia. Mutations have been identified in the genes encoding the LH receptor (LHCGR) [22], INSL3 [23–25], and enzymes involved in testosterone biosynthesis [26–44]. Gene mutations affecting transcription factors associated with disruption of Leydig cell development and/or function in humans have also been described (see below).

3. Regulation of Leydig cell differentiation and function

3.1. Signaling molecules, hormones, neural link, and structural changes

Differentiation of both Leydig cell population is under the paracrine action of Sertoli cell-secreted factors. Two signaling systems are essential: desert hedgehog (DHH) and platelet-derived growth factor (PDGF)-A [45–48]. Sertoli cells produce these ligands, whereas Leydig cells express the receptors (PTC1 for DHH and PDGFRA for PDGFA). Gene inactivation in mice showed that male *Dhh*$^{-/-}$ and *Pdgfa*$^{-/-}$ mice were pseudohermaphrodites with feminized external genitalia [45–48]. A similar human phenotype was described for a 46 XY patient with a *DHH* mutation [49].

In addition to these signaling molecules, the pituitary hormone LH along with other locally produced factors also contribute to ALC differentiation and to the maintenance of their steroidogenic function [12–14,50,51]. For instance, osteocalcin produced by the bone acts on Leydig cell via its G protein-coupled receptor GPRC6A to stimulate steroidogenesis (Fig. 1). Mice deficient in *osteocalcin* or *Gprc6a* display reduced steroidogenesis, feminization, and increased LH production consistent with dysfunctional Leydig cells [52–54]. Similarly, mutations in the *osteocalcin* gene have been associated with primary testicular failure in humans [52]. In Leydig cell, osteocalcin is required for proper expression of several genes involved in testosterone biosynthesis by acting, at least in part, via the CREB transcription factor [54].

Acting in an autocrine/paracrine manner are growth factors and cytokines. Growth factors include insulin growth factor I (IGF-I) and fibroblast growth factor 9 (FGF9), which enhance steroidogenesis [55,56]. On the other hand, the cytokine transforming growth factor beta (TGFβ1), Müllerian-inhibiting substance (MIS or anti-Mullerian hormone, AMH), tumor necrosis factor alpha (TNFα), interleukin 1 (IL-1) and IL-6 have primarily inhibitory effects on steroidogenesis [57–60]. Although their mechanisms of action remain to be fully characterized, TNFα and TGFβ1 were reported to act by interfering with the nuclear receptor NUR77 (NGFI-B, NR4A1) [61,62], a key regulator of several steroidogenic genes (reviewed in [63]). Another essential autocrine/paracrine regulator is testosterone itself. Inactivation of the androgen receptor (AR) gene in the mouse revealed that AR signaling is dispensable for fetal Leydig cell differentiation and function but essential for the maturation of the adult Leydig cell population and for the expression of several steroidogenic genes [64,65].

A pathway independent of the hypothalamo–pituitary axis was also reported to regulate Leydig cell steroidogenesis. It involves a direct, multisynaptic neural pathway between the brain and the testes, which leads to a rapid inhibition of testosterone production [66,67].

Finally, Leydig cell steroidogenesis is also regulated by structural changes occurring at the level of the mitochondria. Mitochondrial fusion and fission, collectively known as mitochondrial dynamics, is essential to maintain the integrity of this organelle in every cell type (reviewed in [68]). In Leydig cells, hormonal stimulation triggers mitochondrial fusion through the up-regulation of the fusion protein Mitofusin 2, a process that is essential for steroid hormone production [69]. Mitochondrial fusion also regulates STAR protein localization at the mitochondria after hormone stimulation [70]. Similarly, association between endoplasmic reticulum and mitochondria leading to the formation of mitochondria-associated membrane was shown to be important for hormone-induced steroidogenesis in Leydig cells [71].

3.2. Intracellular signaling pathways

To function adequately, Leydig cells require the action of pituitary LH. As shown in Fig. 1, LH binds to its receptor, LHCGR, located on the surface of Leydig cells, which triggers activation of adenylate cyclase leading to increased cAMP production [72]. This in turn activates of several kinases, the best studied being protein kinase A (PKA) and PKC (reviewed in [73]).

Another important contributor to LH-induced steroidogenesis is the epidermal growth factor receptor (EGFR) (Fig. 1). Indeed, inhibition of EGFR signaling blunts LH-induced steroidogenesis in Leydig cells [74]. It was later reported that activation of LHCGR leads to a transient cAMP-dependent activation of the EGFR and downstream mitogen-activated protein kinase (MAPK) cascade [75]. The involvement of the MAP kinases extracellular signal-regulated kinases 1 and 2 (ERK1/2) downstream of PKA have long been known to be essential for proper LH-induced steroidogenesis in Leydig cells [76–82] (Fig. 1).

The cGMP signaling pathway was also found to stimulate steroidogenesis. cGMP is produced by the enzyme guanylate cyclase (GC) following exposure of Leydig cells to natriuretic peptides (ANP and CNP) or to nitric oxide. The cGMP pathway was found to act mainly via the protein kinase G at the level of the STAR protein to increase basal steroid hormone production in Leydig cells [83].

In addition to the increase in intracellular cAMP/cGMP levels, a transient increase in cytoplasmic Ca^{2+} concentration following tropic hormone stimulation is also essential for proper steroidogenesis [84] (Fig. 1). Murine Leydig cells contain two main L-type Ca^{2+} channel receptors: the ryanodine receptors I, II, and III, and the inositol triphosphate receptors I, II, and III [85]. While both types of receptors are required for the LH-induced rise in cytoplasmic Ca^{2+} levels, the Ca^{2+} influx was found to originate mainly from activation of the ryanodine receptors located on the endoplasmic reticulum [85,86]. Increased intracellular Ca^{2+} concentration activates the Ca^{2+}/calmodulin kinase kinase (CaMKK) pathway, which in turn leads to activation of the Ca^{2+}/calmodulin kinase I (CAMKI). CAMKI was found to be present in Leydig cells, activated downstream of cAMP following hormonal stimulation, and essential for maximal hormone responsiveness [87,88] (Fig. 1). These data expand the scope of cAMP action in Leydig cells and provide alternate signaling routes leading to proper gene expression in response to cAMP.