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Nonsteroidal Regulation of Testicular Function

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INTRODUCTION

The physiology of an organ such as the testis is significantly more complex than the functions associated with an individual cell type. This intricate physiology reflects the ability of different cell types within the organ to interact and communicate. Therefore, an understanding of the regulation of testis function requires a consideration of the interactions between different cell types. Several cell types make up the testis and the seminiferous tubules where the process of spermatogenesis occurs. The epithelial-like Sertoli cells form the tubule and provide the physical support and microenvironment required for germinal cell development. The mesenchymally derived peritubular cells surround and provide structural integrity to the tubule. Within the interstitium between tubules are the Leydig cells responsible for the production of androgen. The androgens act on the seminiferous tubules to maintain the process of spermatogenesis.

The endocrine regulation of testis function primarily involves the actions of the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). These are two essential nonsteroidal agents required in the regulation of testis function. LH acts specifically on Leydig cells to induce the production of androgen that subsequently acts on the seminiferous tubule at either peritubular cells or Sertoli cells. This action of LH is also required to maintain normal circulating levels of androgens. FSH acts specifically on Sertoli cells to provide cellular differentiation during pubertal development and maintain optimal function in the adult testis. The remaining nonsteroidal agents involved in the regulation of testis function mediate local cell-cell interactions. In general, these cellular interactions are induced or influenced by gonadotropins and/or androgen.

The two primary and essential functional parameters of the testis are the production of sperm and androgen. The complex network of cell-cell interactions mediated, by both steroidal and nonsteroidal agents, have evolved to maintain and control the spermatogenic and steroidogenic processes. The two aspects of cellular physiology that require regulation are growth and differentiation. Growth and differentiation are interrelated, but controlled by distinct regulatory agents. During pubertal development cell growth is required and at the onset of puberty individual cell types have specific growth requirements. A class of nonsteroidal regulatory factors involved in this growth regulation are growth factors. The influence that hormones have on cell proliferation often are indirectly mediated through the local production of these growth factors. Throughout testis development and particularly at the onset of puberty, cellular differentiation is induced and maintained for various cell types to acquire specific functions. Therefore, another class of nonsteroidal regulatory factors are involved in the control of cell differentiation. In considering the regulation of testicular function the factors involved include 1) the ability of gonadotropins to act on the testis, 2) cell-cell interactions mediated by both steroidal and nonsteroidal factors, 3) the control of cellular growth and differentiation, and 4) the maintenance of the spermatogenic and steroidogenic processes. Examples of nonsteroidal factors involved in this process will be discussed.

GROWTH REGULATION

All the cell types in the testis proliferate during fetal development and prepubertally. At the onset of puberty Sertoli cell growth is arrested and cell differentiation is induced. Germinal cell growth is increased and the spermatogenic process initiated. Leydig cell and peritubular cell growth is decreased but continues at a reduced rate in the adult (1). Therefore a number of changes in cell growth are required at the onset of puberty. A number of different growth factors have been shown to be produced in the testis (2) and have been postulated to mediate numerous cell-cell interactions in that gland (Table 1) (3).

The ability of FSH to influence Sertoli cell growth factor production will be used as an example of nonsteroidal growth regulation. Sertoli cells have been shown to produce the growth stimulators transforming growth factor-alpha (TGFα) (4) and fibroblast growth factor (FGF) (5), as well as the growth inhibitor transforming growth factor-beta (TGFβ) (6). Observations have been obtained on the developmental and hormonal regulation of TGFα, TGFβ and FGF expression and action. TGFα expression declines gradually during pubertal development to constant level and is not responsive to hormones. TGFα acts at the epidermal growth factor receptor (EGFR) that was found to be expressed by Leydig, peritubular, Sertoli and germinal cells. Peritubular cells and Leydig cells were responsive to TGFα *in vitro*. FGF

Table 1. *Growth Factors in the Testis*

Growth Factor	Proposed Site Production	Proposed Site of Action	Proposed Function*
IGF-1	Leydig	Leydig	+Steroidogenesis
	Peritubular	Peritubular	+Growth
	Sertoli	Sertoli	+Growth/Differentiation
		Germinal	?
TGF-α	Peritubular	Leydig	–Steroidogenesis
	Sertoli	Peritubular	+Growth
	Leydig	Sertoli	±Differentiation
		Germinal	?
TGF-β	Peritubular	Leydig	–Steroidogenesis
	Sertoli	Peritubular	–Growth/+Differentiation
		Sertoli	+Differentiation
		Germinal	?
IL-1	Sertoli	Leydig	–Steroidogenesis
		Germinal	?
FGF	Sertoli	Leydig	±Steroidogenesis
		Sertoli	+Growth
NGF	Germinal	Sertoli	?

* (+) denotes an increase, (–) indicates a decrease and (?) represents an unknown function.

expression by Sertoli cells remained relatively constant during development and was found to be induced by FSH. FGF has been shown to act on Sertoli cells to promote cell proliferation (5,7) and localize in germinal cells (8). FSH induced Sertoli cell FGF expression may provided an indirect mechanism to control Sertoli and/or germinal cell growth. The three isoforms of TGFβ (TGFβ1, TGFβ2 and TGFβ3) have distinct patterns of expression during development. TGFβ1 remains relatively constant during development and is not hormone responsive. Interestingly, TGFβ3 expression by Sertoli cells has a dramatic transient increase in expression at the onset of puberty that declines after the induction of the spermatogenic process. Therefore, TGFβ3 may be involved in the induction of the pubertal process and germinal cell development. This transient increase in TGFβ3 is not responsive to hormones such as FSH or androgen. TGFβ2 expression by Sertoli cells was only present prepubertally and declined to negligible levels at the onset of puberty. TGFβ2 production was dramatically suppressed by the actions of FSH. Therefore, at the onset of puberty FSH actions on Sertoli cells cause a suppression of TGFβ2 expression and increase in FGF expression. The absence of a growth inhibitor such as TGFβ2 may allow growth stimulators such as TGFα and FGF to promote germinal cell proliferation that is inhibited prepubertally by the growth inhibitor.

This observation of the actions of FSH on Sertoli cells demonstrates that hormones can act on the testis to influence the local production of growth

factors. These growth factors subsequently mediate cell-cell interactions and influence testis growth. Hormones can indirectly through the actions of these nonsteroidal agents, influence cell proliferation and tissue growth.

DIFFERENTIATION REGULATION

During development all testis cell types undergo alterations in cellular differentiation to acquire unique and essential cellular functions. Leydig cells are derived from the precursor mesenchymal cell population in the interstitium during fetal and pubertal development to maintain a high basal androgen production in the adult. Peritubular cells differentiate in response to androgens prior to puberty. Sertoli cells differentiate throughout pubertal development. Germinal cell differentiation and development is initiated at the onset of puberty. Gonadotropins and androgens have an important role in the differentiation process of these cell types. Equally important, however, is the local production of nonsteroidal agents that act as differentiation-type factors and regulate this process Table 2.

Table 2. *Regulation of Cellular Differentiation/Function*

	Source	Agent	Action
Sertoli	Endocrine	FSH	Increase
	Peritubular	PMODS	Increase
	Leydig	Androgen	(?)Increase
		POMC	Modulate FSH
	Germinal	(?) Factors	Increase
Leydig	Endocrine	LH	Increase
	Sertoli	(?)Factors	Increase/Decrease
Peritubular	Leydig	Androgen	Increase
	Sertoli	(?)Factors	Increase
Germinal	Sertoli	(?)Factors	Increase

(?) Represents uncharacterized factors.

The agents that influence Sertoli cell differentiation will be used as an example of nonsteroidal differentiation regulation. The induction of Sertoli cell differentiation results in the expression of a large number of unique secretory products postulated to be essential for germinal cell development. An example of such a Sertoli cell product is transferrin that can bind and transport iron to developing germinal cells. FSH can promote Sertoli cell differentiation and the production of most of these secretory products. Although androgen actions on the seminiferous tubule are essential for the maintenance of Sertoli cell differentiation *in vivo*, the *in vitro* actions of

androgens on Sertoli cells are negligible. Androgens, however, also act on peritubular cells that can produce a paracrine factor termed PModS that induces Sertoli cell differentiation (9). PModS appears to be a differentiation-type factor and has a more dramatic effect on Sertoli cell functions *in vitro* than any individual agent previously identified, including FSH (9,10). A cascade of cell-cell interactions is proposed in that LH acts on Leydig cells to promote androgen production that acts on peritubular cells to promote PModS production which subsequently induces Sertoli cell differentiation and functions required for germinal cell development.

This observation of the regulation of Sertoli cell differentiation demonstrates that hormones can act on the testis to influence the local production of differentiation factors. These differentiation-type factors subsequently mediate cell-cell interactions and influence testis physiology. Through the actions of these nonsteroidal agents, hormones can indirectly influence testis development and cellular differentiation.

SUMMARY

The examples given of the regulation of Sertoli cell growth and differentiation demonstrates that a complex network of cell-cell interactions are mediated by nonsteroidal agents. These nonsteroidal agents play an integral role in the regulation of testis function. The ability of gonadotropins and androgen to influence testis physiology is critical, however, this underlying network of cellular interactions and nonsteroidal growth and differentiation agents provide the actual mechanism by which testis function is regulated. The elucidation of how testis function is regulated will require an understanding of these cellular interactions and the nonsteroidal agents involved.

As previously noted, all of these cellular interactions and this complex regulation of testis function is required to control the steroidogenic and spermatogenic processes. In understanding the regulation of testis function, several aspects of testis physiology must be considered. In the adult testis, spermatogenesis is optimal with minimal regulation and androgen levels within the testis are an order of magnitude greater than that required to stimulate testis function. Therefore, adult testis function is an optimal steady state condition that requires maintenance, but not an active regulation to alter cellular functions. The endocrine regulation and the local network of cell-cell interactions in the adult testis is required to maintain optimal testis function. Factors such as androgens, gonadotropins and paracrine growth and differentiation factors keep testis function maintained. Although an active regulation of testis function is required during fetal and pubertal development, in the adult testis maintenance of optimal function is required. In considering the function of nonsteroidal factors in the testis, this aspect of testis physiology needs to be considered.

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