Follicle stimulating hormone (FSH) plays a critical role in regulating ovarian function and is a key hormone in initiating follicular recruitment and promoting follicular maturation (1). Additionally, it influences differentiation by modulating steroidogenesis, inducing luteinizing hormone receptors and regulating the synthesis of ovarian hormones, such as inhibin and activin. Inhibin is an important regulator of FSH synthesis and secretion in rats (2), while activin appears to be a paracrine regulator of ovarian function (3, 4) that may also be involved in FSH regulation (5, 6). The study of how these two genes are regulated is important for understanding aspects of the reproductive cycle. This report focuses on the regulation of inhibin and activin gene expression in rat granulosa cells (GC) by gonadotropins.

The regulation of inhibin and activin expression is a complex issue since these hormones are structurally related but have functionally opposing effects, at least on FSH secretion. Inhibin is composed of one α-chain and one of 2 β-chains (βA or βB) that associate to form a 32-kD glycoprotein (7–10). Activin is a 28-kD dimer composed of 2 of the β-chains (5, 6). While inhibin suppresses FSH secretion from cultured pituitary cells, activin stimulates it (2). Inhibin and activin have both been identified in follicular fluid and are synthesized by the ovary. The identification of cells that produce inhibin has been facilitated by the availability of antibodies that recognize this protein. Inhibin has been shown to be localized to the GC of developing follicles (11–13), and these cells have also been shown to contain α-, βA-, and βB-mRNAs by in situ hybridization (14–16). It is not clear at present if the same cells can also produce activin and if so, under what conditions.

FSH is an important regulator of inhibin expression both in vivo (17–20) and in vitro (11). In general, FSH acts to increase inhibin secretion and
mRNA levels; however, there are exceptions to this. During much of the rat estrous cycle, FSH induces α- and β-mRNA levels in developing follicles (14, 16). However, the ovulatory surge of LH and FSH strongly suppresses expression of the two mRNA species (20, 21). While α- and β-mRNAs are regulated by FSH in the ovary, α- but not βB-mRNA is regulated by gonadotropins in the testis (22, 23). The net effect of FSH on inhibin synthesis may depend on whether other hormones are present or absent, which tissue is being examined, and what stage of differentiation the follicles have reached.

The individual α- and β-genes appear to be regulated by different mechanisms since there are several conditions in which they are not coordinately expressed. Expression of α-mRNA has been reported in theca interstitial cells and in newly formed corpora lutea, as well as in GC; however, β-mRNA is found exclusively in GC of the ovary (15, 16). Expression of α-mRNA is also seen in follicles that do not express β (15, 16, 20, 21). In the male, α-gene expression occurs predominantly in Sertoli cells but is also found in Leydig cells, while βB-expression is seen only in Sertoli cells (24). Beta mRNA expression has also been seen in tertiary follicles just prior to ovulation (16) and in small antral follicles of the monkey (25) at times when α-expression is not observed. In addition, comparison of the α-genes (26 and Pei, et al., submitted) and βB-genes (26) reveals that the promoter regions have very different structures.

The inhibin α and β genes can be either co-expressed or independently expressed, and it is likely that the regulation of this expression pattern will prove to be complex. We describe here several examples that indicate the important role of FSH in regulating these genes, both in vitro and in vivo. We then briefly consider the use of GC to examine the expression and regulation of cloned and transfected α-inhibin genes.

Materials and Methods

In Situ Hybridization

Twenty-three-day-old female Sprague-Dawley rats (Harlan) were injected subcutaneously with pregnant mare’s serum gonadotropin (PMSG) (10 IU) or vehicle alone. Rats were sacrificed 53 h after the final injection, and ovaries were processed for in situ hybridization analysis as described (15, 27) using 35S-labeled riboprobes specific for the α- and β-cDNAs (14 and Dykema, unpublished).

Granulosa Cell Culture

Granulosa cells were cultured using the procedure described by Epstein-Almog and Orly (28). Ovaries were obtained from immature female Sprague-Dawley rats (23–27 days of age, Harlan). Granulosa cells were cultured in