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COMMENTARY

Regulation of Cellular and System Function by Activin

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ABSTRACT. Activin is an important molecule that regulates hormonogenesis, cellular homeostasis (divide or die pathways), and differentiation programs (developmentally and in adult cells). The cellular mechanisms that integrate an activin signal into a physiological response include a binary receptor complex and tandem serine threonine kinases, intracellular signal mediators, and nuclear transcription factors. Activin antagonists (inhibins) and bioneutralizing binding proteins (follistatins) act as gating molecules to ensure accurate delivery of activin signals to cellular machinery. Correct execution of an activin cue intracellularly permits actions as fundamental as embryonic mesoderm development, neuronal survival, hematopoietic function, and reproductive cyclicity. Absent or incorrect activin signaling results in phenotypes as catastrophic as embryonic lethality, tumor formation, and infertility. The general ways in which a cell senses and responds to an activin signal will be reviewed in the first part of this paper. The role of this ligand in reproductive function will also be examined as a specific example of activin activity. BIOCHEM PHARMACOL **55**;7:953–963, 1998. © 1998 Elsevier Science Inc.

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LIGANDS AND RECEPTORS

Activin, Inhibin, and Related Proteins

Activin is a member of the TGFβ† family of secreted proteins [1]. The extended family members encode dimeric, disulfide-linked ligands that regulate fundamental cellular functions including growth, differentiation, and tissue reorganization [2–5]. In addition to activin, the diverse group of ligands includes five TGFβ isoforms, a group of BMP, MIS, the GDF, OP, nodal, and the distantly related GDNF [3–7]. Non-mammalian homologues are found in *Drosophila* (*dpp*, 60A, and screw), *Xenopus* (*VG-1*, Xnr, and TGFβ$_5$), and sea urchin (Univin) [7, 8]. Assignment to the superfamily is based on a seven-cysteine motif in a conserved C-terminal domain [1, 3].

Activin is synthesized as a homo- or heterodimer of two highly related β-subunits (β_A and β_B), resulting in three possible molecular species [1, 2, 5]. The activin isoform nomenclature reflects the type of subunit assembly: activin A (β_A-β_A), activin B (β_B-β_B), and activin AB (β_A-β_B). Three additional β-subunits have been cloned: mammalian β_C- and β_E-subunit and *Xenopus* β_D-subunit [9–11]. Assembly of these subunits into active dimers has not been demonstrated to date. Inhibin is an activin antagonist composed of an activin β-subunit (β_A or β_B) and a dissimilar α-subunit [1, 5]. This heterodimeric protein is distinct among the TGFβ family members, and the α-subunit bears little sequence similarity to other superfamily members.

Activin and most members of the TGFβ family are produced as precursor proteins, which are cleaved to form bioactive ligands [1, 2]. Uncleaved precursor regions of TGFβ act as bioneutralizing binding domains allowing latent protein to be produced but not activated until processed at a cell surface [12, 13]. A separate bioneutralizing binding protein, follistatin, exists to biomodulate activin activity [14]. It is likely that activin and follistatin participate in ultra-short loop regulation pathways in many tissues (for example, in the pituitary) [14, 15]. Activin also binds the broad spectrum protease inhibitor α2-macroglobulin with low affinity [16, 17].

Overall sequence similarity between TGFβ family members is as low as 29%; however, analysis of the crystal structures of TGFβ$_2$ and OP-1 suggests a three-dimensional fold that may confer topological similarity between superfamily members [18–20]. A central cysteine knot is gener-

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† *Abbreviations:* TGFβ, transforming growth factor β; BMP, bone morphogenic protein(s); MIS, Müllerian inhibiting substance; GDF, growth and differentiation factor(s); OP, osteogenic protein; GDNF, glial-derived nerve growth factor; *dpp*, decapentaplegic gene product; RI, activin type I receptor; RII, activin type II receptor; GDNFR-α-GDNF receptor alpha subunit; FTNA, alpha-subunit of the small G-protein p21ras farnesyltransferase; RIII, activin type III receptor; ARE, activin response element; IP3R, inositol phosphate receptor; RyR, ryanodine receptor; FAST-1, forkhead activin signal transducer-1; FGF, fibroblast growth factor; *Mad*, *mothers against dpp*; PCOS, polycystic ovarian syndrome; FSH, follicle stimulating hormone; LH, luteinizing hormone; GnRH, gonadotropin releasing hormone; and IGF, insulin-like growth factor.

ated by disulfide bridges between 6 of the 7 conserved cysteine amino acids in the mature (C-terminal) portion of the protein. Four antiparallel β-sheets emanate from the knot, suggesting finger-like projections. Facing perpendicular to and opposite the cysteine knot is an α-helix, which is the anthropomorphic "heel" of an overall structure that can be described as a left hand. The individual subunits of OP-1 or TGFβ$_2$ are joined by the seventh unpaired cysteine and fit together as two hands shaking. It is proposed that all members of the TGFβ superfamily will have similarly ordered three-dimensional structures. Based on the structural information provided by the crystal structure of TGFβ$_2$, functional assignments of some TGFβ$_1$ and TGFβ$_2$ receptor binding domains have been made and exist outside the cysteine knot [21, 22]. Generation of activin mutants will provide additional information regarding the structure/function relationship of this ligand to its receptor or binding protein.

Cell Surface Receptors

The receptor for the TGFβ family of ligands is binary, composed of two single membrane spanning serine-threonine kinases designated type I and type II [2–8]. Receptor similarity exists between members of the superfamily; however, discrimination between diverse ligands is high and is predicated on differential ligand binding to RII [23–27]. The activin receptor complex is composed of two single membrane spanning subunits designated type RI (50–55 kDa) (RI) and type RII (70–75 kDa) (RII) [28–31]. Dominant negative and constitutively active receptors have been generated and used to elucidate important structure/function characteristics of these membrane bound adapter proteins. First, RII is constitutively phosphorylated, and phosphorylation of RII is not necessary for activation of downstream signaling activities [31–33]. Second, RII can bind activin in the absence of RI; however, signal transduction pathways are not initiated solely by ligand binding to this receptor subunit [29, 30, 33]. Instead, ligand-RII binding activates RII serine-threonine kinase activity of the receptor subunit [29, 30, 33]. Mutations and truncations of the RII serine-threonine kinase domain block signaling pathways in a dominant-negative fashion [34, 35]. RI phosphorylation occurs in a juxtamembrane 30 amino acid region described as the GS box [33, 36]. The GS box is glycine and serine rich, and mutation of all serine and threonine residues abrogates phosphorylation and inhibits downstream signal transduction activity [33, 36]. Interestingly, mutations of single residues fail to inhibit activity of RI. Conversely, mutations resulting in phosphorylation mimetics constitutively activate signal transduction cascades in a ligand- and RII-independent manner [33, 36]. Analogous conclusions can be drawn from mutational analysis of TGFβ receptor subunits [37–42]. Thus, the functional holoreceptor complex consists of specific ligand and kinase-intact RII and RI subunits.

Five activin RII isoforms exist which have different affinities for activin A (affinity for activin B and activin AB are untested) [25, 31]. In addition, two activin RI genes exist, and the combinatorial association of various RII(B) with RI subunits provides an added layer of complexity and cellular signaling specificity [43, 44]. Exceptionally broad distribution patterns of receptors are found in the embryo [45–47] and brain [48, 49]. The cellular logic preserving conserved receptor subunits differing by ligand affinity and expression pattern has not been clearly delineated. Redundancy, tight control of embryonic development using a few conserved ligands to maximize cellular control, plasticity in embryonic to adult transition of activity, and multifactorial regulation of expression patterns (by growth factors, by steroids, by self) are plausible reasons for this diversity.

Genetic Deletion of the Activin System

Confounding the ability to clarify the role of the ligands and receptors in specific cellular functions is the ambiguity of results generated from genetic deletion of activin or its receptors [50–52]. Activin A and activin AB deficient mice develop normally through fetal life, and then die at birth with defects in craniofacial development (cleft palate and lack of whiskers) [53]. Activin B deficient mice grow to adulthood and have no eyelids and reduced reproductive function [54]. Curiously, activin RII subunit deficient mice develop normally through adulthood with reduced fertility due to lower FSH production [55]. These results suggest several possibilities. First, activin may not be essential for prenatal development of all organ systems in which RII/RI is expressed. Second, RII/RI may utilize a ligand other than activin. A candidate ligand that binds the activin RII subunit is OP-1 [56]. OP-1 regulates bone formation, is expressed in the embryo, and may account for the normal development of the null mice in the absence of an activin signal. Further adding to the complexity of this system is the phenotype of animals deficient in the activin binding protein follistatin [57]. Loss of follistatin function results in widespread defects in muscle, skin, bone, and tooth development. Modulation of previously uncharacterized ligands may produce this phenotype. The best example of overlap between receptor, ligand, and predicted physiology is the *MIS* gene family [58]. *MIS* RII knockout animals and *MIS* knockout animals develop into pseudohermaphrodites. Functional assignment for the activin system awaits further study.

TGFβ Family Member Receptor Classes

TGFβ causes oligomerization of two type II receptors and two type I receptors [59]. Whether this tetrameric protein structure is required for activin signal propagation is unknown. The association of two dissimilar serine-threonine kinase receptor subunits into an active complex is required for all members of the TGFβ family except for GDNF [2, 6, 8, 60, 61]. GDNF, a distant TGFβ family member, binds a distinct receptor class. GDNF binds GDNFR-α, which is

anchored in the cell membrane through a glycosylphosphatidyl inositol anchor [61]. The GDNF–GDNFR-α complex then binds and activates a membrane spanning tyrosine kinase receptor called Ret tyrosine kinase [62]. A third class of receptor in the TGFβ family is the type III receptor (RIII). TGFβ RIII was first identified as a β-glycan present on the surface of most cells [63]. RIII has no signaling motif; however, it is required for $TGF\beta_2$ activity in a cell. Cell surface, membrane anchored binding proteins for activin have not been identified.

Inhibin will bind (with low affinity) to activin RII [2, 31]. It is therefore likely that some of the antagonism between inhibin and activin is based upon competition for the RII subunit. The existence of a separate, inhibin-specific receptor or signal transduction pathway has been hypothesized but is difficult to prove. The activity of inhibin in biological assays may be interpreted as an inhibition of basal activin activity; however, inhibition of pituitary FSH release by inhibin ($ED C_{50}$ = 8.3 pM) cannot be explained by the lower affinity interaction of inhibin for the activin receptor (20- to 200-fold lower) [64, 65]. Supporting the hypothesis that a separate inhibin receptor exists, an inhibin specific binding moiety has been identified in a hematopoietic cell line (K562), and inhibin specific binding is noted in the ovary and testes [66–68]. This putative receptor protein indicates that inhibin activity may involve proteins in addition to the receptor subunits of the activin signaling pathway. By analogy to the classes of TGFβ family receptors described above, inhibin may bind a yet unidentified type II receptor, may bind and activate an RI (similar to BMP), may have a structurally dissimilar receptor (similar to GDNF), or may use an adapter protein such as RIII to act in concert (or competition) with activin type RII or RI receptors. The existence of an inhibin-specific receptor and competition with the activin receptor are not mutually exclusive conclusions. The complete elucidation of the functional relationship between inhibin and activin receptors awaits the cloning of the inhibin receptor.

INTRACELLULAR MECHANISM OF ACTION

Receptor-Proximal Protein Partners

The mechanism by which activin and other TGFβ family members exert tight and specific control over multiple intracellular processes involves signaling components shared with other receptors as well as components that appear to be uniquely activated by this group of ligands. Rapid progress in delineating post-receptor signaling cascades has been made using the "yeast two-hybrid" system, wherein proteins that interact with the various receptor subunits have been identified. Thus far, only one protein that interacts with RII (TRIP-I) has been identified, and it binds only TGFβ receptors and does not associate with activin receptors [69]. More success has been achieved using the signaling TGFβ/activin RI moiety as the protein bait. This strategy has resulted in the identification of two proteins: an immunophilin called FKBP-12 [70] and the FTNA [71]. Both proteins bind the RI subunit of activin and TGFβ. FKBP-12 is a protein that has rotamase activity, which assists in protein folding and inhibits the intracellular calcium receptors IP3R and RyR [72, 73]. While the mechanism is not clear, FKBP-12 has been shown to inhibit TGFβ, activin, and MIS signaling pathways, perhaps acting as a docking protein for a cytoplasmic inhibitor [70, 74]. A candidate inhibitor is the serine/threonine phosphatase calcineurin, which is a known FKBP-12 interacting protein and also participates in calcium-dependent pathways in a variety of cells. The role of activin in calcium mobilization has been studied in several cell systems; however, a unified theory of activin intracellular actions in this area is still lacking [75, 76].

A second interaction with TGFβ and activin RI involves the FTNA [71, 77, 78]. FTNA activates both wild-type and oncogenic *Ras* by stabilizing Ras membrane localization (enzymatically attaching a 15-carbon farnesyl residue to a cysteine near the carboxyl-terminus) [79]. The alpha-subunit is not catalytic, but stabilizes and regulates the specific beta-subunit. It is possible that in some cells binding of ligand to RII causes phosphorylation of RI and the release of an active p21ras alpha-subunit. The ability of activin to regulate Ras may provide an explanation for the growth modulatory role of the ligand. Consistent with Ras acting downstream of activin, overexpression of a dominant-negative p21ras mutant in *Xenopus* embryos blocks activin-induced mesoderm induction, while a constitutively active p21ras-alpha-subunit mutant mimics activin action [80]. However, neither Raf nor MAP kinase is activated by activin, implying a novel activin signaling pathway distal to Ras or suggesting the necessity of a secondary factor (perhaps FGF in the case of mesoderm induction) to activate the system fully [81]. TGFβ signaling pathways involving Ras and ERK1 have been delineated; however, there are no data supporting a role for activin in modulating Ras-dependent pathways [82]. Clearly, additional research is needed to define the role of activin in modulating Ras-dependent activities in a variety of cell systems.

Direct Kinase Substrates of Activated RI

Target cytoplasmic substrates for activated TGFβ superfamily receptors have been identified using *Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis*, and mouse genetic systems. In *Drosophila*, complementation studies have identified the most likely intracellular modifier of the TGFβ family member, *dpp* [83]. Homologues of the gene, identified as *Mad* (*mothers against dpp*), have been identified in *Xenopus*, *C. elegans*, and mammals (*Smads*, *Xmads*, *Madrs*) [84, 85]. After initial confusion in the literature due to obtuse nomenclature of homologous factors isolated from various species, a consensus document was published, which renamed the cytoplasmic factors Smad [86]. Additionally, numerical assignments coordinating the appropri-

ate Smad with its ligand were established [86]. By convention, then, Smad1 is the BMP-associated co-factor [85–88]. Smad2 is activin and TGFβ specific [85–91]. Smad3 is TGFβ specific [92]. Smad4 was identified as a deleted gene product in spontaneously arising pancreatic cancers that were TGFβ insensitive [92–96]. This tumor suppressor gene (also known as a member of the *dwarfin* gene family) is required for both activin and TGFβ activity. Both *Smad2* and *Smad4* reside on human chromosome 18q21, which is a region deleted in human pancreatic and colorectal cancers [90, 93].

Smad1, Smad2, Smad3, and Smad4 have conserved domains in the N-terminus (MH1) and C-terminus (MH2) and are separated by a dissimilar serine-threonine hinge region. C-terminal serines have been defined as phosphorylation targets in Smad2, and phosphorylation of Smad2 is required for concentration of Smad2 into the nucleus [91]. Injection of Smad2 into *Xenopus* embryos causes a secondary axis, a result identical to that obtained after activin injection [85]. Furthermore, co-injection of activin mRNA enhances the lacZ-Smad2 nuclear localization in *Xenopus* embryos. Additionally, excision of the C-terminal domain of the Smads confers transcriptional activation on synthetic targets, suggesting that the Smads are present in the cytoplasm as inactive, latent proteins. Within the nucleus, the Smads are functional co-activators or co-repressors of DNA-binding proteins and activin/TGFβ-dependent gene transcription. Assembly of heterodimeric and homodimeric Smad proteins has been demonstrated following receptor activation by activin, BMP, or TGFβ, indicating that intracellular specificity of response is dependent on the type of Smad activated by ligand [89, 92].

The implication that these cytoplasmic regulators of TGFβ or activin action act as tumor suppressors may be an important clue in the study of the etiology of human cancer. Indeed, loss of function at any level of the activin/TGFβ-dependent cell regulation can result in abnormal cellular proliferation. Smad4 was identified as a loss of function gene in spontaneously arising pancreatic cancers. Further, cell lines that are TGFβ insensitive or activin insensitive have been studied, and they lack either an intact receptor system or intracellular Smad [97]. Conversely, overexpression of Smad4 causes cell cycle arrest in some cells [95]. Imbalance in the local activin milieu can also result in tumor development. Genetic elimination of the inhibin α-subunit, thereby creating an unopposed activin signal, results in gonadal tumors. This result led to the assignment of the α-inhibin subunit as a tumor suppressor gene. Clearly, the study of activin and receptor targets as mediators of cancer phenotypes merits further analysis.

Nuclear DNA Binding Proteins That May Associate with Active Smad

Two downstream DNA-binding proteins that interact with the Smad cofactors have been identified. Using similar complementation strategies as those used to define Smad, the Drosophila genes *schnurri*, *sal*, and *salr* have been identified as candidates for transmission of co-activator signal to the DNA transcription machinery [98–100]. *Schnurri* is homologous to the mammalian zinc finger protein MBP-1 (or PRDII-BFI or HIV-EP1) and binds κB sites present in enhancer regions of many cellular (and viral) genes [101]. Expression of inflammatory or immune response genes is enhanced by occupation of the κB DNA site. NF-κB (a Rel family heterodimeric protein complex) also binds the decapeptide nuclear κB sites. Signal propagation via κB sites is multifactorial, with extracellular stimuli capable of modulating genes through direct activation of MBP-1 or NF-κB or by phosphorylation and degradation of a cytoplasmic inhibitor of NF-κB, IκB. Activin-regulated gene transcription by mammalian homologues of *schnurri* is still under investigation.

In early *Xenopus* development, activin regulates a homeobox gene element called the ARE (core element 5′-AAATGT-3′) [102]. By yeast-one hybrid analysis of proteins that bind the ARE, a DNA-binding protein of the forkhead family was identified [102]. FAST-1 was cloned and is a basic leucine zipper protein capable of transducing an activin signal. Importantly, Smad2 binds FAST-1, clearly relating the events at the level of the receptor to nuclear transcription. It is anticipated that mammalian homologues of FAST-1 and related AREs will be described. However, FAST-1 and the ARE of development cannot be the only transducers of activin signal. First, FAST-1 is expressed only in the oocyte and early development and then is not detected in total mRNA isolated from later developmental stages. Therefore, Smad must act with other FAST-like molecules or other DNA-binding proteins to transduce signals in adult tissues. Second, genes that activin regulates (such as the immediate early gene *junB*) do not contain the ARE of development. Consensus TGFβ response elements (5′-TGGCC · $N_{3–5}$ · GCC-3′) have been identified in a variety of genes, but no TGFβ-responsive factor (TβRF) has been identified for these gene elements [103]. Among the potential target genes for Smad-FAST or other activin-induced transcription factors is a component of the AP1 transcriptional regulator unit, *junB*. Activin stimulates the *junB* gene in activin-treated pre-erythrocytes (K562 cells), preneuronal cells (PC12) [104], and granulosa cells.

ROLE OF ACTIVIN AND INHIBIN IN REPRODUCTIVE FUNCTION

Role in Reproduction

While similar in structure at the ligand and receptor level, and utilizing similar intracellular signaling molecules, the TGFβ superfamily exhibits an enormous range of cellular actions that profoundly alter developmental programs, left-right axial symmetry, cellular homeostasis, hormone secretion, and neuronal survival. Most members of the family act primarily as paracrine or autocrine factors. The mechanisms

and absolute functional difference between activin and TGFβ are under active investigation. A clear role for activin in the reproductive axis has been identified and will be reviewed. The inhibins represent a branch of the family tree that evolved to provide information to the pituitary from the ovary via classic endocrine pathways. The mechanism by which inhibin acts independent of or via activin has not been clearly determined.

Activin and inhibin were originally purified from ovarian follicular fluid as proteins that stimulated or inhibited, respectively, pituitary FSH release. As these proteins are potential regulators of the pituitary gonadotrope, numerous studies have been performed to examine the reproductive cycle-dependent changes in tissue expression of the subunit mRNA and circulating levels of hormone. More recently, recombinant human preparations of activin and inhibin have been administered *in vivo* to examine the effect of exogenous ligand on reproductive indices.

Tissue-Specific Expression of Subunit and Receptor mRNA

While the subunits that make an activin (β_A or β_B) or inhibin (α, β_A, or β_B) molecule are expressed more broadly than in the ovary and pituitary, the focus of this section will be on the regulation of these ligands in the reproductive tissues of the female. The normal reproductive cycle in the female is established by regulated follicle growth. It has long been known that growing follicles produce non-steroidal hormones at various times during their lifespan to dictate pituitary release of regulatory hormones. This feedback system between the ovary and pituitary is controlled, in part, by inhibin [105]. The regulation of inhibin subunit expression in the follicle and the subsequent production of hormone that regulates FSH has been studied intensely. Because the ovary is composed of follicles at various stages of development, many studies have used cellular mapping methods (such as *in situ* hybridization and *in situ* ligand binding) to determine expression source and abundance of subunit mRNA or protein. In the rat, inhibin and activin subunit mRNA is expressed exclusively in the granulosa cells of follicles that have been recruited into the preovulatory pool [5, 105]. The abundance of the subunit mRNAs increases during the normal estrous cycle and declines following the preovulatory gonadotropin surge. Similarly, in the human and nonhuman primate ovary, the cumulus and granulosa cells of growing follicles express the mRNA for the α-, β_A-, and β_B-subunits [106, 107]. Unlike those of the rat, human theca cells express the α-subunit. In the dominant follicle, α- and β_A-subunit are expressed; however, the β_B-subunit is not detected. PCOS is a disease of follicle maturation in the human, and the expression pattern of inhibin and activin subunit mRNA has been examined in follicles collected from ovaries with this disease. The follicles have similar subunit mRNA abundance and localization relative to those from normal menstrual cycles; however, α-subunit protein is not produced [108]. This circumstantial evidence points to a defect or imbalance in the inhibin and activin system in the polycystic ovary.

Receptors and binding proteins for activin and inhibin have also been identified in ovarian follicles. In the rat, follistatin mRNA accumulation mirrors inhibin subunit mRNA accumulation in the developing follicle [109]. Similarly, in the human, follistatin mRNA is expressed in the developing and dominant follicle; however, it is absent in follicles of PCOS women [107, 108]. Receptor mRNA has also been detected in the ovarian granulosa cell, corpus luteum, and oocyte [49, 107–109]. A paracrine or autocrine role for activin or inhibin in controlling ovarian function locally has not been delineated clearly; however, the presence of both receptor and bioneutralizing binding protein predicts some level of activity at the local level.

Endocrine Role for Activin and Inhibin in Regulating Pituitary FSH

The inverse relationship between circulating, immunoreactive inhibin and immuno- and bioactive FSH during the menstrual cycle, an increase in pituitary FSH upon infusion of an immunoneutralizing inhibin antibody, and the regulation of FSH by injection of recombinant human inhibin support the conclusion that inhibin is an endocrine regulator of FSH secretion.

The circulating pattern of inhibin is inversely correlated to FSH. Two forms of inhibin circulate in females: inhibin A and inhibin B [110]. Interestingly, the patterns of the two hormones are different from each other, supporting *in vitro* data of differential regulation of subunit expression by hormones and growth factors [5]. The patterns of human and rodent inhibin A and inhibin B levels, with respect to the reproductive cycle, are very similar [110–112]. For descriptive purposes, the patterns of inhibin will be described relative to the human menstrual cycle. Inhibin A is low in the circulation of women during the early follicular phase and increases in concentration in the days preceding ovulation, in keeping with the expression pattern of this molecule in the dominant follicle [107, 110]. Inhibin A levels fall slightly during the early luteal phase and then increase again in the luteal phase. Luteal inhibin A production is positively regulated by LH [113], and luteal phase inhibin A levels are associated with low levels of FSH [114, 115]. FSH levels then increase during the luteal-follicular transition at the time of luteolysis and decreased luteal inhibin A [116]. Similarly, inhibin B levels increase during the follicular phase and are associated with low FSH levels [111]. Little inhibin B is detected during the luteal phase of the cycle. The production of inhibin B during the follicular phase and of inhibin A during the luteal phase suggests that FSH secretion is differentially regulated by inhibin B and inhibin A depending upon the stage of the menstrual cycle and that the factors that control inhibin A and inhibin B subunit expression are cycle-stage dependent. Suppression of the hypothalamic-pituitary-gonadal

axis by GnRH-agonists or age causes suppression of inhibins in the female [117, 118]. Injection of FSH-like preparations causes an increase in peripheral inhibin [117]. In the male, inhibin B is the isoform involved in FSH regulation [119, 120]. Further solidifying the endocrine role of inhibin is the increase in FSH following immunoneutralization of inhibin activity in rodents [121] and a suppression of FSH following hormone injection into rats and monkeys [122–124]. Thus, a classic negative feedback loop is established between the pituitary and ovarian inhibin. The cellular mechanism by which inhibin mediates FSH secretion from the pituitary gonadotrope is not known. As discussed previously, inhibin may act as an antagonist of activin action in the pituitary by binding activin RII and blocking tonic activin action. Alternatively, inhibin may occupy a distinct receptor that regulates FSH through Smads or some other cascade of intracellular mediating factors.

A role for activin as a positive regulator of FSH secretion is supported by an increase in FSH in response to exogenous activin [123–128] and a decrease in FSH in response to follistatin [129]. Activin A has been detected in the circulation of women during the normal menstrual cycle; however, the assay used to detect this ligand is able to measure follistatin-bound activin, which could represent bioinactive ligand [130]. Binding-protein-free assays have been developed but do not detect activin peripherally. This finding may be due to the sensitivity of the assay, and further studies are required before conclusions can be drawn concerning the physiological implications of circulating activin during the reproductive cycle. It is likely that activin is a paracrine acting factor, produced in and acting on the pituitary gland itself rather than regulating gonadotrope activity in an endocrine fashion. Regulation of pituitary FSH by immunoneutralization of pituitary activin [131] and by follistatin [15] *in vitro* supports this hypothesis.

Intriguingly, activin A is detected in a number of pathophysiological conditions including hyperthyroidism, renal failure, and some solid cancers [132]. The role of the ligand in the pathology of the disease and the utility of the assay as a diagnostic tool for disease states are areas of active investigation. Binding-protein-free activin is also detected in the serum of women in the third trimester of pregnancy [133]. Pregnancy-associated activin and inhibin are outside the scope and page limitation of this review; however, it is likely that activin and inhibin have important roles in the establishment of pregnancy [134, 135], as diagnostics of fetal anomalies [136, 137] or preterm labor [138], or as regulators of parturition [139].

Role of Activin and Inhibin in Follicle Function

Inhibin and activin also have important effects on the follicle, oocyte, and corpus luteum of rodents and primates. Activin inhibits and inhibin augments LH- and IGF-stimulated androgen production by human thecal cells via modulation of 17β-hydroxylase activity [140]. Moreover, inhibin overrides the inhibitory action of activin in these cells [141]. In granulosa cells from immature follicles, activin is an autocrine modulator of FSH-induced aromatase activity in nonhuman primates; however, in mature follicles that have acquired LH-responsiveness, activin inhibits steroidogenesis [142]. Dimeric activin A also suppresses progesterone synthesis by luteinizing granulosa cells *in vitro* [143, 144].

Collectively, these data led to the hypotheses that activin promotes follicular maturation in an autocrine/paracrine manner by simultaneously suppressing theca and augmenting granulosa cell function, but perhaps prevents final differentiation or follicular luteinization [145, 146]. As the dominant follicle matures, inhibin production increases while activin decreases, which further enhances follicular estradiol synthesis by promoting increased production of androgen substrate [147]. The dramatic ability of activin A to cause reorganization of cells from primary rodent follicles to develop into large antral, oocyte-containing follicles and the ability of activin to stimulate immature granulosa proliferation support a role for activin in early follicular development *in vitro* [148, 149]. The inhibition of final differentiation or follicular luteinization [145, 146] is supported by the potent induction of follicular atresia or lack of follicle development following intrabursal administration in rats [66] and in monkeys [123]. Mechanisms that could provide an explanation for this inhibition include (a) depleted thecal androgen production, thereby depriving follicles of substrate for estradiol; or (b) induced production of follistatin in excess of activin, which would bioneutralize local activin effects. Effects of activin and inhibin on luteal cell function have also been investigated in primates. While progesterone production by macaque luteal cells was suppressed by activin A, but not inhibin A *in vitro* [150], intraluteal infusion of activin A at midluteal phase did not alter luteal function *in vivo* [123]. Conversely, systemic infusion of rhesus monkeys with activin A during the midluteal phase caused premature luteolysis that was not observed with inhibin A [123]. These data support the important role of inhibin, activin, and follistatin actions in ovarian function.

Other TGFβ family members, including TGFβ, MIS, BMP, and GDF-9 are expressed at high levels in the ovary [151–155]. The function of these proteins in modulating follicle function is currently under investigation. Perhaps the most interesting factor in regard to follicle function is GDF-9. GDF-9 is expressed exclusively in the oocyte, and genetic deletion of this protein results in animals without follicles [154, 155]. This indicates that GDF-9 is a candidate oocyte-derived growth factor and is central to the organization and growth of follicles in the ovary. Additional details on the roles of these factors in ovarian follicle function are essential to the understanding of follicle health and ovarian disease.

FUTURE DIRECTIONS

Activin is an important factor that regulates numerous cellular functions including pituitary FSH secretion and granulosa cell growth. Many advances have been made in understanding the mechanism by which activin causes cellular responses, but many questions remain unanswered. What cellular mechanisms transduce antagonistic activin and inhibin signals? Why has such a large and, in some cases, overlapping gene family evolved? What are the implications of loss of functionality within this gene family for the onset and progression of tumorigenesis? What lessons can be learned from genetic deletion studies in animal models? The answers to these problems will provide the insight necessary to understand the fundamental role these genes play in cellular function, and the approaches to the same problems will lead to more questions.

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