—Review—

Activin, an Intraovarian Peptide, Has a Role in Oocyte Maturation

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Activin A, a dimer of the inhibin β-subunit, was originally identified in porcine ovarian fluid. This agent exerts biological effects on the release of follicle stimulating hormone (FSH) by pituitary cells *in vitro* which oppose those of inhibin; while inhibin suppresses FSH release, activin A stimulates it [1, 2]. Activin A has been shown to regulate hemopoiesis [3], lymphocyte proliferation [4], spermatogenesis [5], and early development [6–8]. Recent evidence indicates that activin A can act on oocytes [9, 10], granulosa cells, and thecal cells [11–13], suggesting that it has an intraovarian paracrine effect. In addition to inhibin and activin, follicular fluid contains the polypeptide follistatin, a binding protein of activin A that suppresses FSH release in a manner similar to inhibin [14, 15]. The development of follicle and maturation of oocytes are initiated by the secretion of FSH from the pituitary glands followed by a surge of luteinizing hormone (LH) that is mediated by follicle cells. Inhibin, activin and follistatin are believed to regulate follicular development. However, little information is available about the roles played by these and other factors in mammalian oocytes and little is known about the existence and roles of activin in humans. This review focuses on the role of activin A in rat oocyte maturation with an emphasis on the expressions of activin and its receptor in the oocytes themselves. This review also examines the presence of activin A and the activin-binding protein, follistatin, in human follicular fluids.

Structure and Intraovarian Function of Activins

Activin and inhibin are closely related members of the TGF superfamily, formed by either homo- or heterodimerization of the subunits (activin A=βA,βA; activin AB=βA,βB; activin B=βB,βB) or heterodimerization of the inhibin α subunit with one of two β subunits (inhibin A=α,βA; inhibin B=α,βB) (Fig. 1). It is of interest to note that when each of the β subunits is combined with α subunit the resulting molecule inhibits the release of FSH, whereas any two β subunits (βA,βA, βA,βB and βB,βB) joined together in a molecule release FSH. Such a precedent can be found in the case of platelet-derived growth factor (PDGF), a heterodimer of two closely related A and B subunits, the same PDGF-like biological activity being exhibited by a homodimer of either the A or B subunits [16, 17].

Data from rat and bovine granulosa cell models suggest that activin regulate granulosa cell differentiation in an autocrine fashion and that this action of activin is related to the stage of granulosa cell differentiation and, hence follicular maturity. In the presence of FSH, activin enhances aromatase activity, estradiol production, and progesterone production [13, 18–20], with no effects on the levels of these parameters in the absence of FSH. Activin also increases FSH receptor numbers, with no change of in binding affinity [21]. These *in vitro* obser-

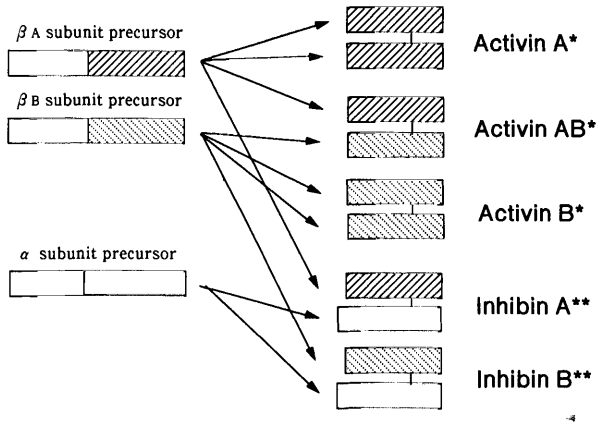

Fig. 1. Diagrammatic representation of how the α- and β-subunits of inhibin can be combined to yield FSH releasers* and FSH suppressors**.

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vations are consistent with activin as an autocrine regulator promoting folliculogenesis during the preantral or early antral stages of growth of follicles. In cultured luteinizing human granulosa cells, activin increases mitogenesis and decreases both basal and gonadotropin-stimulated aromatase activity and progesterone production [22, 23].

The action of activin on the granulosa cells are saturable and display the specificity expected of a high affinity receptor. Messenger RNAs encoding two activin receptors, designated type IIA and type IIB, have been cloned [24, 25] and they are expressed in the ovary [26]. As is the case for the all of the receptors, however, little is known about the structure of those proteins or about the second-messenger signaling systems that they employ. Follistatin, a structurally unrelated isomer originally identified in follicular fluids as an inhibitor of FSH release [14, 15], is a high affinity activin-binding protein [27], but it does not appear to be involved in transmitting an activin signal across the cell membrane. It likely prevents the action of activin via its role as an activin-binding protein [19].

Activin and Follistatin in Oocyte Maturation

Nuclear maturation events, such as breakdown of germinal vesicles, progress spontaneously *in vitro* when oocytes are obtained from mature follicles [28, 29]. Oocytes in maturing and mature follicles were induced to undergo spontaneous meiotic maturation being incubated as cumulus-oocyte complexes [10]. As shown in Table 1, 35.8% of the oocytes from mature follicles obtained 48 h after PMS treatment lost their germinal vesicle in 1 h of incubation. Percentages of germinal vesicle breakdown (GVBD) in oocytes obtained 24 and 36 h after PMS, however, were significantly lower than that in oocytes obtained 48 h after PMS. Activin A, at a dose of 10 ng/ml, served as a potent inducer of oocyte maturation, increasing the percentage of GVBD in the rat oocytes (Table 1). Induction of oocyte maturation by activin A was dose dependent (Fig. 2). The effect was significant and maximal at a dose of 1 ng/ml. Since activin A regulates follicular development [11–13,18–20], these data provide a close link between follicular development and oocyte maturation. Activin A exerts its stimulatory effects at 1 ng/ml, which is less than the physiological concentrations found in follicular fluids of several species [30, 31]. It is possible that activin A is a paracrine factor in the acquisition of meiotic competence of oocytes, a process that depends primarily on FSH.

Follistatin blocks the maturation-inducing action of activin A when both are added to oocytes *in vitro* (Fig. 3). Activin A increased the percentage of GVBD significantly in 1 h; the addition of follistatin at doses of 10 ng/ml to the incubation medium in the presence of activin A, 10 ng/ml, inhibited its stimulatory action. Follistatin is capable of binding same dose of activin A [27] and it antagonizes the effects of activin on steroidogenesis of rat granulosa cells, presumably by competing with activin for binding to its receptors [19]. Follistatin may have inhibitory effects on cumulus-oocyte complexes in the same manner. Interestingly, the addition of follistatin alone to the medium significantly decreased the percentage of GVBD in 2 h of culture (Fig. 3). This finding raises the possibility of a source(s) of activin A in the cumulus-oocytes complexes other than the granulosa cells shown to produce activin A [22, 32].

Table 1. Germinal vesicle breakdown as a function of time after PMS treatment and the effects of activin A on cumulus-oocyte complexes

Hours after PMS	Percentage of Germinal Vesicle Breakdown	
	Control	Activin A, 10 ng/ml
24	15.9	31.7*
36	17.8	33.0*
48	35.8$	47.1

*, $p<0.05$ compared with untreated controls.
$, $p<0.05$ compared with that obtained 12 h earlier.

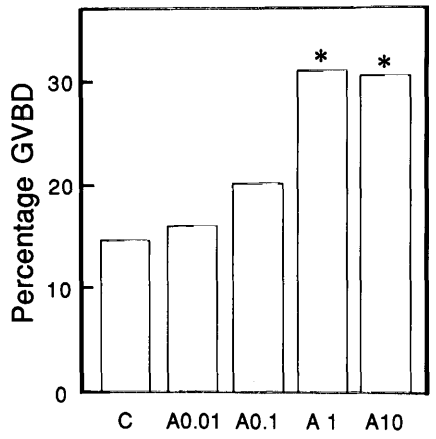

Fig. 2. Concentration-dependence of activin A induced maturation of cumulus cell-enclosed oocytes. GVBD was analyzed in oocytes obtained 24 h after PMS treatment and incubated for 1 h in the presence of the indicated concentrations (ng/ml) of activin A (A). *, $p<0.05$ compared with untreated controls.

Expressions of Activin A and its Receptor in Oocytes

A total of 100 cumulus-oocyte complexes or denuded oocytes obtained from the ovaries of PMS-treated rats were washed five times with PBS [10]. PolyA+ RNA was extracted by use of the Micro-Fast Track mRNA isolation kit (Invitrogen, San Diego), which allowed the direct isolation of polyA+ RNA from a very small number of cells. Purified mRNA was isolated by our RNA extraction procedure. Contamination of genomic DNA was ruled out by PCR amplification of each cDNA preparation using primers that flank a target sequence of the actin gene that contains an 87 bp intron. If genomic DNA were present in the cDNA, a 330 bp target sequence would be amplified as well as the 243 bp fragment representing the cDNA. No 330 bp PCR product for β-actin was detected in any of the RT-PCR reactions with our RNA samples [33]. To offset the efficiency difference in RNA extraction, an internal standard, i.e., 10^6 copies of a synthetic polyA+ RNA pAW109 that was transcribed from the plasmid pAW109, was added to each batch that was lysed in a detergent-based buffer. The lysates of 100 oocytes or cumulus-oocyte complexes and internal standard RNA were then applied directly to oligo (dT) cellulose for adsorption. The resulting polyA+ RNA pellet was resuspended in 6 μl of 10 mM Tris-HCl, pH 7.6, 1 mM EDTA and the two 2 μl aliquots were used for each RT-PCR reaction, one for the activin βA mRNA assay and the other for assay of activin type IIA receptor mRNA and internal standard RNA.

RT-PCR was performed on mRNA samples from rat cumulus-oocyte complexes and from denuded oocytes. Ten microliters of the PCR-amplified products were electrophoresed in 1% agarose gel containing 0.5 μg/ml ethidium bromide and were then photographed. The identity of the amplified products was confirmed by correct sizing on agarose gel. As shown in Fig. 4, both cumulus-oocyte complexes and denuded oocytes expressed activin βA and activin type IIA receptor transcripts as evidenced by the presence of amplified cDNA fragments of the correct sizes. The specificity of these bands was determined by cloning and sequenc-

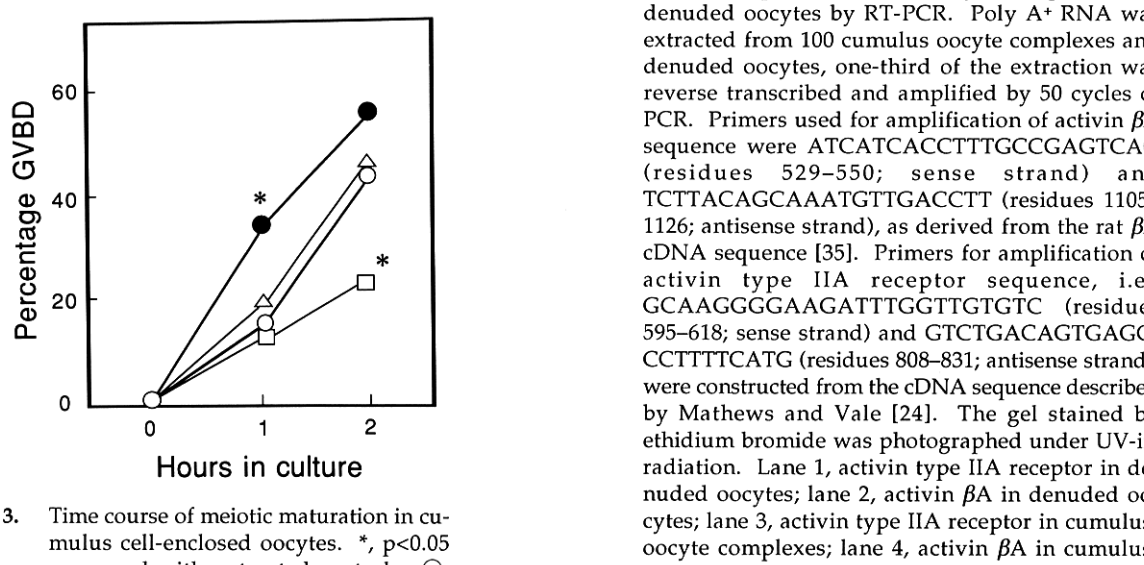

Fig. 3. Time course of meiotic maturation in cumulus cell-enclosed oocytes. *, $p<0.05$ compared with untreated controls. ○, controls; ●, activin A 10 ng/ml; □, follistatin 10 ng/ml; △, activin 10 ng/ml+follistatin 10 ng/ml.

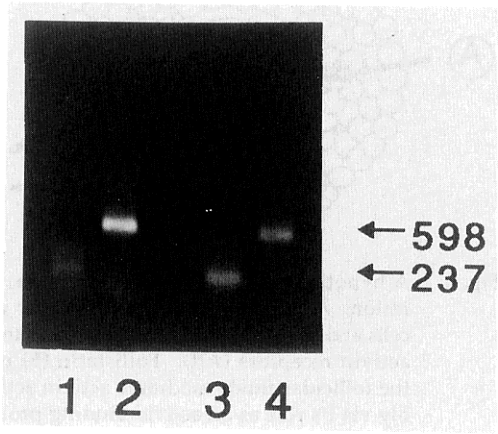

Fig. 4. Detection of activin βA and activin type IIA receptor transcripts in cumulus-oocyte complexes and denuded oocytes by RT-PCR. Poly A+ RNA was extracted from 100 cumulus oocyte complexes and denuded oocytes, one-third of the extraction was reverse transcribed and amplified by 50 cycles of PCR. Primers used for amplification of activin βA sequence were ATCATCACCTTTGCCGAGTCAG (residues 529–550; sense strand) and TCTTACAGCAAATGTTGACCTT (residues 1105–1126; antisense strand), as derived from the rat βA cDNA sequence [35]. Primers for amplification of activin type IIA receptor sequence, i.e., GCAAGGGGAAGATTTGGTTGTGTC (residues 595–618; sense strand) and GTCTGACAGTGAGCCCTTTTCATG (residues 808–831; antisense strand), were constructed from the cDNA sequence described by Mathews and Vale [24]. The gel stained by ethidium bromide was photographed under UV-irradiation. Lane 1, activin type IIA receptor in denuded oocytes; lane 2, activin βA in denuded oocytes; lane 3, activin type IIA receptor in cumulus-oocyte complexes; lane 4, activin βA in cumulus-oocyte complexes. The positions of the expected 237-bp and 598-bp PCR products for activin type IIA receptor and activin βA, respectively, are indicated.

ing the PCR products, which showed a 100% nucleotide sequence identity with the cloned sequences reported.

It is likely that activin A present in follicular fluids directly stimulates oocyte maturation via its receptors on the oocytes. We also present evidence that activin βA is expressed in both cumulus-oocyte complexes and in the oocytes themselves. Since activin A is a homodimer of the βA subunits, while inhibin is a heterodimer consisting of the βA subunit together with an α subunit [1, 2], our data indicate that the oocyte contains mRNA encoding both activin A and inhibin.

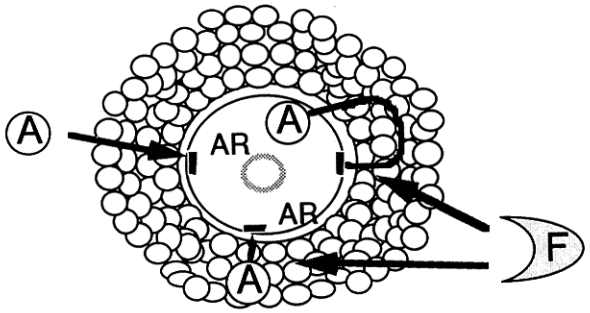

Fig. 5. A hypothesis for a role of activin in oocyte maturation. Activin (A) produced either by granulosa cells and oocytes themselves act on oocytes through activin receptors (AR). Follistatin (F) present in the follicular fluids modulate activin action possibly via its role as an activin binding protein.

Based on our data that follistatin added to the medium exerts inhibitory effects on oocyte maturation (Fig. 3), it may be at least in part activin A that is present, reflecting the presence of βA subunit. A recent study on activin expression during early mouse development reported agreement with our idea that α subunit protein cannot be detected from the fertilized egg stage onwards, and that the presence of β subunits reflects the presence of activin rather than of inhibin [35]. These results suggest that activin A, produced by oocytes themselves along with granulosa cells under modulation by follistatin, may play an important role in oocyte growth and maturation in autocrine and paracrine fashion leading finally to acquisition of fertilization competence (Fig. 5).

Presence of Activin A in Human Follicular Fluid

The activity of activin A was determined with a bioassay based on the induction of differentiation in Friend cells, according to a previously reported method [31, 36]. Concentrations of 0.16 to 5 ng/assay of standard recombinant activin A produced from Chinese hamster ovary cells increased the percentage of hemoglobin-positive cells in a dose dependent manner (Fig. 6). At a dose of 5 ng, activin A induced differentiation in 53% of Friend cells. Hemoglobin inductive activity was minimal when untreated follicular fluid was added to the assay

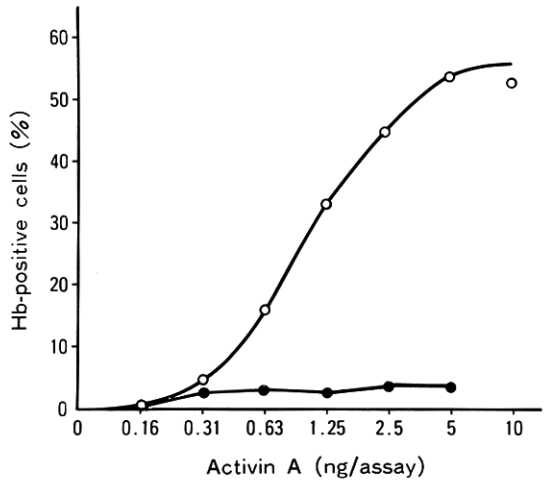

Fig. 6. Standard activity curve for activin A (○) and the displacement curve generated by the addition of 20 μl of unpurified follicular fluid to standard activin A (●). The induction activities of activin A were inhibited the follicular fluid (possibly follistatin).

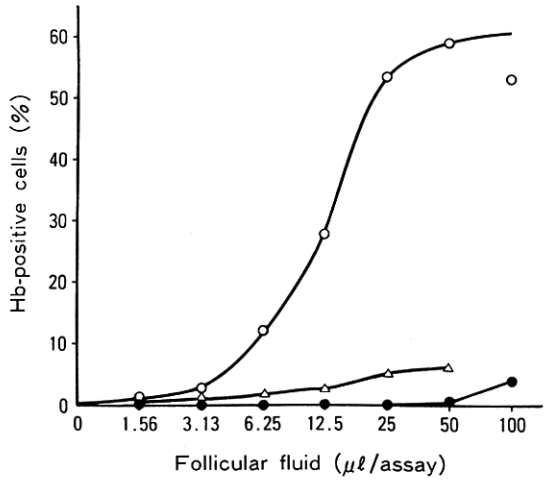

Fig. 7. Dose-response curve of induction activity of purified follicular fluid (○) and unpurified fluid (△) and displacement curve induced by the addition of 100 ng of follistatin to purified follicular fluid (●). The curve (○) was parallel to the standard curve for activin A (Fig. 5) and the activities were abolished by the presence of follistatin (●).

Table 2. Comparison of concentrations of activin A and other parameters according to the number of follicles

N of follicles per patient	Activin A (ng/ml)	Follicle volume (ml)	Estradiol (ng/ml)	Progesterone (μg/ml)
<8 (39)*	161 ± 35	3.5 ± 2.0	665 ± 361	5.3 ± 3.7
>8 (55)	106 ± 25	3.4 ± 1.7	704 ± 545	6.8 ± 3.7

The results are mean ± SD. *Number of follicles examined is shown in parentheses.

mixture. The addition of 20 μl of follicular fluid inhibited the activity of activin A as follistatin exerted (Fig. 6). Purified follicular fluid increased the percentage of hemoglobin-positive cells in a dose dependent manner, similarly to standard activin A (Fig. 7). The addition of 100 ng follistatin to the assay inhibited differentiation activity of purified follicular fluid; it was hardly detectable at lower doses and only 4% at a dose of 100 μl of the purified fluid (Fig. 7).

We detected the presence of activin A in human follicular fluid with biological activities similar to those of recombinant activin A. Purified follicular fluid induced differentiation of Friend cells in a dose-dependent manner, similarly to activin A (Figs. 6 and 7). The similarity of this substance to activin was further confirmed by the fact that its activity was neutralized by follistatin (Fig. 7). It is of interest, however, that activin A activity was barely detectable in unpurified follicular fluid although a considerable amount of activin-like substance was present. Untreated follicular fluid inhibited the activity of activin A when added to the standard assay. These results suggest that follicular fluid contains a specific inhibitor of activin A, or follistatin, which is consistent with the data obtained in a radioimmunoassay study [37].

Activin A and Follicular Development

Activin concentrations were estimated from a titration curve as the percentage of hemoglobin-positive cells in serial dilution of authentic activin A. The initial level of activin in the fluid was estimated to be 131 ± 40 ng/ml. The concentration of the activin-like substance in human follicular fluid was approximately 100-fold higher than that of serum activin A determined by the same assay [36], suggesting that activin A may be produced in the ovary in humans as it is in other species of animals [38, 39]. Earlier study which could not identify the presence of activin in human follicular fluids [40] may be due to the coexistence of follistatin in the fluid. Activin A expression has been identified in the human ovary [22]. The high concentration of the substance in follicular fluid indicates that it exerts autocrine or paracrine effects on follicular cells, such as granulosa cells, that have been shown to possess activin A receptors [41].

There were no significant correlations between the concentration of activin A and the volume of follicular fluid, concentrations of estradiol, progesterone or prolactin, or the maturity of oocytes retrieved. In contrast, concentration of the activin-like substance differed significantly according to the number of developed follicles (Table 2), suggesting that it may have a local regulatory function. Since the number of developed follicles per ovary increased as the concentration of activin-like substance decreased, it is conceivable that the lower concentration of activin-like substance is responsible for the larger number of follicular development or vice versa. In normal cycle without stimulation, it may be possible that activin A plays a role in selection of follicles for ovulation because follicles containing granulosa cells that produce the greatest amount of activin would be expected to be most responsive to FSH [42].

Acknowledgments

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