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INVITED REVIEW

Expression and secretion of activin A: possible physiological and clinical implications

Stefano Luisi, Pasquale Florio, Fernando M Reis[1] and Felice Petraglia

Chair of Obstetrics and Gynecology, University of Siena, Siena, Italy and [1]Department of Obstetrics and Gynecology, University of Minas Gerais, Belo Horizonte, Brazil

(Correspondence should be addressed to Felice Petraglia, Chair of Obstetrics and Gynecology, University of Siena, Viale Bracci, 53100, Siena, Italy; Email: petraglia@unisi.it)

Introduction

Activins, first isolated in 1986 from porcine follicular fluid (1, 2), are gonadal proteins which stimulate pituitary follicle-stimulating hormone (FSH) synthesis and secretion. However, from 1986 to the present day, several lines of research have found that activins are not only synthesized in the ovaries and testes, but also in other tissues where they function as paracrine and autocrine factors to regulate a number of processes within and outside of the reproductive axis. The evidence of expression of activin mRNA in a wide variety of tissues – including placenta, pituitary, adrenals, spleen, bone marrow and specific regions of the brain – and the diverse set of biological functions in these tissues suggested a possible role for activin as a growth factor and a cytokine (3–6). Furthermore, the availability of a suitable assay developed in the last few years has made it possible to measure activins in several biological fluids. In particular, the discrete amount of activin A circulating in the systemic bloodstream suggests a possible endocrine role, but up to now the possible source(s) and target(s) are still debated. The present review summarizes the various activin sources and functions in humans, and the data obtained on activin A measurement in various biological fluids which suggest possible physiological and clinical implications.

Structure, synthesis and receptors

Activins are dimeric proteins, members of the transforming growth factor-β (TGF-β) superfamily, a group of structurally similar but functionally diverse growth factors (2). This group includes the multiple TGF-βs, bone morphogenetic proteins, the *Drosophila* decapentaplegic gene product, Mullerian duct-inhibiting substance, and Vg-related gene products (6–8).

Activins are homodimers of the β-subunit, and the differential disulfide-linked dimerization of subunits gives rise to three protein factors: βA-βA (activin A), βB-βB (activin B), and βA-βB (activin AB) (1, 2). Recently, another three β-subunits have been cloned, βC, βD and βE, but no information is available on dimeric proteins (9, 10). However, there are data showing the formation of βC activin heterodimers in human liver and prostate (10). Of these, activin B is the only dimeric form that has not been identified in gonadal fluids in its native form, but recombinant βB-βB is biologically active, like the other two activins, in increasing the release of FSH from cultured pituitary cells. For this reason the three forms of activin were at first considered to be members of the hypothalamus–pituitary–gonadal axis (7, 11). They were thus named activins because, contrary to inhibins, they showed a stimulatory action on the pituitary secretion of FSH.

Subsequently, the expression of activin subunit mRNAs was found in several organs other than gonads: brain, pituitary, thyroid, adrenal cortex, pancreas, liver, bone marrow, and female and male reproductive organs (12) (Table 1), and the possible role of activins as growth factors was suggested.

In addition, a recent review provides a brief overview of activins and their receptors, including their structures, expression, and functions in the female reproductive axis as well as in the placenta (13).

To complete this picture, another FSH-suppressing protein was discovered and named follistatin. Follistatin is a monomeric glycoprotein structurally unrelated to activins, but with high affinity to activins, neutralizing the biological effects of activins, and acting as a major activin binding protein (14–17). The biological effects of follistatin are opposite to those of activins, and in many cases similar to those of inhibins. Follistatin is present in high concentrations in human serum and follicular fluid and is probably the major inhibin/activin binding protein in the gonads, where it modulates local paracrine and autocrine functions, although in serum, α2-macroglobulin is the most abundant binding protein for activins (18). These binding proteins are important because both follistatin and α2-macroglobulin alter the bio- and immunoactivity of activins and, in part, of inhibins.

Over the last few years, receptors which bind activins and other TGF-β superfamily members have been discovered (19). Both type I and type II activin receptors appear to be involved in the signal transduction pathway, and both are transmembrane proteins with serine-threonine kinase domains. The type II receptor confers ligand specificity and the type I receptor, in combination with the type II receptor, transmits the phosphorylation signal (19–21). To date,

Table 1 Sources/targets of activins and concentrations of activin A in various biological fluids. The range of values (in ng/ml) is shown in parentheses. (Data adapted from (12) and (114).)

Sources/targets of activins	**Concentrations of activin A** (ng/ml)
Brain	Cerebrospinal fluid (0.20–1.50)
Pituitary	Serum (0.52–1.18)
Thyroid	
Adrenal cortex	Peritoneal fluid (1.52–1.74)
Bone marrow	
Liver	Coelomic fluid (0.45–5.20)
Pancreas	
Ovary	Amniotic fluid (0.20–0.77)
Testis	
Placenta	Follicular fluid/seminal plasma (3.50–10.75)

there are two type II activin receptors (type IIA and type IIB) and at least three type I receptors, which can bind activins in concert with the type II receptors *in vitro* (19, 20). Expression studies suggest that activin receptor type II is the major receptor regulating activin signaling in the reproductive axis (22).

Assay

The evolution of the knowledge about activins has been delayed for some years by the lack of a suitable assay. In fact, activin assay development has been difficult largely due to the near 100% conservation of the molecule between the two β-subunits (23).

First, a radioimmunoassay (RIA) for activin was developed, but this assay showed significant cross reactivity with inhibin A, due to the fact that the βA subunit recognized by the RIA antibody is, clearly, part of both inhibin A (α-βA) and activin A (βA -βA) (24). In 1991, a two-site assay for inhibin A and activin A was developed, using antibodies raised against synthetic peptides of the α and βA subunits (25), and so providing the means to assay the proteins separately. With respect to the other forms of activin, in 1993 Wong *et al.*, using hypogonadal mice as host species, were able to generate a panel of monoclonal antibodies that were used in generating specific, sensitive, and independent activin A and activin B two-site assays (26); these were used to demonstrate measurable concentrations of these hormones in pregnant women (27). It soon became clear that the performance of existing bioassays and immunoassays for activin were compromised in biological fluids by the presence of activin-binding proteins such as follistatin and α2-macroglobulin.

To overcome this problem, in 1991 Groome developed a new two-site enzyme immunoassay procedure for activin A which incorporated an analyte denaturation and oxidation step with the scope of reducing the interference of the binding proteins and other inhibin-related proteins. This assay resulted in a reliable method for quantifying total activin A concentrations in a variety of biological samples (25).

One year later, the same author's group developed a sensitive and specific ELISA to measure total activin AB, and preliminary results showed a more restricted distribution of this isoform compared with activin A (28).

Regarding activin B, recently Vihko *et al.* used an immunoenzymometric assay to measure this protein in human serum during ovarian stimulation and late pregnancy. The assay is based on a monoclonal antipeptide anti-βB (29).

Sources, secretion and putative regulatory roles

The demonstration that activin subunits are expressed by various tissues poses the question of the source(s) of activin A in the circulation. The present evidence indicates that serum concentrations of activin A are not significantly different between men and women when studied before puberty (8–15 years) (30) or between 20 and 50 years of age. Starting from the age of 50 years, serum activin A concentrations are significantly greater in men than in women at the corresponding age (31). In fact, while activin A concentrations remain constant in women, a significant increase in men occurs, reaching peak values between 70 and 90 years of age (Fig. 1). Moreover, no significant correlation between concentrations of activin A and FSH has been found in either gender (31), suggesting that the changes in this circulating protein do not influence FSH secretion.

Activin and brain

Activin subunit mRNA levels vary considerably throughout the various brain regions, and are differentially expressed. In fact, activin βA mRNA levels are highest in the olfactory bulb, striatum, and pons/medulla, and gonadotropin-releasing hormone (GnRH)-secreting neurons also express activin βA subunit mRNA (32).

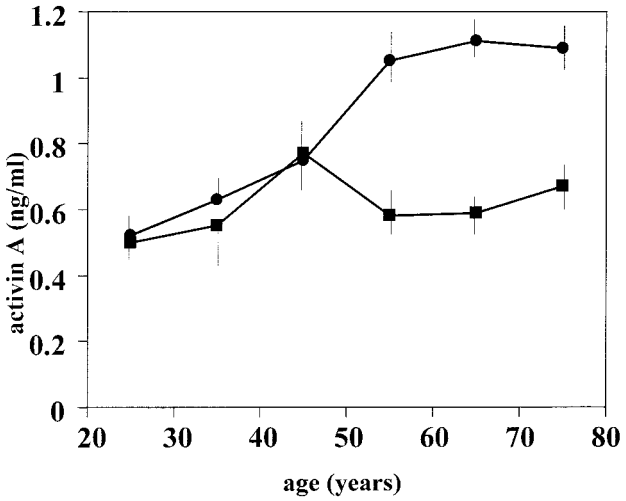

Figure 1 Changes in serum activin A levels in healthy women (■) and men (●) during life. Results are means±S.E.M. (Data adapted from (31)).

Intense βA staining is also detected in oxytocin-rich regions of the paraventricular and supraoptic nuclei of the hypothalamus, suggesting the presence of activinergic synapses on oxytocin containing cells (33). Furthermore, activin βA subunit immunostained fibers are distributed in the hypothalamus in close association with GnRH immunoreactive fibers (34).

Activin βB subunit mRNA is localized in the nucleus of the solitary tract (NTS) projecting to oxytocinergic neurons of the magnocellular neurosecretory system (33). Other βB perikarya were identified in the cerebellum, substantia nigra, caudate, putamen, red nucleus of the stria terminalis, and various hypothalamic areas. The presence of β-subunit staining in the preoptic area of the hypothalamus raised the possibility that activin might be involved in the regulation of GnRH production. Indeed, activin A increases GnRH release from rat cultured hypothalamic cells, and the secretory effect of activin A is also associated with a change in the cellular morphology (35).

Cultured human olfactory neurons release activin A in medium under different stimuli. In fact, progesterone and GnRH significantly increased the release of activin A from FNC-B4 cells (32). Because these cells secrete both GnRH and activin A in culture medium, a reciprocal autocrine regulation is suggested in the fine tuning of GnRH secretion, supporting the functional interaction between activin and the GnRH neuronal system in the human hypothalamus. Activin A has been shown to modulate oxytocin release (33), and to regulate the neurotransmitter phenotype in peripheral neurons (36). No data report the presence of activin A in portal vessel circulation. The presence of activin A in cerebrospinal fluid has been shown in women and men.

Therefore, brain activin A is a candidate for a role as neurotransmitter/modulator. The action on neural differentiation probably involves the synthesis and release of follistatin, which counters the activity of activin (37). An emerging role of activin A as neuroprotector is suggested by the evidence of its action as a nerve survival factor (38), or as an inhibitor of neural differentiation (39), as well as a mitogen (40), or as a potent survival factor for neurogenic clonal cell lines, retinal neurons and midbrain dopaminergic neurons (36). Furthermore, activin A modulates the survival of specific populations of injured neurons (41), and induction of activin A is essential for the neuroprotective action against traumatic brain injury (42). Additionally, it was suggested that treatment with activin A may help to prevent the degeneration of vulnerable striatal neuronal populations in Huntington's disease (43).

Activin and pituitary

Besides being one of the sites of action for activin A, the pituitary is also a source for this protein (44) even though there is no evidence for secretion into the bloodstream. Local autocrine/paracrine actions have been described. Highly purified activin A is a potent and selective FSH secretagogue, and activin A treatment in rats elevates the levels of FSH β-subunit mRNA and serum FSH levels (45). In primary cultured rat pituitary cells, treatment with activin A increases FSH concentration by an increase in the number of FSH secreting cells, while it does not affect luteinizing hormone (LH) secretion (46). Moreover, activin A interacts with androgen steroids in modulating either basal or GnRH-mediated FSH release (47).

Although activin stimulates FSH β-subunit biosynthesis and secretion, a large percentage of human gonadotrope tumors have been demonstrated to be non-responsive to characterized activin effects. This phenotype may indicate a loss of functional cell surface receptors and/or intracellular signaling mediators of activin responses (48), as suggested by the study of D'Abronzo *et al.* which demonstrated that somatic mutations within the intracellular kinase region of type I/type II receptors are rare in human pituitary tumors (49).

The pituitary action of activins is not restricted to gonadotropes, activin A being able to inhibit basal growth hormone (GH) and adrenocorticotropin (ACTH) secretion (1, 50–53), as well as GH-releasing hormone (GHRH)-stimulated GH secretion (51), and intracellular cAMP levels (54). Furthermore, activin A has an antimitogenic action on rat somatotrope cells by inhibiting GHRH-stimulated proliferation of these cells (55). In the pituitary cell line AtT20, an established mouse corticotropin cell line, activin inhibits pro-opiomelanocortin mRNA biosynthesis and ACTH secretion (53). In addition, activin A significantly reduces

the thyrotropin-releasing hormone-mediated prolactin release in rats (50).

Activin and thyroid

Immunoreactive activin A is localized in the cytoplasm of the thyroid follicle cells, indicating an active synthesis, even though there is heterogeneity in the expression level, mainly between different follicles but also among different cells in the same follicle (56, 57).

In vitro studies on cultured porcine thyroid cells have shown that activin A induces a potent stimulation of DNA synthesis. This effect is abolished by the addition of follistatin, and additively enhanced by epidermal growth factor (EGF) (57). These data, together with the evidence that immunostaining for activin A in thyroid follicular cells is more intense in patients with Graves' disease than in normal subjects, and the fact that iodine metabolism (reuptake and release of iodide) and cAMP accumulation in porcine thyroid cells (58) is regulated by activin A, suggest a suppressive effect of activin on the function of porcine thyroid cells. Similarly to TGF-β, activin A inhibits both the basal and the EGF-stimulated proliferation of thyrocytes or porcine thyroid cells *in vivo*. The simultaneous expression of TGF-β, activin A and their receptors suggests an interesting autocrine role for these factors in the thyroid gland (54). The indication that activin A stimulates thyroid growth (59) further suggests that activin A may contribute to goitrogenesis.

Serum activin A levels are slightly elevated in patients with hyperthyroidism (59), while a significant increase is shown in hypothyroid patients (60). However, it is not clear whether the changes in activin A in hyperthyroid patients result from thyroid secretion or from another source.

Activin and adrenal cortex

Activin βA subunit has been detected in human fetal and adult adrenal cortex, suggesting a role in the development and in the functional regulation of this gland (61, 62). Recent studies have demonstrated a diffuse immunopositivity for βA subunit in the normal and hyperplastic human adult adrenal gland and in most adrenocortical tumors (61). Recombinant activin A inhibits mitogenesis and enhances ACTH-stimulated cortisol secretion from cultured human fetal zone cells, but it has no effect in adult adrenal cells (62). Moreover, activin A selectively suppresses fetal zone proliferation and enhances the ACTH-induced shift in the cortisol/dehydroepiandrosterone sulfate ratio of fetal zone steroid production. These data indicate that activin A may be an autocrine or paracrine factor regulated by ACTH, involved in modulating growth and differentiated function of the human fetal adrenal gland (63). Moreover, βA subunit mRNA is up-regulated by ACTH, suggesting that this protein is an autocrine/paracrine mediator of ACTH action (64). Activin A modulates the functional response of human fetal adrenal cortical cells to ACTH: it inhibits proliferation of cultured human fetal adrenal cortical cells and it also modulates ACTH-stimulated steroidogenesis. In fact, gonadectomized inhibin-deficient mice later develop adrenal cortical tumors (65). There are no data on the possible contribution of adrenal glands to circulating activin A.

Activin and pancreas

Rat pancreatic islets express mRNA for activin βA subunit (66) and immunohistochemistry revealed the presence of βA subunit in both B cells (67, 68) and non-B cells (68), while in human pancreas activin A is localized in the insulin-positive B cells (56).

Some experimental studies showed an involvement of activin A in glucose homeostasis. In fact, activin A stimulates rat insulin secretion at either 3.0, 8.3 or 16.7 mM glucose, an effect which is concentration dependent (67, 69), mediated by Ca^{++} entry (70, 71) and counteracted by reduction of extracellular Ca^{++} (67). These actions are mediated by signaling of activin A to a significant number of activin receptors. In fact the lack of activin effect in MIN 6 cells (cells not expressing activin receptors) is abolished when cells are transfected with Act R II (72). The evidence that activin A and glucagon are pooled in the same granules (69) suggests that both may be released into the portal vein, modulating liver function by acting on activin receptors abundantly expressed in the liver. The observations that activin A augments glucose production in isolated rat hepatocytes (73), and that an acute intraperitoneal administration of activin A causes hypoglycemia in mice (68), further support the concept that activin A may play a role in glucose metabolism. In addition, transgenic mice with a mutation of the receptor for activin have a lower survival rate, smaller islet area, and lower insulin content in the whole pancreas, with impaired glucose tolerance (74).

With regard to human glucose homeostasis, it has been shown that activin A acts on cultured human pancreatic islets to stimulate insulin secretion in the presence of glucose (75). Although in the absence of glucose activin A is not able to increase insulin secretion, very low concentrations of activin A potentiate glucose-mediated insulin secretion in a dose-dependent mode starting from the lowest concentrations (75). Taken together, these data may suggest a role for activin A in endocrine pancreas, as an autocrine or paracrine factor, modulating islets function as a local regulator.

When measured in the serum of patients with diabetes mellitus, activin A levels are not significantly different from those in healthy controls (59). Higher levels of activin A circulate in the serum of pregnant women with gestational diabetes (76) and a decrease

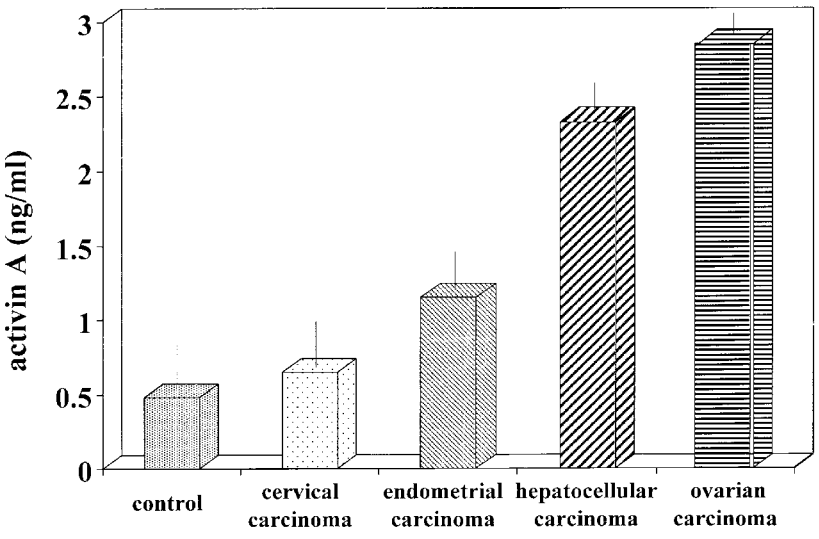

Figure 2 Serum activin A levels in epithelial malignant tumors. (Data adapted from (77), (99) and (105)).

was observed after insulin therapy and normalization of maternal glycemia. The amplitude of the pulsatile secretion of maternal serum activin A is also increased in women with gestational diabetes (76).

Activin and liver

The expression of inhibin βA subunit mRNA has been found in liver tissue with focal nodular hyperplasia and in the tumor and non-tumor tissue obtained from patients with hepatocellular carcinoma superimposed on liver cirrhosis. On the other hand, in patients with hepatocellular carcinoma but without cirrhosis neither the tumor nor the non tumor tissue expresses mRNA for inhibin βA subunit (77). Therefore, the tumor tissue as well as the surrounding cirrhotic tissue seems to contribute to the elevated serum activin A levels found in patients with hepatocellular carcinoma (Fig. 2). It was previously described that mRNA for βA subunit is expressed in focal nodular hyperplasia (77), a densely fibrotic lesion that is hormone-dependent; this finding is interesting, since several of activin-A producing tissues are estrogen-dependent (78).

Activin A acts as an autocrine negative regulator of DNA synthesis in rat parenchymal liver cells and plays a significant role in the regulation of growth in hepatocytes (79), together with follistatin (80); indeed, when recombinant human activin A is administered to rats, there is a marked reduction in liver mass; histopathological evaluation of liver specimens reveals extensive cell death in the centrilobular region, with the dying cells fragmented into apoptotic bodies (81). *In vitro* studies have shown that in human hepatoma cells both production and action of activin A are reduced: this alteration may be, at least partially, responsible for accelerated cell growth *in vivo* (82).

The contribution of liver to circulating activin A is unknown. Patients with liver cirrhosis have elevated serum activin A levels and patients with hepatocellular carcinoma show serum activin A levels higher than patients with uncomplicated cirrhosis. Therefore, an involvement of activin in the nodular regeneration of cirrhotic liver has been proposed. In fact, patients with acute or chronic hepatitis had serum activin A levels which were no higher than in normal subjects (59). Taken together, this evidence suggests a role for activin A in modulating liver growth, and a role for activin A measurement as a possible useful complementary test, in conjunction with α-fetoprotein, in the diagnosis of hepatocellular carcinoma (77). Serum activin A levels have also been found to be high in two patients with cholangiocarcinoma and metastatic liver cancer of unknown origin, similar to the levels found in pregnant women at term (59).

Activin and bone marrow

Activin A is expressed by human bone marrow cells and monocytes and is regulated by inflammatory cytokines and glucocorticoids (83). Human marrow fibroblastoid cells contain immunoreactive activin A, and the production of activin βA RNA is up-regulated by pro-inflammatory cytokines/regulators such as interleukin 1 alpha, tumor necrosis factor-α, lipopolysaccharide or 12-O-tetradecanoylphorbol 13-acetate (84). On the other hand, hydrocortisone and dexamethasone inhibit both the constitutive and the cytokine-stimulated expression of activin βA RNA (84). The predominance of βA-subunit mRNA in the bone marrow suggests a specific role for activin A in the local process of osteoclast differentiation (85). Activin A stimulates the formation of osteoclasts, but not osteoclast activation (86). Activin A blocks the activity of key inflammatory cytokines such as interleukin, and at the same time a complex regulatory loop is operable to modulate the effects of activin A during

inflammation (87, 88). Secretion of activin A from immune cells has been suggested by the evidence that fever is associated with increased levels of activin A. This release of activin A in inflammatory reaction may be local as well as systemic.

Activin and female reproductive organs

Ovary Ovary is the organ from which activins were first isolated. mRNA for βA subunit has been found in granulosa cells, in the thecal cell layer, and in luteinized granulosa cells (89). Furthermore, the staining of the dimeric activin A has been shown in the granulosa and cumulus cells of human ovarian follicles and in granulosa-lutein cells of the human corpus luteum (90).

The immunostaining for activin βA subunit changes according to the menstrual cycle, being positive in the granulosa cells of preantral and small antral follicles, mainly in the cumulus cells (91).

A lot of data suggest an autocrine/paracrine role of activin A within the ovary, able to modulate the development and luteinization of the follicles and also the production and secretion of ovarian steroid hormones, decreasing progesterone and estradiol secretion, both basal and FSH-stimulated (92). The role of activin A in oocyte maturation is supported by reports of the expression of βA subunit (93) and type II receptors in rat (94) and mouse (95), and by a potent stimulatory effect on the *in vitro* rat oocyte maturation, being able to increase the signs of nuclear maturation within 48 h of *in vitro* culture (92).

High activin A concentrations are measurable in follicular fluid, but only small changes are seen according to the ovarian follicle maturational events (96).

Small changes of circulating serum activin A levels are described during the menstrual cycle, with nadir values at the mid-follicular phase (97). Because serum levels of activin A do not change in pubertal maturation (30) nor in women with premature ovarian failure or after physiological menopause, the ovaries are not considered a major source of activin A (31, 98).

Active secretion of activin A has been shown in women with ovarian cancer. Indeed, serum activin A levels are frequently elevated in women with epithelial ovarian cancer (99) (Fig. 2). Moreover, a recent study demonstrated that activin A levels correlate with recurrent or persistent disease in patients with epithelial ovarian cancer and suggests a role for this protein as a serum marker for this pathology (100). Most primary epithelial ovarian tumors synthesize and secrete activin protein *in vitro*. Specific immunostaining for the βA subunit of activin has been observed in the tumor cells of mucinous adenoma and cystic tumor with borderline malignancy, as well as in the tumor cells of mucinous adenocarcinoma (101). Positive staining for the βA subunit has been observed in serous tumor cells (101). Imbalanced expression of inhibin and activin subunits in ovarian surface epithelium may represent an early event which leads to epithelial proliferation, and this suggests a possible autocrine and/or paracrine role for activin in the regulation of ovarian epithelial tumors (102). Secretion of activin A has been shown within ovarian cystoadenoma (103).

Uterus/endometrium Immunoreactive βA subunit is localized in the luminal and glandular epithelium and in migratory cells, while the endometrial stromal cells, decidua, vascular smooth muscle and endothelium are devoid of immunoreactivity (104). However, both cultured glandular and stromal cells express activin A mRNA and secrete inhibin A (105).

Immunostaining for the βA subunit and activin A is present in the cytoplasm of human endometrial glands throughout the menstrual cycle and in decidua during early pregnancy. The intensity of immunostaining for the βA subunit is strong during the menstrual phase, becomes weaker during the early proliferative phase, and is intense again at the mid and late proliferative phase. The stromal cells are weakly immunoreactive with antibodies against the βA subunit or dimeric activin A from the menstrual to the midsecretory phase and become stronger in the late secretory phase (104, 106).

Secretion of endometrial activin A into the circulation is suggested by two different pathological conditions: endometriosis and endometrial carcinoma. Endometriotic cells express mRNA for activin βA- and βB-subunits, and for activin type II and type IIB receptors (107). High concentrations of activin A have been found in endometriosic cysts and in peritoneal fluid of women with endometriosis (107). However, since peritoneal fluid activin A is high both in controls and in patients with endometriosis, ectopic endometrial tissue does not appear to contribute significantly to the protein content in the peritoneal fluid (107). High concentrations of activin A have been measured in ovarian endometrioma, higher than in serum (108).

Endometrial and cervical carcinoma cells express the gene for activin A. Activin A is secreted from HEC-1A and HEC-1B cells in culture medium and its measurement in uterine fluid of women with endometrial carcinoma suggests that this protein is secreted from tumoral cells into extracellular fluid (105). Serum levels of activin A are increased in women with endometrial or cervical carcinoma and they decrease 1 month after the surgical removal of the tumor, suggesting that uterine tumors may be a source of circulating activin A (105) (Fig. 2).

Placenta and pregnancy Human placenta and fetal–maternal intrauterine membranes express activin as well as activin type II receptors, and βA mRNA expression levels increase throughout pregnancy, with

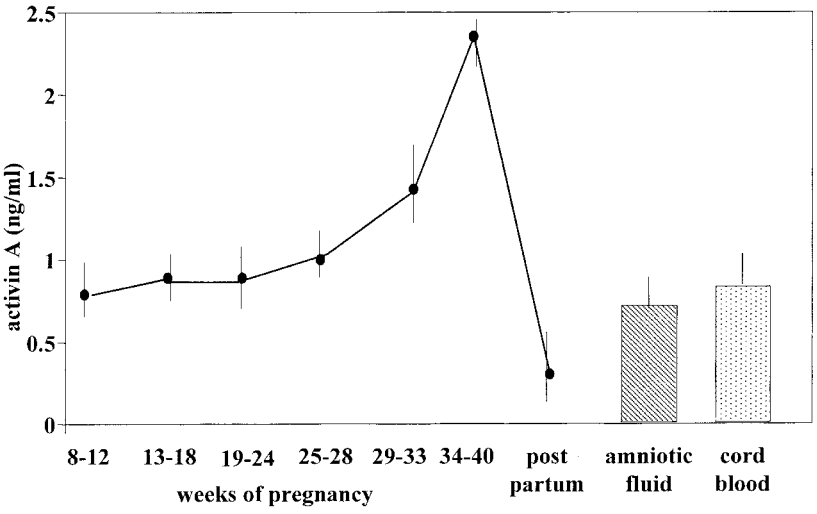

Figure 3 Changes in activin A concentrations in maternal serum throughout healthy gestation, and in amniotic fluid and cord serum at term. Results are means ± S.E.M. (Data adapted from (27)).

the highest levels found at term (109). *In vitro* studies have shown that activin A has a stimulatory effect on human chorionic gonadotropin secretion from trophoblast cultures (110). These tissues are an important source of activin A in pregnant women.

High concentrations of activin A are measurable in maternal serum throughout healthy gestation (27, 109), with an increasing pattern from early gestation until term (Fig. 3). The rapid decrease following placental delivery suggests that, during pregnancy, human placenta is the major source for activin A. Maternal serum activin A levels are higher in healthy women who undergo vaginal delivery than in those who deliver by elective caesarean section (111). An increased secretion and expression of activin A-subunit mRNA has been reported in the chorion and amnion of women delivering at term or preterm (76, 112). The finding that activin A may stimulate the release of prostaglandin from fetal membranes and of oxytocin from cultured placental cells has led to the suggestion that this protein could be involved in the mechanisms of labor (109).

Activin A is measurable in amniotic fluid and also in cord blood. During the second and third trimester of pregnancy, the concentrations of activin A in amniotic fluid tend to increase. In any case, abnormally high activin A levels in amniotic fluid at midtrimester have been described in a group of women who later suffered fetal demise (113). At early gestation, activin A concentrations in coelomic fluid are significantly higher than in maternal serum and amniotic fluid (114) (Table 1).

Coelomic fluid is an important reservoir of activin A and is probably used by the embryo for its development even though several fetal tissues express mRNA for the βA-activin subunits (115).

Maternal serum concentrations of activin A are elevated in several gestational diseases such as gestational diabetes and pre-eclampsia (75). In the latter, the ratio between activin A and follistatin is markedly increased, indicating that high amounts of free activin A are available in the maternal circulation and suggesting a role for activin in the maternal adaptive response to the disease. Of particular interest are the data showing that increased activin A levels at midtrimester may predict those patients who will later develop pre-eclampsia (116, 117).

Breast Activin βA subunit is expressed in human mammary gland, with a predominance in the epithelial cells of ducts and lobules, while dimeric activin A is detectable in the cystic fluid of breast fibrocystic disease (118). Local synthesis of activin A in the human breast is further indicated by the identification of βA mRNA in breast tissue using reverse transcriptase-polymerase chain reaction (119).

The presence of activin also in this tissue could be linked to an effect on cell growth. In fact, activin has an effect on the differentiation of mammary epithelial cells (120) and in promoting the growth and morphogenesis of primary or transformed mammary epithelial cells (121). Furthermore, the proliferation of breast cancer cell lines *in vitro* may be inhibited by activin A and this mechanism is dependent on estrogen receptor expression (122). The contribution of breast to circulating activin A is under investigation.

Activin and male reproductive organs

Testis In the adult human testis, the βA subunit is expressed by both Sertoli and Leydig cells (123) and also by the lumen of the tubules (124), indicating that it is likely to be a secreted product. Activin A has stimulatory effects on Sertoli cells reaggregation and germ cell proliferation (125, 126) and some of these effects are presented by follistatin (127). These data are consistent with regulatory effects on the interaction

between Sertoli cells and developing germ cells and possibly also Leydig cell steroidogenesis (128).

Testis may be one of the sources of activin A in the systemic circulation as well as in seminal plasma of both normal and oligo/azoospermic men but it is undetectable in all post-vasectomy samples (in particular Sertoli cells).

Prostate Human prostate shows immunoreactive activin A (124). In particular, immunohistochemistry of prostate epithelium predominantly stained activin A while that of stroma cells stained mainly follistatin. In benign prostatic hyperplasia tissues, activin and its binding protein have an adjacent localization, suggesting that a paracrine interaction occurs between the activin ligands and follistatin–binding proteins both in normal and pathological prostate (129). However, in benign prostatic hyperplasia the expression of βA subunit mRNA is variable among patient samples (130).

Activin A is synthesized in prostatic tissue of men with high grade prostate cancer, and the activin βA and follistatin mRNA and proteins are expressed and localized to poorly differentiated tumor cells. In the non malignant region, activin βA-subunit mRNA and protein are predominantly localized to the epithelium. In the progression to malignancy, follistatin and activins are colocalized to the tumor cells (130). The interpretation of data about the roles played by activin A and follistatin in human prostate is complicated by the evidence of different effects shown by the different *in vitro* models used. Activin A is a potent growth inhibitor of LNCaP cells; moreover, these cells also produce activin A, suggesting that locally derived activin may play a role in regulating cell proliferation (131). In fact, in androgen-responsive prostate cancer cell lines (LNCaP cell), androgen withdrawal reduces cell growth, and the decline in circulating follistatin increases local activin A tone and further inhibits cell proliferation. In contrast, the growth of androgen-independent cancer is not affected by the loss of androgens, and the high local production of follistatin prevents a change in activin action in response to reduced circulating levels of androgens.

Conclusion

The discovery of activin A in follicular fluids years ago and the first data obtained on LH/FSH regulation pictured activin A as a gonadal hormone, mainly involved in the fine tuning of follicular development, and suggesting the ovary as the main source of activin A in circulation. However, a growing amount of evidence as listed in the present review indicates that activin A is detected in several organs which may contribute to circulating protein levels. To date, it is impossible properly to answer the question which is the main source of circulating activin A in humans.

At present, the evidence that activin A and activin receptors are frequently co-expressed in some tissues suggests a large spectrum of local actions. Activin A may be defined as an important regulator of several physiological and developmental processes, including reproduction. The role of circulating activin A remains uncertain. Contrary to inhibin, which acts as a hormone of the reproductive axis in women and men and during gestation, activin A can be considered a mainly autocrine/paracrine factor which acts as a local regulator of cellular growth and differentiation. However, because of the high concentrations in the systemic circulation it will be of interest in the future to understand the significance of changes in activin A levels in patients with benign and/or malignant tumors.

In conclusion, the variety of actions of activin A and the possibility of measuring activin A will probably offer new diagnostic and therapeutic tools in clinical medicine in the years to come.

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