INHIBIN - SYNTHESIS, SECRETION AND PRACTICAL USES

Structure of inhibin

Inhibin is a protein secreted by granulosa (female) and Sertoli (male) cells in response to FSH, and its major action is the negative feedback control of pituitary FSH secretion (ref 1). It is found in blood plasma, although difficult to detect until recently. It is found in great quantities in seminal plasma and follicular fluid. Inhibin is a dimeric protein of great complexity. The ‘mature’ form of inhibin has a molecular weight of 32,000 daltons, and consists of one alpha-chain (approx 18 kDa) and one beta-chain (14 kDa) linked by disulphide bridges. The subunits alone possess no known biological action.

Each subunit is produced from a separate gene, and is produced as a large precursor protein. Each subunit has multiple cleavage sites, such that subunits of different size are routinely found in follicular fluid. Various combinations of alpha-beta subunits appear possible, giving rise to a number of different dimeric inhibin forms. Commonly found forms in bovine follicular fluid are approx 29, 34, 48, 58, 68, 77, 122 and >160 kDa (ref 2). While the biological significance of these multiple forms is not clear, the dimeric forms appear to have similar biopotencies.

The inhibin family is further complicated by the existence of two separate beta-subunit genes, and thus two distinct proteins. These have been termed the beta-A subunit and the beta-B subunit. Thus there are two ‘types’ of inhibin, inhibin-A or inhibin-B, and each may exist in 7 to 9 different molecular forms. To date, the inhibin-B forms have been detected and measured in humans and other primates, but have not yet been detected in ruminants. While inhibins are dimers of alpha and beta subunits, dimers of beta-beta subunits form another hormone, activin. Activin is a general growth-stimulating factor, and appears to have local effects on many cell types. Additionally, activin stimulates FSH secretion, thus antagonizing the main biological action of inhibin.
Not all of the circulating inhibin is in the biologically active (dimeric) form. In humans and ruminants, there is a vast amount of ‘free’ alpha subunit in the blood and follicular fluid. This has posed a major problem for the measurement of inhibin, as most antibodies used recognize the alpha subunit only, and thus measure ‘free’ alpha as well as dimeric inhibin. The free alpha subunit has no known biological action.

In order to get around the problem of measuring free alpha subunit, a two-site ELISA was developed for use in humans (ref 3). One antibody raised against the alpha subunit is bound to the bottom of microtitre plate wells. The samples are added and free alpha and dimeric inhibins bind to the antibody. Then a second antibody is added, that is raised against the beta subunit. This antibody will bind to dimeric inhibin but not the free alpha subunit. Variations on this theme have been used by different laboratories to measure dimeric inhibin in follicular fluid of many species, but the assay is not sensitive enough for use in blood of ruminants.

**Secretion of inhibin**

Most information regarding changes in circulating inhibin concentrations thus comes from human or rat studies. In rats, inhibin-A concentrations are low on the day of metestrus, and rise towards a maximum on the evening of proestrus. Concentrations fall sharply on the morning of estrus. This mirrors the changes observed in plasma estradiol concentrations. In contrast, inhibin-B concentrations are high on metestrus, decline slightly on proestrus, and fall a little further on the morning of estrus. The decline of both inhibins on the morning on estrus coincides with the peak of FSH, consistent with the negative feedback relationship between FSH and inhibin.

In human follicular fluid, concentrations of inhibin-A change little with follicular diameter (and thus development), whereas inhibin-B concentrations rise markedly as follicles develop. In ruminants, inhibin-A concentrations are most notably related to follicle health: regressing or atretic follicles contain more inhibin overall than healthy follicles, although the different molecular weight forms change differently, most decreasing as follicles become atretic.
During the primate menstrual cycle, inhibin-A concentrations are low during the follicular phase, increase after ovulation and increase to a maximum during the mid-luteal phase. This is in fact consistent with the lack of correlation of inhibin-A and follicle development, and suggests that inhibin-A is primarily of luteal origin. In contrast, inhibin-B concentrations are high in the follicular phase, and fall progressively towards ovulation. This is the inverse of changes in estradiol secretion, but loosely follow the changes observed in FSH secretion. A recent study has reported inhibin-A in sheep blood, and little changes were noted during the follicular phase, other than a sudden decrease immediately following ovulation (ref 4). In cattle, dimeric inhibin-A has only been measured in the plasma of superovulated animals (ref 5). Exactly how changes in measurable inhibin concentrations relate to bioactivity is not clear, due in part to the different molecular species, and also owing to the presence in plasma of inhibin binding proteins. There are two major binding proteins, follistatin and alpha 2-macroglobulin. Alpha 2-macroglobulin is a high-capacity, low affinity binding protein, whereas follistatin is a high-affinity, low-capacity binding protein.

In rats and humans, females secrete both forms of inhibin. Males, however, only appear to synthesize and secrete inhibin-B.

Control of inhibin secretion

In accordance with the negative feedback relationship between inhibin and FSH, inhibin secretion is increased by FSH. This has been observed in most species studied. In ruminant, FSH stimulated plasma inhibin-A concentrations in vivo, and inhibin-A secretion from granulosa cells in vitro. In rats, FSH stimulated both inhibin-A and inhibin-B, but inhibin-A was more sensitive to FSH than was inhibin-B. In women given single injections of FSH, both inhibin forms are stimulated, whereas during a FSH-stimulated cycle, plasma concentrations of inhibin-B but not inhibin-A were stimulated; this is consistent with the follicular source of inhibin-B.
Other factors have been shown to influence inhibin secretion. Estradiol stimulated inhibin-A but not inhibin-B in rats, and IGF-1 increased alpha subunit expression.

Inhibin as markers of health and disease

Within the human reproductive disease field, the measurement of inhibin has become a catchy, new way to monitor reproductive function (ref 6). While not always better (and certainly more expensive) than monitoring steroid secretion, it is currently more popular. Such studies have enlarged our knowledge of inhibin in primates, and may offer a glimpse of potential applications of the inhibin craze to domestic animals.

A considerable concern these days is reproductive aging. Most obvious is the decrease in fertility before ‘aging’ women reach menopause. This has been related to an increase in pituitary FSH output in older women, which is not explained by changes in ovarian steroid hormone secretion. Inhibin is thus a potential mediator of this age-related rise in FSH secretion. In general, the data suggests that inhibin-B concentrations are lower in older women, inhibin-A and follistatin concentrations change little, and activin concentrations are higher. The combined effect of these changes can explain the increase in FSH. Similar data have been produced in ewes, which shows that ewes when young secrete more inhibin-A than when older.

Another effect of aging is observed in men. As men get older, libido declines significantly. This too is associated with elevated FSH concentrations, although both testosterone and inhibin-B decline with age in similar patterns. Although testosterone is responsible for male libido, inhibin-B has been suggested as a marker of testicular function. Serum inhibin has been positively correlated with sperm count, testis volume and testis histology.

In women, inhibin-A is secreted by the feto-placental unit, as evidenced by increased concentrations during pregnancy. Significant increases are observed by 6 - 8 weeks. Inhibin-A is a potential marker of progression of pregnancy following IVF and embryo transfer. Multiple pregnancies result in higher inhibin-A concentrations than singleton pregnancies at 8 – 10 weeks.
Additionally, in embryo transfer, a number of fetuses are lost early in pregnancy, but this loss is
sometimes not detected by traditional hCG testing and/or ultrasound. There is evidence that
inhibin-A secretion is dependent on the presence of a live fetus, as concentrations do not increase
as normal in cases of early fetal loss; it may thus be a good marker for monitoring IVF
pregnancies.

Inhibin has also been evaluated as markers of ovarian disease. In some but not all studies,
inhibin-A and inhibin-B concentrations were lower in follicles from women with polycystic
ovarian syndrome (PCOS) compared to healthy follicles. Plasma inhibin-A and inhibin-B
concentrations were reported to be higher in women with PCOS compared to controls. It has also
been suggested that the pattern of secretion of inhibin is altered in PCOS. It was suggested that in
normal women, inhibin-B is secreted in pulsatile manner (corresponding to FSH pulses), whereas
this pulsatile pattern is lost in PCOS.

Inhibin concentrations may also be a marker for progression of certain ovarian cancers.
Inhibin-B concentrations are significantly elevated in patients with granulosa cell tumors, but
normal in patients in clinical remission. In contrast, inhibin secretion is not affected by epithelial
cell cancers (as epithelial cells do not synthesize inhibin).

Uses of inhibin in domestic animals

As inhibin acts systemically to inhibit FSH release, it follows that a reduction of inhibin
secretion would increase FSH concentrations and thus offer potential for increased fertility.
Domestic ruminants have been immunized against a variety of inhibin preparations, and small
increases in ovulation rate have been reported in cattle, sheep and goats. However, the responses
are far from superovulatory, and this approach to fertility management is not widely used.

Key references


