Inhibin: Central, Gonadal And Extragonadal Actions*

İNİİİBİN: SANTKAL, GONADOL VE EKSİ'RAG ONA DA L ETKİLERİ

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SUMMARY

Inhibins' existence was first proposed over 50 years ago. It was recently purified and synthesized. Tlie initial application of inhibin into clinical practice as a tumor marker for granulosa cell tumors and hydatiform moles was recently reported. Inhibin with its wide range of central, gonadal and extragonadal actions is likely to be a hormone of major interest in the future.

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ÖZET

***Inhibinler** varlığı 50 yıl önce ileri sürülmekle beraber ancak yakın zamanlarda saflaştırılmış ve senlezlenmişiir. Granuloza lıiicreli tümörler ve ınol hydatiforın da tümör markeri olarak klinik uygulamaya ait ilk çalışmalar yeni başlamıştır. Santral, gonadal ve ekstragonadal geniş etkileri ile inlıibin gelecekte en çok ilgi çekecek hormonlardan biıi olarak görünmektedir.*

Anahtar Kelimeler: inhibin

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Over 65 years ago it was proposed that in addition to steroid hormones, proteins could also play a role in the regulation of gonadotropin secretion from the anterior pituitary.

Motram and Cramer reported the appercance of castration cells with pituitary hypertrophy after the treatment of testes with radium (1). This observation suggested that there is an inverse relationship between the testes and the pituitary and testes may secrete substances that arc inhibitory. Martins and Rocha showed that these effects could be reversed by injection of extracts of bull or goat testes (2). MeCullagh, by injecting the aqueous extracts of testes into castrated male rats was able to prevent the appearance of the so called castration cells in the pituitary (3). He concluded that the testis had a dual endocrine activity and he named the content of the aqueous extract "inhibin". This extract was able to prevent the pituitary hypertrophy in the male rats after castration in contrast to the fat-soluble hormone later known as testosterone which was responsible for maintaining the accessory sex organs.

After the identification of the gonadotropins in 1931, it was demonstrated that a substance in the seminiferous tubules might influence the secretion of FSH because men with oligozoospcrmia or azoospermia showed elevated levels of FSH in their urine (4,5).

Interest in this water soluble substance regulating the secretion of FSH dwindled and was not revived until the early 1970s. A major obstacle in the inhibin research was the inaccuracy of the bioassays

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CANADA

for FSH. After the development of radiommunoassays for the gonadotropins inhibin research gained pace (6,7). Isolation of the Luteinizing Hormone Releasing Factor (LRF) by Schally in 1971 increased our understanding of the control of reproduction (8). LRF was later found to mediate the secretion of both LH and FSH and for this reason it was renamed GnRH (9). After the isolation of GnRH the science world witnessed the appearance of over a hundred different agonists or antagonists of GnRH. However, neither the native hormone nor the analogs have demonstrated selective modulation of FSH. Moreover, the long proposed FSH-rcleasing factor of hypothalamic origin that preferentially affects FSH release has not been isolated and characterized. The search of the mechanism of selective control of FSH release rekindled inhibin research.

Isolation of Inhibins and Activins

Direct evidence for the existence of inhibin emerged from several studies between 1970 and 1980 when the extracts of seminal plasma, testis extracts and ovarian follicular were shown to have the capacity to suppress FSH secretion.

The initial isolation of inhibin was achieved from bovine follicular fluid as a 58 kDa glycoprotein consisting of two disulphide linked subunits of molecular masses of 43 and 15 kDa (10). Ling and colleagues isolated two forms of inhibin, termed inhibin A and inhibin B, identified by the differing NH_2 terminal aminoacid sequences of their p-subunits, now termed pA and pB (11). All subunits arc synthesized as large precursor proteins and each precursor contains several potential proteolytic processing sites and in each case the cleavage of one of these sites yields the mature pA, or pB subunit as the earboxyl terminal fragment of the precursor (12). There appeared to be a great degree of homology between the p subunits of inhibin and TGF-p. These led the investigators to wonder if inhibin had similar FSH stimulating activity as the TGF-p. Subsequently two forms of proteins consisting of two p subunits of inhibin linked by an interchain d-sulphidc bond was synthesized (13,14). Because these proteins elevate the basal secretion of FSH by pituitary cells they were designated as activins. The FSH stimulatory effect of activins distinguishes them from the action of GnRH: Activins do not modify LD secretion, require a latent period of several hours before stimulating the secretion of FSH, act on both release and synthesis of FSH, and bind to different membrane receptors from those of GnRH.

Assays For Inhibin

Attempts to isolate inhibin depended on the development of spesific assays based on the suppression of FSH. Many of the bioassays developed were not adequately defined in terms of sensitivity and specificity, and precision and failed to lake into account that the use of inhibition of FSH as the endpoint of the assay could lead to nonspecific effects (15). Following the purification of inhibin, radioimmunoassays have now been developed that offer relatively rapid, practical and sensitive methods to measure inhibin.

In-vitro bioassays

1. The rat pituitary cell bioassays

a) Measuring the release of FSH into the medium

b) Measuring the changes in the FSH cell content

2. Ovine pituitary cell culture assay

Radioimmunoassay: The demonstration that the synthesis of inhibin subunits arc controlled by separate genes raises strong possibility that free alpha and beta subunits may exist at the site of production and may pass into the circulation. Furthermore the identification of the beta subunit dimcrs of inhibin also raises questions of specificity in the radioimmunoassays for the inhibin molecule. It is important that any assay system developed should clearly specify the cross-reactivities of free alpha or beta subunits and inhibin related proteins. Two approaches have been used in the development of radioimmunoassays for inhibin-immunization with the native molecule and immunization with short synthetic peptides derived from the sequence of inhibin (12). A higly specific and sensitive radioimmunoassay for inhibin was developed using antibodies to (Tyr30)-In-a(1-30), ^{125}l-cyclic (Cys , Tyr^{7}) human in-a (6-30) as radiotracer, and purified porcine inhibin as standard reference (12).

Sites and Mechanisms of Action of Inhibin

FSH and LH arc under the control of GnRH. Their secretion is modulated by feedback effects of gonadal steroids and peptides with inibin being one of them.

Pituitary Action of hihibin: There is evidence that FSH and LH arc differentially regulated. In men with seminiferous tubule failure leading to infertility, FSH levels may be elevated in the presence of testosterone and LH concentrations within the normal range (16,17). This suggests the presence of an FSH regulating feedback factor, the secretion of which is reduced as a result of seminiferous tubule damage.

In certain circumstances, FSH is eonstitutively secreted by the pituitary gland and shows little short term dependence on GnRH. In ovarieetomi/ed ewes which have undergone HP disconnection, administration of GnRH maintains normal castrate levels of FSH and LH. Following the cessation of GnRH pulses LH becomes undetectable within 6 hours but FSH secretion continues relatively unaltered for some days (IS). When dispersed anterior pituitary cells are cultured for over a period of 10-28 days FSH continues to be secreted into the medium at a relatively constant rate up to 28 days whilste the LH levels fall rapidly. Addition of inhibin to these cultures produces a major inhibition of FSH (19). Thus FSH and LH arc regulated differentially in some circumstances, particularly in the absence of GnRH.

Although a number of investigators have demonstrated suppression of FSH in-vivo and in-vitro by impure preparations of inhibin, these results should be viewed with caution since aetivin and FSH supporting protein (FSP) are present in the follicular fluid, which commonly was used as the source of the impure inhibin preparations. In a recent study the infusion of pure 31 kDa bovine inhibin to castrated sheep demonstrated a dose related FSH suppression (20). Inhibin was showed to have inhibitory effect on FSH release, cell content and synthesis, with minor effects on LH, TSH, Prolactin and growth hormone. Studies using purified inhibin from follicular fluid have confirmed these effects on pituitary cells in culture (10).

In experiments done on pituitary cells in culture it was shown that there were two separate mechanisms of action of inhibin on FSH release (19). At low concentrations, inhibin rapidly suppresses FSH release and synthesis; at higher concentrations, the cell content of both gonadotropins are affected by the degradation of intracellular stores of FSH and LH. These effects arc reversible after the removal of inhibin from the culture medium.

Studies using pure inhibin have shown that inhibin exerts suppressive effects on the release of FSH and LH following GnRH stimulation (10,19). This inhibition involves a decrease in the sensitivity of cells to GnRH. It was of interest that this effect of inhibin was antagonized by a GnRH agonist. Recent studies have also shown that inhibin decreases the number of GnRH receptors on pituitary cells in culture and also diminishes the up-rcgulation of GnRH receptors by GnRH (22,23).

Hypothalamic Action of Inhibin: Franchimont el al. have demonstrated that inhibin preparations extracted from the rclc testis fluid and human seminal plasma decrease the endogenous GnRH content of isolated rat hypothalami after short term incubation with varying inhibin concentrations (24). Lumpkin ct al also showed that inhibin preparations purified from the rctc testis fluid preferentially inhibit FSH secretion in the adult male rat by a hypothalamic mechanism (25). The injection of inhibin into the third ventricle of castrated rats resulted in a decrease in plasma FSH levels during the 24 hour poslinjection period whereas FSH levels increased steadily in controls. When compared with control the plasma LH levels were not significantly different for any given period of time. In contrast, FSH and LH releases induced by a GnRH challenge administered 6 hour post-injection were similar in inhibin and control groups, thereby arguing against a pituitary site of action of inhibin injected into the third ventricle.

Gonadal Actions of Inhibin: Several paracrine actions on steroidogenesis in the ovary and testis have been discovered for inhibin, aetivin and TGF-B. Recently, it was demonstrated that recombinant porcine inhibin directly inhibits FSH-induced aromalization by rat granulosa cells (26). In primary cultures of testis cells, the alpha-beta hcterodimer of inhibin enhances Leydig cell androgen biosynthesis stimulated by LH, whereas the bcta-A-beta-A aetivin homodimcr suppresses androgen production (27).

Sources of Inhibin

Inhibin is produced by the Sertoli cells in the male and granulosa cells in the female.

Inhibin has been detected in ovarian follicular fluid and ovarian homogenatcs from all mammalian species studied so far. FSH, testosterone and

somatomcdin-C stimulate inhibin production (28,29). FSH is the principal controller of inhibin secretion. Inhibin activity was higher in the venous effluent from the side of the dominant follicle (30). Studies also have shown that the amount of inhibin produced by granulosa cells from larger follicles is greater than that from small follicles (31). Tsonis ct al have also showed that inhibin activity correlated significantly with the estradiol concentration and aromatase activity of the granulosa cell (31). McLaehlan et al. showed that a good correlation can be obtaiend between the circulating levels of estradiol and ihnibin in women undergoing ovarian hypcrstimulation for IVF (32).

Recent studies have also shown that the corpus luleum is a significant source of inhibin in the luteal phase of the menstrual cycle (33). This view has also been supported by in-vitro studies demonstrating that human granulosa cells allowed to luteinize in culture have the capacity to produce inhibin: These cells can no longer be stimulated by FSH but respond to LH and testosterone (31). Additional evidence that the luteal cells can produce inhibin comes from the demonstration that the rat and human luteal cells contain mRNA for the alpha subunit of inhibin (34).

Confirmation that the ovary is the major source of inhibin comes from the rapid disappearance of scrum inhibin levels following oophorectomy (35).

Bicsak ct al studied the hormonal control of granulosa cell inhibin biosynthesis (36). They showed that FSH stimulation of inhibin production was through the cAMP-mcdiated pathway. FSH, LH and hCG all increased inhibin production by granulosa cells. PRL and lerbutaline were shown to have no effect on inhibin production. Effects of various growth factors were also studied and GnRH and EGF were found to be inhibitory while IGF I and VIP enhanced inhibin production.

Inhibin in Menstrual Cycles

The availability of sensitive radioimmunoassay has enabled the measurement of circulating levels of inhibin during the menstrual cycle (33). Serum inhibin was inversely correlated with serum FSH in the mid to late follicular phase. Serum estradiol rose in paralellel with inhibin in the late follicular phase, although estradiol peaked 1 day earlier. Serum inhibin increased late in the follicular phase reaching a peak coincident with the LH surge. After the midcycle rise, scrum inhibin levels fell approximately 50% to a nadir on day 2, although this fall was not significant. Subsequently the inhibin levels rose during the luteal phase to values higher then seen in the midcycle. Both FSH and LH levels declined rapidly in the early luteal phase and remained low until the perimenstrual period. In the early to midluateal phase inhibin was inversely correlated with FSH and directly correlated with progesterone. A significant fall in inhibin occured in the 2 days before the onset of menses, while FSH rose in the same period. Inhibin was inversely correlated with FSH and directly correlated with progesterone in this phase of the menstrual cycle.

Mais ct al. studied the hormonal dynamics during luteal-follicular transition in women (37). During cstradiol-progestcronc withdrawal, there was a selective increase in mean serum FSH levels beginning 24 h before and reaching a peak 24 hours after the onset of menses. The frequency of LH pulses increased slightly but not significantly during this period, with a significant rise in mean serum LH levels on the day of menses. Thus an acute rise in FSH occurred the day before and rise in LH occurred the after the onset of menses in the phase of luteal-follicular transition. The dissociation between the secretion of FSH and LH during this period is probably the result of additional neuroendocrine events other than the changes in the pulsatile secretion of GnRH. In further studies Roseff ct al. showed that the secretion of inhibin by the corpus luteum appears to be coupled with E2 and P4, and that the time course of luleolylic processes is intimately associated with the degree of inhibin decline and rise of FSH levels (38). They concluded that follieulogcnesis is determined by dimished inhibin suppression of FSH secretion occurring 5 days before the onset of menses. Thus the ovary appears to participate actively in regulating its follieulogcnesis by using inhibin as a controlling signal.

Inhibin in Estrus Cycles

Using a heterologous radioimmunoassay system Hasegawa et al. noted that inhibin levels in the circulation of rats during the estrus cycle were higher during the follicular phase but dropped sharply during proestrus, suggesting that the proestrus rise of FSH can be attributable to a fall in

inhibin secretion (39). This view would be in accord with a recent study by Woodruff ct al. demonstrating that mRNA for the alpha and beta subunit of inhibin rises steadily during dicstrus to a peak late in the afternoon of procstrus (40).

Inhibin in Pregnancy

Data from the 1VF programs indicate that the decline in inhibin levels late in the luteal phase of normal menstrual cycles is not seen in conception cycles, the levels of inhibin being maintained and in fact increasing significantly (41). Studies in three women with no ovaries who achieved pregnancy after oocyte donation showed that the inhibin levels during early pregnancy are similar to those women with ovaries who achieve pregnancy (42). These results suggests that: 1.Maternal ovary does not eontribute significantly to inhibin secretion during pregnancy 2. The trophoblast is the likely source of inhibin during pregnancy 3. Inhibin may have a role in the regulation of FSH during pregnancy and/or a local role within the fcto-placcntal unit.

An embryonic source of inhibin is supported by the studies of McLachlan et al demonstrating that the placenta has the capacity to produce both immunoaclive and biouctive inhibin (43). Davis et al. showed that the placenta contained mRNA for the alpha and BA subunits of inhibin (44). Petraglia et al. demonstrated that the placental cultures have the capacity to produce inhibin and this substance can be localized immunocylochemically to the cytotrophoblasl of the placenta (45).

Although the role of inhibin in early pregnancy remains to be elucidated, the significant homology that was noted between inhibin and the decapentaplcgie gene complex in Drosophilia and the VG1 g'mc of xenopus, both of which are involved in early embryonic differentiation. This view is supported by the recent demonstration that TGF-B, a protein with significant homology to inhibin, plays an important role in various embryonic events (2).

Inhibin in Puberty

During normal human puberty, inhibin levels rise in parallel with those of gonadotropins and sex steroids (46). With rising FSH levels in early puberty, there is stimulation of the development of seminiferous tubuli epithelium and ovarian follicular development. This stimulation results in a rise in inhibin secretion. Once serum inhibin rises to adult eoncentraitons, the inhibin-FSH negative feedback relationship becomes established. This is a postulate in view of the data and there is no direct evidence at the moment to prove it.

Inhibin and PCO

Several investigators have speculated that in patients with polycystic ovarian syndrome (PCO) elevated serum inhibin levels may be involved in producing the elevated LH and somewhat suppressed FSH levels (47). They postulated that high levels of androgens drived by the increased LH levels, may stimulate inhibin secretion from the granulosa cells which in turn, inhibits the FSH secretion by the pituitary. The elevated levels of androgens secreted by the ovary and perhaps the adrenal glands also contribute to an increased estrogen pool by extraovarian tissues and could serve to augment pituitary sensitivity to GnRH with a resultant secretion of more LH than FSH,

In a recent study on 5 subjects, Buckler et al, demonstrated that inhibin levels are not significantly different in the early or midfollieulur phase and that the inhibin levels in the late-luteal phase, at midcycle and during the mid-luteul phase are significantly lower than in normal cycles (48), Furthermore, the normal subjects and those with PCO showed no differences in inhibin levels following the endogenous gonadotropin rise and effective gonadotropin withdrawal that occurs after continued administration ol'GnRH agonists.

These later studies suggests there is no primary defect of inhibin secretion in the PCO patients.

Inhibin and Tumors

Recently Lappohn el al. reported that inhibin could serve as a useful marker for granulosa cell tumors (49). Serum immunoreactivc inhibin levels were measured in 6 women with such lumors, 3 women had been treated surgically. In two women with residual or recurrent disease, the scrum inhibin levels were elevated 5 and 20 months before the clinical manifestations of recurrence became evident, It was evident that these tumors secreted inhibin autonomously. The inverse correlation that was also demonstrated between FSH and inhibin concentaritons suggest that serial determinations of FSH levels may also serve as a grunalosa-cell tumor marker.

Scrum inhibin measurements have also been suggested as a useful marker for another tumor, the hydaliform mole (50). Inhibin levels were much higher in almost all patients with hydaliform moles than in normal pregnant women. In some patients, inhibin was a more specific marker for such tumors than was hCG. After resection of the molar tissue, inhibin levels fell rapidly (in less than 10 days) if tissue removal was complete. In contrast, levels of hCG did not decrease to nonpregnant levels until approximately 10 weeks after the successful removal of a mole. Thus, inhibin appeared to be a better early indicator of the presence of residual molar tissue and therefore of the need for chemotherapy.

Extragonadal Actions oflnhibins and Related Substances

Observations by Mcunicr cl al. have supported the view that inhibin and aclivin may have more widespread actions than those in relationship to the reproductive system (51). Alpha and beta subunils of inhibin were demonslraled in a large number of tissues such as the brain, spleen, adrenal, pituitary, kidney and the bone marrow.

1. Hemopoesis: Activin was snown to synergizc with erythropoietin in stimulating erythroid differentiation. Inhibin was noted to oppose this action (52).

2. Lymphoid tissue: It was recently demonstrated that inhibin stimulates the uptake 3H-lhymidinc into rat thymocytes (15). This action of inhibin supports an immunoreglutaory role for this protein.

3. Neural tissue: The demonstration of inhibin in the brain and spinal cord strongly suggests a regulatory role for these proteins in the central nervous system (51).

The story of development pertaining to inhibin is reminiscent of others in endocrinology. That is, a substance studied because of one biologic activity and one potential clinical application in this ease FSH suppression and male contraception-turns out to have many other roles and potential applications, some totally unsuspected. The first clinical application for inhibin is likely to be a serum marker for cancer. The administration of inhibin and related substances is also likely to be important in the control of animal and human reproduction. Together with the potential roles of inhibin in hematology, immunology and local control of tissue growth, these arc wide horizons for a humble gonadal product thought initial to have a relatively minor feedback influence on one pituitary hormone (54).

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