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MINI REVIEW

Inhibins and Activins: Chemical Properties and Biological Activity (42611A)

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Abstract. The long-sought, nonsteroidal, gonadal inhibitor of the secretion of FSH has been isolated, characterized, and the primary structure in several species (human, porcine, bovine, murine) has been deduced. Inhibins are proteins consisting of two subunits (18-kDa α- and 14-kDa β-subunits) linked by disulfide bridges and two forms of inhibins were observed in human, porcine, and murine, but only one in bovine. Each form of inhibin (A and B) has a common α-subunit, but a highly homologous, distinct β-subunit (β_A and β_B). The β-subunits and the α-subunit are linked to form inhibins A and B which exert an inhibitory effect on basal FSH secretion, but the dimer formed by either two β_A-subunits or two distinct β_A- and two β_B-subunits (homoactivin-A and activin, respectively) possess FSH-stimulating activity. Inhibin secreted in response to FSH from the pituitary originates primarily from the granulosa cells of the ovary and the Sertoli cells of the testes, thus demonstrating a reciprocal feedback relationship.

Inhibin was reviewed previously in this journal by Channing *et al.* (1) in which earlier studies on the isolation of inhibin from follicular fluid (FF), difficulties encountered in isolating this molecule, chemistry of a small peptide isolated from seminal plasma claimed to be inhibin, and some physiological properties of inhibin were discussed. During the ensuing 4 years, exciting new information has been gained concerning the isolation and characterization of inhibin from FF, and rapid progress has been made toward elucidating the biological and immunological activities of this molecule. The major problems in the isolation and characterization of this protein has been the self-aggregation or attachment of inhibin to large carrier proteins and lack of a commonly accepted efficient bioassay specific for inhibin activity. To circumvent the latter, an assay system using pituitary monolayer culture originally designed for monitoring the activities of hypothalamic-releasing factor was modified and universally adopted for inhibin bioassay. This assay has been described by Channing *et al.* (1) and Baker *et al.* (2) in detail. Such a bioassay was used for the first successful isolation of inhibin from porcine (3–5) and bovine (6) FF. Subsequently, the complete amino acid sequences of inhibin were deduced from the cDNA clones encoding inhibin using the N-terminal sequence data so obtained to design synthetic oligonucleotide probes (7–10).

Further observations revealed a previously unrecognized protein hormone, named *activin,* which is structurally related to inhibin, but with biological activity opposite to that of inhibin (11, 12). This molecule, recently isolated and characterized from FF, may be of physiological importance in controlling the secretion of FSH (follicle-stimulating hormone). Specific, highly sensitive radioimmunoassays (RIAs) for inhibin (13–16) have been developed and applied to the study of hormonal factors governing the secretion of these proteins.

Much of the recent progress stems from the work of four groups (3–6) who adopted the above-mentioned assay method for inhibin activity and from the development of procedures to process the FF in a denaturing agent to prevent aggregation of, as well as to purify, this hormone with high-performance liquid chromatography (HPLC) and SDS–PAGE, thereby allowing purification to homogenous stable product. Furthermore, with the knowledge of the sequence of the N-terminal of the inhibin molecule the total amino acid sequence of inhibin was deduced using recombinant DNA methodology. The complete primary structures of the precursors of inhibins have been characterized from porcine (7, 8), bovine (9), human (10), and recently murine (17) ovarian tissues. All these molecules show extensive common amino acid sequences and are closely related. There are two forms of porcine, human and

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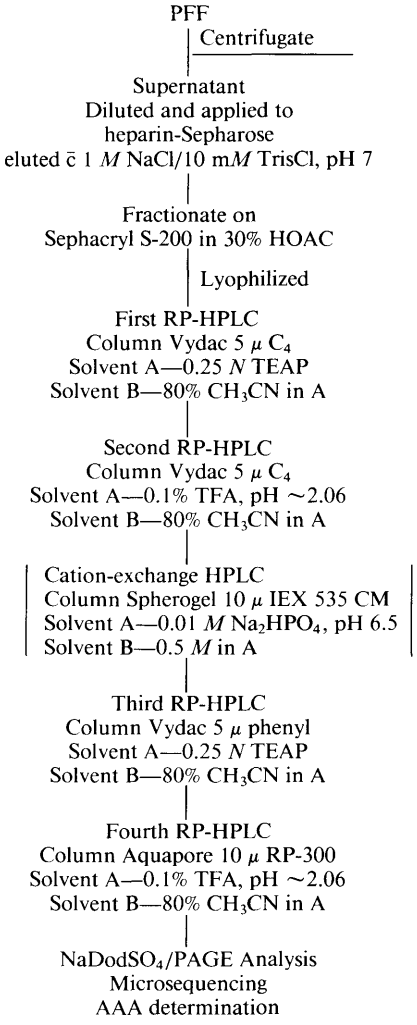
murine inhibin (A and B) of M_r ~32,000 (32 kDa) and each is found to be a dimer comprised of a common α-subunit (M_r ~18,000) and one of two similar, but distinguishable β-subunits (M_r 14,000 and 14,700 respectively). These dimeric subunits ($\alpha\beta_A$ and $\alpha\beta_B$) are held together by disulfide bridges. So far only one form of 32 kDa inhibin has been isolated from bovine FF which is equivalent to the dimer of $\alpha\beta_A$ of other species (18–19). In addition, a mol wt ~56,000 (56 kDa) species composed of a mol wt 44,000 α-subunit and a mol wt ~14,000 β_A-subunit was isolated from bovine FF (6, 20). This may suggest that the 56-kDa inhibin has been further processed to the 32-kDa biologically active molecule in the FF.

Isolation and characterization of inhibins and activins. The isolation of inhibins and activins from porcine FF (pFF) in our laboratories was performed as outlined in Table I. For details, see a recent review by Ling *et al.* (21). In 1985, four groups were successful at purifying inhibins from FF to homogene-

TABLE I. ISOLATION OF pFF INHIBINS AND ACTIVINS



Note. This scheme yields pure inhibins and activins of homogeneity. Homoactivin A was purified by an additional step of cation-exchange HPLC which is enclosed in parentheses.

TABLE II. PROCEDURES FOR ISOLATING FOLLICULAR FLUID INHIBINS

Procedures	References
Heparin–Sepharose affinity chromatography Sephacryl S-200 superfine gel filtration RP-HPLC $NaDodSO_4$/PAGE analysis	Ling *et al.* (3)
Matrex gel red A affinity chromatography Phenyl–Sepharose hydrophobic interaction chromatography Sephacryl S-200 superfine gel filtration DEAE–Sepharose CL6B anion-exchange chromatography RP-HPLC $NaDodSO_4$/PAGE analysis	Miyamoto *et al.* (4)
1-Propanol or $(NH_4)_2SO_4$ precipitation Preparative gel filtration RP-HPLC $NaDodSO_4$/PAGE analysis	Rivier *et al.* (5)
Gel filtration on Sephacryl S-200 superfine Gel filtration on Sephadex G-100 superfine Gel filtration on Sephadex G-200 superfine RP-HPLC Preparative $NaDodSO_4$/PAGE	Robertson *et al.* (6)

ity, and Table II shows these isolation procedures which all involve the submission of the inhibin molecules to a denaturing reagent (4 *M* or 30% HoAC or 6 *M* urea). The molecular weight and homogeneity of the isolated molecule, as well as the subunits linked by disulfide bridges, were ascertained with SDS–PAGE analysis. The purification factor ranges from 4000 to 8000 with a yield of less than 15%. All purified preparations of inhibins suppressed the basal secretion of FSH in a monolayer pituitary culture system with a ED_{50} of $\sim$1 ng/ml. The N-terminal amino acids of the inhibin subunits were determined (Table III) and synthetic oligonucleotide probes were designed accordingly for isolating cDNA clones encoding the α-, β_A, and β_B-chains of the porcine inhibin (7, 8) and the α- and β_A-chains of the bovine inhibin (9). By this means the biosynthetic precursors and corresponding amino acid sequences of the subunits of each inhibin were deduced (7–10, 17).

Primary structure of inhibin. The precursor of the common α-subunit of porcine inhibin is a protein of 364 amino acids. Preceding the carboxyl-terminal 134 residues there is a pair of arginines representing the proteolytic cleavage site to yield a mature 18-kDa α-subunit. Additional arginine pairs at the proregion of the α-precursor suggest potential cleavage sites to produce an α-subunit with a molecular weight greater than 18 kDa. Indeed, cleavage after one of these arginine pairs in the proregion of the precursor yields a 44-kDa α-subunit as that observed in bovine inhibin (6). There are two glycosylation sites, one at the proregion of the α-subunit precursor and the other within the α-subunit of mature 32-kDa inhibins. However, only one possible N-linked glycosylation site occurs in the proregion of the β-subunit precursors, and no glycosylation site is found in the mature 14-kDa β-subunits. That is to say, only the mature α-subunit contains a sugar moiety. Similarly, the precursor of the β-subunit of inhibin A ($\alpha\beta_A$) is a protein of 424 amino acids with a set of five conservative arginines preceding the carboxyl-terminal 116 residues; proteolytic cleavage at this position produces the mature 14-kDa β_A-subunit. There are three basic amino acids (Arg–Lys–Arg) preceding the carboxy-terminal 115 amino acid residues for the proteolytic clevage sites to yield a 14-kDa β_B-subunit. The α- and β-subunits form a dimer of 32-kDa inhibins A and B ($\alpha\beta_A$ or $\alpha\beta_B$) (Fig. 1).

TABLE III. *N*-TERMINAL SEQUENCE OF SUBUNITS OF INHIBINS DETERMINED BY FOUR DIFFERENT GROUPS IN 1985

Ling *et al.* (3)	
Porcine inhibin A α	Ser–Thr–Ala–Pro–Leu–Pro–Trp–Pro–Trp–Ser
Porcine inhibin A β	Gly–Leu–Glu–Xaa–Asp–Gly–Lys–Val–Asn–Ile
Porcine inhibin B α	Ser–Thr–Ala–Pro–Leu–Pro–Trp–Pro–Trp–Ser
Porcine inhibin B β	Gly–Leu–Glu–Xaa–Asp–Gly–Arg–Thr–Asn–Leu
Miyamoto *et al.* (4)	
Porcine α	Ser–Thr–Ala–Pro
Porcine β	Gly–Leu–Glu–Cys
Rivier *et al.* (5)	
Porcine α	Ser–Thr–Ala–Pro–Leu–Pro
Porcine β	Gly–Leu–Glu
Robertson *et al.* (6)	
Bovine α 56 kDa	Asn–Ala–Val
Bovine β	Tyr–Leu–Glu
Miyamoto *et al.* (20)	
Bovine α 32 kDa	Ser–Thr–Pro–Pro
Bovine β	Gly–Leu–Glu–Cys

Note. Xaa denotes a residue which was not identified in the microsequencing analysis. These and additional N-terminal amino acid sequence data on the subunits of each inhibin were used, based on the "long-probe" approach, to identify cloned complementary DNAs encoding the biosynthetic precursors and deduce the corresponding amino acid sequences of the subunits of each inhibin (Mason *et al.*, 1985, 1986; Mayo *et al.*, 1986; Foage *et al.*, 1986; Esch *et al.*, 1987).

McLachlan *et al.* (14, 22) have proposed that a pair of arginines at positions preceding the carboxyl-terminal 208 residues (positions 59–60) of the α-precursor were processed to form a 44-kDa α-subunit of the 56-kDa bovine inhibin and this high molecular form further processed to the 32-kDa inhibin. Similarly, proteolytic cleavage of arginine pairs at positions 55–56 may yield even greater molecular forms of inhibin (Fig. 2). However, Miyamoto and his co-workers (20) have proposed a model to a three-subunit complex to explain the inhibins of high molecular form. They have isolated six molecu-

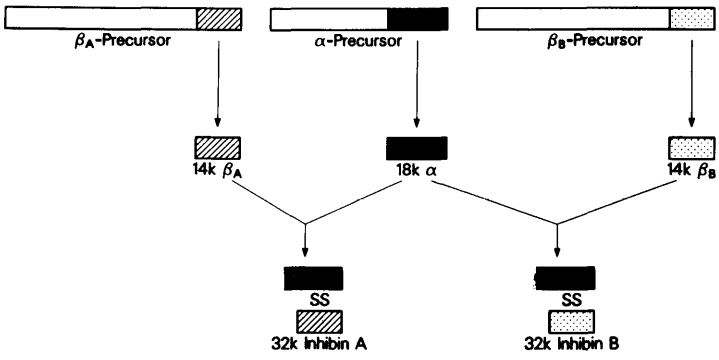


FIG. 1. Inhibins A and B are formed by proteolytic cleavages of α-, β_A-, or β_B-precursors, yielding a common 18-kDa α-subunit and two distinct but highly homologous 14-kDa β-subunits (β_A or β_B). α- and β-subunits form 32-kDa inhibins A ($\alpha\beta_A$) and B ($\alpha\beta_B$). One glycosylation site (not shown) is at the mature α-subunit of mature 32-kDa inhibin.

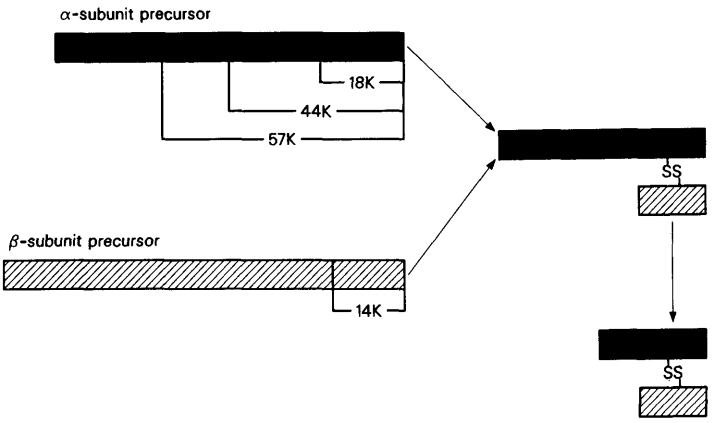


FIG. 2. Proteolytic cleavage of arginine pairs at positions 51–56 of the α-subunit precursor yields a 56-kDa inhibin which is composed of a 44-kDa α-subunit and a 14-kDa β-subunit. this 56-kDa inhibin further processes to form the 32-kDa inhibin. Glycosylation sites, two at the precursor of α-subunit and one at that of β-subunit, are not shown.

lar forms of biologically active inhibins from bovine FF by immunoaffinity chromatography (120, 108, 88, 65, 55, and 32 kDa). The 55- and 32-kDa inhibins are processed by proteolytic cleavage as described above. The 65-kDa inhibin is composed of a 14-kDa β-subunit linked by disulfide bridges to a 52-kDa α-subunit, presumably corresponding to the whole precursor minus the signal sequence. The higher molecular forms are comprised of three subunits. An additional 62-kDa subunit, immunologically related to the mature β-subunit, is attached by disulfide bridges to the respective 65-, 55-, and 32-kDa inhibins.

The β_B-subunit (115 residues) is one amino acid residue shorter than β_A (116 residues) and shows 70% homology with β_A. Mature β_A- and β_B-subunits contain nine cysteine residues which are remarkably similar to the distribution of cysteine residues in the TGFβ (transforming growth factor β) (112 residues), a growth factor originally isolated based on its ability to promote anchorage-independent growth (23). This cysteine distribution pattern was later found in the carboxyl-terminal of Müllerian-inhibiting substance (24). The α-subunit contains seven cysteine residues. Therefore, inhibins A and B are dimers composed of a common α-chain (18 kDa) and one of two distinct but highly homologous β-chains. These dimers of $\alpha\beta_A$ and $\alpha\beta_B$ are held together by disulfide bridges (Fig. 2).

The α-precursor mRNA in the ovarian tissue (~1.5 kDa) is more abundant than that of the two main species of β_A-precursors mRNAs (~4.5 and 7.2 kDa) and the β_A-precursor mRNA is more abundant than that of the β_B-precursor mRNA (~4.5 kDA) according to Southern and Northern analyses (7, 17).

Porcine, bovine, human, and murine inhibins. The cloned cDNA sequences encoding the biosynthetic precursors of α-, β_A-, and β_B-subunits of the porcine (7), human (10), and murine (17) inhibin, and the α- and β_A-subunits of the bovine (9) inhibin have been identified. An equivalent β_B-subunit for the bovine species has not yet been identified. Inhibin α-subunit cDNAs from porcine ovarian and human placental libraries were also cloned and sequenced (8).

There are slight species differences in the α-subunit, but virtually no differences in the β-subunit, indicating a high degree of conservation of amino acid sequence in this molecule. There is an approximate 80% homology in the α-subunits of these four species. Across all four species there are amino acid differences at only four positions among the mature human, porcine, and rat β_A-subunits and the mature β_B-subunits are completely identical. With the exception of a po-

tential additional N-linked glycosylation site in the mature human α-subunit (two in human vs one in the other three species), all other potential N-linked glycosylation sites (each one in the proregion of α, β_A, and β_B precursors) are preserved in four species. The sugar moiety in the mature α-subunit probably accounts for the fact that the molecular weight of inhibin observed in SDS–PAGE is different from that deduced from the amino acid sequence.

Activins—Novel proteins with structures related to inhibin, but biological activity opposite to that of inhibin. In our original report on the isolation of the two forms of inhibin from pFF (3), two FSH-stimulating activities were observed in the side fractions of the first RP-HPLC purification. When the complete primary structures of the inhibin α-, β_A-, and β_B-subunits were deduced, the striking homology and the identical cysteine distribution among β-subunits and TGFβ led us to carry out experiments showing that TGFβ is a potent stimulator of the secretion of FSH and that this stimulatory activity of TGFβ could be overridden by an effective dose of inhibin (25). Further purification of the FSH-stimulatory fractions using procedures described in Table I yielded two forms of homogenous protein. Microsequencing of the amino-terminus of these purified proteins revealed one as a single amino acid sequence which is identical to that of the β_A-subunit (26) and the other two amino acid sequences as if $\beta_A\beta_B$-subunits were sequenced together (11). When these proteins were subjected to NaDodSO$_4$/PAGE under reducing and nonreducing conditions and compared with inhibins A and B, they were found to be dimeric proteins comprised of the β-subunits of inhibin A and inhibins A and B, respectively. These $\beta_A\beta_A$ and $\beta_A\beta_B$ dimeric proteins are linked by interchain disulfide bridges. Both dimeric proteins stimulate the basal secretion of FSH, an activity opposite to that of inhibin. For this reason, they are named homoactivin-A and activin. Conceivably, a homodimer of $\beta_B\beta_B$ could produce similar FSH-enhancing activity (Fig. 3). The dimeric protein comprised of β_A (homoactivin-A) was also isolated and characterized by Vale *et al.* and designated as FRP (follicle-stimulating hormone-releasing protein) (12).

When the subunits of inhibin (α, β_A, β_B) were isolated and characterized as the product of different mRNA, we postulated the existence of multiple combinations of inhibin subunits (7). The isolation and characterization of activins further extended this hypothesis that rearrangements of several gene products from a limited number of genes results in considerable diversified final products with opposite biological activity. Furthermore, each one of these final products may be processed in different types of cells and may have different functions at different target cells. This hypothesis is further reinforced by a very recent observation (27) that a monocyte cell line originally derived from a 1-year-old boy with leukemia secretes extremely high quantities of a protein as stimulated by 4β-phorbol 12-myristate 13-acetate (PMA). This protein designated EDF (erythroid differentiation factor), when characterized by microsequencing and SDS–PAGE analysis, was found to be the very same molecule comprised of two β_A subunits of inhibin—*homoactivin A.* The physiological significance of this new discovery *in vivo* remains to be elucidated.

Immunocytochemical localization of inhibins. Inhibin-like immunoreactivity was detected in rat ovaries and testes as determined by antiserum to synthetic fragment of ^{30}Tyr-inhibin α-chain(1–30). This study indicates the Sertoli cells in the testes, and the granulosa and luteal but not the thecal cells in the ovary, are specifically stained and the intensity of the staining correlates with the stages of the development of the follicles. Recently, preliminary studies by B. Bloch *et al.* (unpublished data) using antiserum to [^{30}Try]-IN-α(1–30) also showed immunoreactive inhibin in the brain, in particular, the hypothalamus. The majority of immunoreactive areas are identical to those identified with a somatostatin antibody by immunohistochemical methodology. This observation, if confirmed and validated, suggests that the inhibin-like substance in the hypothalamus may have either direct effects on the basal secretion of FSH by the pituitary or different functions in the brain.

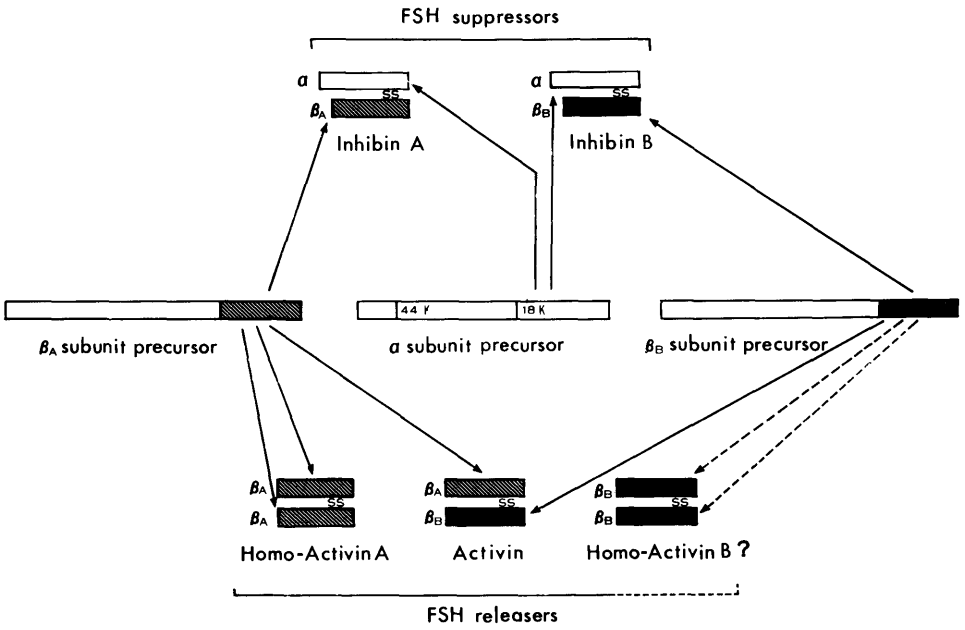


FIG. 3. Different dimeric proteins as products of gene rearrangement result in opposite biological activities. Heterodimeric inhibins A and B comprised of a common α-subunit and one of the two similar but distinguishable β-subunits (β_A or β_B) suppress the secretion of FSH, whereas the dimeric proteins formed by two β-subunits (either $\beta_A\beta_A$, $\beta_B\beta_B$, or $\beta_A\beta_B$) stimulate the release of FSH.

Measurement of mRNA encoding the precursor of inhibins and activins. Davis *et al.* (29) reported that PMSG (pregnant mares' serum gonadotropin)-stimulated ovaries showed higher levels of mRNA encoding the α-subunit precursor, which is consistent with early studies (30) reporting that PMSG stimulated the release of bioassayable inhibin. Substantial mRNA encoding the α-subunit precursor was also detected in rat corpus luteum; this is in accord with the immunohistochemical localization of inhibin in the corpus luteum, indicating luteal tissue may be an additional source of inhibin. Recently, Esch *et al.* (17) showed the presence of mRNAs encoding α- and β_B-chains, but no detectable mRNA encoding the β_A-chain in the rat testes, whereas all mRNAs encoding α-, β_A-, and β_B-chains were detectable in the ovaries. This suggests that there may be specific and distinct physiological roles for inhibins A and B. In addition, if there is no extratesticular source of β_A-mRNA, then the male rat may be devoid of the stimulators of the secretion of FSH, i.e., activin ($\beta_A\beta_B$) and homoactivin A ($\beta_A\beta_A$), which are derived from the β-subunits of the two inhibins. Furthermore, this information also implies that differential expression of gene product may account for the well-documented different pattern of gonadotropin secretion between the male and the female, i.e., cyclic vs tonic secretion of gonadotropins. Indeed, this is pertinent if the immunoreactive inhibin in the hypothalamus is ascertained and found to play an important physiological role.

Effects of inhibins and activins in vitro. The purified inhibins A and B are statistically equipotent in suppression of the basal secretion of FSH with a half-maximal inhibition at concentrations of 0.5–1.0 ng/ml ($\sim 5.3 \times 10^{-11}$ *M*) (Fig. 4a). The secretions of LH and other pituitary hormone are unaffected. A similar potency of a 32-kDa bovine inhibin was reported.

This specific inhibition of FSH secretion was observed only after at least 18 hr of incubation and the inhibitory action is clearly demonstrated after 48 hr incubation. This type of mode of action is different from that of all hypothalamic-releasing factors which only take minutes to a few hours to act. In

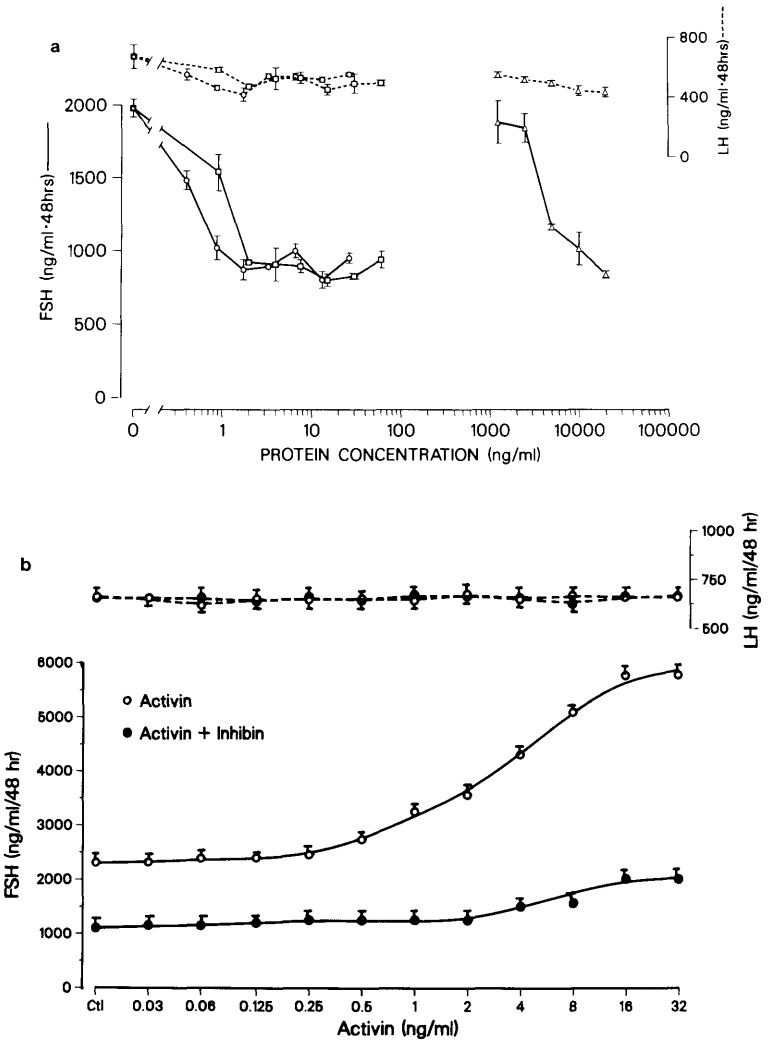


FIG. 4. Dose–response curve of (a) inhibin and (b) activin on the basal secretion of FSH *in vitro*.

addition, inhibins also suppress the intracellular level of FSH, but have no effect on that of LH during the periods tested.

Contrary to inhibins, activin and homoactivin A elevate the basal secretion of FSH by pituitary cells *in vitro* with an ED_{50} of 3.7 $\pm$ 0.5 ng/ml ($\sim 1.5 \times 10^{-10}$ *M*), while not altering the secretion of other pituitary hormones (Fig. 4b). Again, this stimulatory effect of activin and homoactivin A on basal secretion of FSH is different from that of a proposed hypothalamic FSH-releasing factor.

In cells pretreated with activins, LRF-mediated secretion of both LH and FSH is enhanced, a phenomenon opposite to that of cells pretreated with inhibin. Contrary to inhibins, activins enhance intracellular FSH in the pituitary cells and potentiate the FSH-induced aromatase activity in granulosa cells which cannot be suppressed with an effective dose of inhibin (31).

Radioimmunoassay for inhibin. Antisera have been raised for use in inhibin RIA. McLachlan *et al.* (14) prepared antisera to the purified bovine 58-kDa inhibin and used

the labeled 32-kDa inhibin as tracer for the RIA that could be used to assess the levels of inhibin in human plasma. Hasegawa *et al.* (13) have developed an RIA using inhibin antiserum against porcine 32-kDa inhibin that can measure the inhibin levels in the rat biological fluid. However, the theoretical possibility exists that antiserum to inhibin or antiserum to activin may fail to discriminate between inhibin and activin when native proteins are used as antigens due to the similarity of inhibin and activin. To circumvent this potential problem, inhibin antiserum has been raised against a synthetic fragment of the α-chain of inhibin and a specific RIA for inhibin has been developed (15, 16). ^{30}Tyr-inhibin-α(1–30) (16) or ^{26}Gly, ^{27}Tyr-inhibin-α(1–27) (15) conjugated to a carrier protein was used as antigen to elicit antiserum which was subsequently selected for its ability to bind to the native 32-kDa inhibin. This RIA can distinguish inhibins from activins and TGFβ with a sensitivity of 42 pg/tube 32-kDa inhibin.

Hormonal regulation of inhibin production. The granulosa cells of the ovary and the Sertoli cells of the testes are primarily responsible for inhibin production although the luteal cells were also observed to be a source of inhibin as determined by immunohistochemical technique (28) and detection of mRNA encoding the α-subunit precursor of inhibin (29).

FSH stimulates the secretion of inhibin by granulosa cells, and its production in cultured rat granulosa (16, 32) and Sertoli cells (16, 33) *in vitro* is in proportion to the concentration of FSH as determined by specific RIAs using synthetic fragments of the N-terminal amino acid sequence of the α-subunit of porcine inhibin (15, 16). This FSH-mediated release of inhibin is mediated through agents that increase intracellular cAMP levels, including a phosphodiesterase inhibitor, cholera toxin, forskolin, or dibutryl cAMP. Under certain circumstances, LH (13, 32) was found to enhance the secretion of inhibin.

In vivo studies with inhibin. There are very few reports on *in vivo* studies with purified inhibin because of the paucity of inhibin which now only can be obtained by a tedious purification procedure. A preparation of pure inhibin isolated on the basis of an *in vitro* bioassay and characterized a chemically, specifically suppressed serum FSH level, but not that of luteinizing hormone, when it was injected at a dose of 24 μg per injection at 1 min and 4 hr after ovariectomy in metaestrous rats (16) (Fig. 5). This observation provides evidence that the inhibin isolated and characterized chemically and

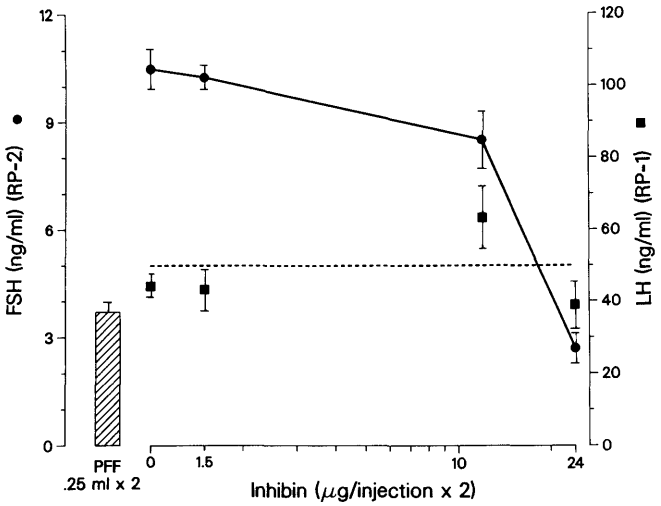


FIG. 5. *In vivo* suppression of FSH secretion by inhibin isolated from pFF.

through methods of molecular biology are the molecules that account for the inhibin activity in pFF.

Treatment with PMSG increased the level of inhibin mRNA in the rat ovaries (29) and the plasma inhibin as measured with the specific inhibin RIA (15). This immunoreactive inhibin increased corresponding to the time of PMSG treatment and disappeared when the ovaries were removed. Furthermore, Rivier and Vale (34) have reported that the progressive increase of inhibin secretion in immature rats is concurrent with a dramatic fall in plasma FSH levels, suggesting a negative feedback mechanism such that increased pituitary FSH elevates the inhibin release by the ovary, and, subsequently, the follicular inhibin suppresses the basal secretion of FSH.

These observations are in accord with the report that plasma inhibin levels in women whose follicles were stimulated with clomids and HMG (human menopausal gonadotropin), as measured by an RIA using an antiserum against bovine 56-kDa inhibin with the ^{125}I-labeled 32-kDa bovine inhibin as tracer, rose progressively in parallel with estradial production and the number of follicles stimulated (14).

Inhibin-like peptides from seminal fluid are not identical to inhibins from FF. As indicated in the review on inhibins by Channing *et al.* (1) and subsequent reports (35–37), two groups have isolated and characterized two inhibin-like single-chain polypeptides from human seminal plasma, named α-inhibin and β-inhibin, using a bioassay for inhibin activity substantially different from the universally recognized and ascertained pituitary monolayer culture system (1, 2). The α-inhibin consists of 92 amino acids. The N-terminal portion (1–31), COOH-terminal portion (41–92), and whole molecule of α-inhibin have been claimed to suppress FSH levels of LRF-stimulated mouse hemipituitaries as determined by radio receptor assays (36). The β-inhibin (1–94) and β-inhibin (67–94) have been reported to be equipotent on a molar basis (37). However, Lilja and Jeppsson (38) demonstrated that α-inhibin is identical to the major degradation product of the gel-forming protein secreted by the seminal protein (39). The β-inhibin was found to be identical to a sperm-coating antigen originated from prostate epithelium (40). In addition, α-inhibin, β-inhibin, and their synthetic fragments containing the putative biologically active core region show no inhibin activity in the pituitary monolayer *in vitro* system. The fact that α-inhibin and β-inhibin are not active in cultured pituitary cells has been concluded by Li and Ramashara (41). These findings indicate that the bioassay used for monitoring the inhibin activity during the process of isolation affects the type of protein or peptide isolated and biological activities retained. Thus, the significance of human seminal plasma α- and β-inhibins needs to be reassessed.

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