Purification of a candidate gonadotrophin surge attenuating factor from human follicular fluid

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Gonadotrophin surge attenuating factor (GnSAF) is a new non-steroidal ovarian substance, different from inhibin, which attenuates the pre-ovulatory luteinizing hormone (LH) surge in superovulated women. Human follicular fluid (FF) was used as a source for the isolation of GnSAF, the activity of which was monitored in an in-vitro pituitary bioassay. Primary rat pituitary cells were incubated with test substances for 48 h and subsequently washed and incubated with 0.1 µmol/l gonadotrophin releasing hormone (GnRH) plus test substances for 4 h. GnSAF activity was expressed as the reduction of GnRH-induced LH secretion in the 4 h incubation. GnSAF was purified from 250 ml of FF which was heat-treated at 80°C for 5 min. Heparin-sepharose chromatography, Con-A sepharose chromatography, reversed-phase high-performance liquid chromatography (HPLC) and preparative native gel electrophoresis were used for GnSAF fractionation. Using these purification steps, we have obtained an apparently homogeneous preparation that stains as a single band on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. GnSAF has an apparent molecular weight of 12.5 kDa and was identified by amino acid sequence (mass spectrometry) to be the C-terminal fragment of human serum albumin.

Key words: gonadotrophin surge attenuating factor/human follicular fluid/luteinizing hormone

Introduction

Evidence has been accumulated during the past decade that the ovary produces a putative hormonal factor named gonadotrophin surge inhibiting (Sopelak and Hodgen, 1984) or attenuating (Messinis and Templeton, 1989) factor (GnSIF/AF), which blocks the luteinizing hormone (LH) surge in monkeys and rats (Littman and Hodgen, 1984; Koppenaal et al., 1991) or attenuates the endogenous LH surge in superfused ovaries (Messinis and Templeton, 1990a,b). GnSAF exerts its action at the pituitary level by reducing pituitary responsiveness to gonadotrophin releasing hormone (GnRH) and thus attenuates the pre-ovulatory LH surge (Messinis and Templeton, 1990a). Evidence has also indicated that the production of GnSAF is stimulated by follicle stimulating hormone (FSH) (Koppenaal et al., 1991; Messinis et al., 1993).

GnSAF reduces only the GnRH-induced secretion of LH without affecting basal gonadotrophin secretion. It is a non-steroidal factor different from inhibin (Fowler et al., 1990). The latter is a gonadal protein involved in the control of FSH secretion from the pituitary (Ying, 1988). Non-steroidal gonadal factors involved in the regulation of pituitary LH secretion have not yet been identified. It is likely that GnSAF is a new ovarian hormone that may play a role in the control of LH secretion during the mid-cycle LH surge in the female (Fowler and Templeton, 1996).

By monitoring the change in the response of LH to GnRH in various in-vitro bioassay systems GnSAF/IF bioactivity has been demonstrated in ovarian follicular fluid of various species such as pigs (Danforth et al., 1987), rats (Busbridge et al., 1988), monkeys (Schenken and Hodgen, 1986), cows (Danforth and Cheng, 1994) and humans (Busbridge et al., 1990; Fowler et al., 1990; Knight et al., 1990; Mroueh et al., 1996). During the last few years two publications have reported the purification of GnSAF/IF bioactivity to homogeneity. A 37 kDa protein from rat Sertoli cell-conditioned medium was isolated (Tio et al., 1994) while a 69 kDa protein with GnSAF/IF activity from bovine follicular fluid has been purified (Danforth and Cheng, 1995). So far, purification of GnSAF to homogeneity from human follicular fluid (FF) has not been reported.

In the present study we report the isolation, purification, and amino acid sequence of a polypeptide with GnSAF activity from FF.

Materials and methods

Rat pituitary cell cultures

Short-term cultures of rat pituitary cells were carried out by established methods (Pappa et al., 1999). Pituitary cells were prepared to give 200 000 cells/well on 24-well tissue culture plates (Corning, Bibby Sterlin, Staffordshire, UK). Plating was achieved by culturing the cells under sterile conditions for 48 h in Dulbecco’s modified Eagle’s medium nutrient mixture F-12 Ham serum-free culture medium (SFDM; 0.1% BSA fraction V, 10 µg insulin/ml, 5 µg...
transferrin/ml, 4 nmol/l dexamethasone, 50 pmol/l 3,3,5-triiodo-L-thyronine, 1% penstrep (Sigma-Aldrich, Deisenhofen, Germany) and 500 µl/l gentamycin (Gibco BRL, Karlsruhe, Germany) supplemented with 10% steroid extracted (×2) fetal calf serum (Gibco BRL).

**GnSAF and inhibin bioassays**

At the end of the plating period the cells’ medium was replaced with SDFD and the cells were incubated in triplicate (on at least two separate cultures) in the absence (control) or presence of test substances for 48 h. Media were collected and stored at −20°C (basal secretion). Inhibin bioactivity is defined as inhibition of basal FSH secretion over this 48 h period. To assay for GnSAF the cells were washed with fresh medium and then incubated with 0.1 µmol/l GnRH plus test substances and they were incubated for an additional period of 4 h. After incubation, media were removed and frozen at −20°C (GnRH-induced LH secretion) until measurement for the levels of LH and FSH by enzyme linked immunosorbent assay (ELISA) methods. GnSAF activity is defined as the suppression of GnRH-stimulated LH secretion over this 4 h interval. One unit of GnSAF is the amount required to inhibit 50% of GnRH-induced LH secretion.

**ELISA assays for the measurement of rat gonadotrophins**

Rat gonadotrophins in cell culture media were measured in a competitive ELISA format, using reagents supplied by the NIDDK, as has been described (Pappa et al., 1999). Briefly, rat LH ELISA used hormone preparations NIDDK-rLH-RP3 and rLH-I-9 and antisera NIDDK-anti-rLH-S-11. Rat FSH ELISA used hormone preparations NIDDK-rFSH-RP3 and NIDDK-rFSH-I-8 and antisera NIDDK-anti-rFSH-S-11. The range of the assay for rat LH ELISA and rat FSH ELISA was 0.5–50 ng/ml and 1.25–40 ng/ml respectively. In both ELISA methods the optical density decreases as a linear function of the LH or FSH concentration. The intra- and interassay coefficients of variation of sample pools containing high, medium and low LH concentrations were 8.8, 5.1 and 3.8 respectively in rat LH ELISA. The intra- and interassay coefficient of variation of the same pools containing high, medium and low FSH concentrations were 13.6, 6.9 and 7.7% respectively.

**Heat treatment after dextran-coated charcoal**

Human follicular fluid (FF) was obtained from superovulated women who had participated in an in-vitro fertilization programme in the Department of Obstetrics and Gynaecology, University of Ioannina, Greece. Informed consent was obtained from the women and the study was approved by the scientific committee of the hospital. Approximately 250 ml of frozen FF were thawed, centrifuged to remove precipitated proteins, and subjected to two-fold steroid extraction treatment as previously described (Danforth et al., 1987). After steroid extraction, FF was divided in 25 ml aliquots and heated in sealed flasks for 5 minutes at 80°C and centrifuged.

**Heparin-sepharose chromatography**

The heat treated FF was applied to five 1×20 cm heparin-sepharose (Pharmacia Biotech, Vienna, Austria) columns at 8 ml/h. Non-bound proteins were eluted with 20 mmol/l Tris-HCl buffer, pH 7.0 at 25°C and collected as a pool. The bound proteins were eluted at 8 ml/h with 20 mmol/l Tris-HCl buffer, pH 7.0 containing 1 mol/l NaCl. The pooled non-bound fractions from heparin-sepharose column were brought to a volume of 30 ml using an Amicon concentrator (UM2, Amicon Ltd, Stonehouse, Glos., UK). Aliquots of the pooled bound and non-bound fractions from the column were tested for GnSAF and inhibin activity.

**Con A sepharose chromatography**

The unbound fractions from heparin-sepharose chromatography containing GnSAF activity were applied to three 1×10 cm Con A sepharose chromatography (Pharmacia Biotech) columns developed in 20 mmol/l Tris-HCl/0.5 mol/l NaCl at 18 ml/h. After the columns were washed, the absorbed proteins were eluted by a linear gradient of 0.00–0.25 mol/l α-D-methylmannoside in starting buffer. Individual fractions (4 ml each) were collected and equilibrated against SFDM medium using Centricon-3 microconcentrators (Amicon) prior to testing for GnSAF and inhibin bioactivity.

**Vydac C4 reversed-phase high-performance liquid chromatography (HPLC)**

The biologically active fractions obtained from the above step were pooled, concentrated and lyophilized, then resuspended in 5:1 solvent A/solvent B (solvent A: 0.1% TFA/H2O, solvent B: 0.1% TFA/CH3CN) at a final volume of 50 µl and loaded onto a Vydac reversed-phase HPLC column (4.6×250 mm internal diameter) at a flow rate of 1 ml/min. Bound fractions were eluted using a linear gradient of 23–75% solvent B over a period of 45 min. Fractions of 1 ml were collected. Aliquots of these fractions were lyophilized and resuspended in culture medium prior to GnSAF and inhibin bioassays.

**Preparative native rod gel electrophoresis**

Fractions with maximum GnSAF activity after RP-HPLC were pooled and separated by native polyacrylamide gel electrophoresis (PAGE) onto a 7% rod polyacrylamide gel (inner diameter of gel tube:

![Figure 1](https://academic.oup.com/humrep/article-abstract/14/6/1449/2919252/1450)
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0.5 cm, running conditions: 2 mA, 1 W max power). Following electrophoresis the rod gel was rinsed in PBS. Then, it was cut in 0.25 cm slices and each slice was halved. One half was used for the detection of GnSAF activity [SFDM culture medium (1 ml) was added to the gel pieces and after allowing the proteins to diffuse overnight at 4°C, the products of diffusion were tested in the bioassay]. The other half was used for protein analysis by sodium dodecyl sulphate (SDS)–PAGE.

SDS–PAGE and microsequencing

The halves of each 0.25 cm gel piece kept for analysis by SDS–PAGE were immersed in 30 μl sample buffer (0.0625 mol/l Tris/HCl pH 6.8, 2.3% SDS, 0.025 mol/l dithiothreitol, 10% glycerol, 0.05% bromophenol blue) for 30 min at room temperature and analysed under reducing conditions in 1 mm thick 15% acrylamide gel, according to established methods (Laemmlli, 1970). The proteins were revealed by silver staining. The following molecular weight standards were used to calibrate the gel: bovine albumin (M, 66 000), egg albumin (M, 45 000), glyceraldehyde-3-phosphate dehydrogenase (M, 36 000), carbonic anhydrase (M, 29 000), trypsinogen (M, 24 000), trypsin inhibitor (M, 20 100), α-lactalbumin (M, 14 200). The GnSAF protein band was excised from the gel and was immersed in 1% acetic acid. It was then sent to EMBL (Heidelberg, Germany) for sequence analysis by the method of mass spectrometry as previously described (Shevchenko et al., 1996).

Incubation with N-glycosidase F

Partially purified GnSAF (2.5 μg: the active fractions after the HPLC step) was incubated with N-glycosidase F in 50 mmol/l sodium phosphate buffer in the presence of 10 mmol/l EDTA, 50 mmol/l HEPES, 0.035% β-mercaptoethanol, 0.5% NP-40, pH 7.5, in a final volume of 100 μl, at 37°C for 36 h. Ten units of N-glycosidase F were added at 0, 12 and 24 h in order to obtain extensive deglycosylation of proteins. Rabbit IgG (~1 μg) was used as control as it was subsequently subjected to SDS–PAGE. The treated GnSAF fractions were tested for activity in the rat pituitary bioassay.

Statistical analysis

Analysis of variance, followed by Dunnett’s or Bonferroni tests, was used to analyse all purification and dose response experiments. Significance was set at P < 0.05.

Results

Heat treatment

Based on previous observations that GnSAF activity is resistant to heating (Danforth et al., 1987; Knight et al., 1990) we used heat treatment in the purification procedure, taking advantage of the fact that under these conditions inhibit activity is dramatically reduced. Heat treatment also resulted in the

Figure 2. Con A sepharose chromatography. The GnSAF active fractions after heparin-sepharose chromatography were pooled, dialysed and applied to three 1×10 cm Con A sepharose columns in 20 mmol/l Tris–HCl/0.5 mol/l NaCl (starting buffer) at 18 ml/h. Bound proteins were eluted with linear gradient of 0.00–0.25 mol/l α-D-methylmannoside in starting buffer. Fractions of 4 ml were collected. (A) GnSAF and inhibit bioactivity of Con A sepharose bound fractions. Aliquots of every fraction were dialysed using Amicon 3 microconcentrators before determination of GnSAF activity ( – – ) or inhibit activity ( – – – ) in the rat pituitary cell bioassay. Each point represents the mean ± SEM of triplicate determinations in the bioassay. Fractions 5–9 indicated GnSAF activity (α-D-methylmannoside (mol/l) 0.5, 0.25) and inhibit activity (α-D-methylmannoside (mol/l) 0) caused significant reduction of GnRH-induced luteinizing hormone (LH) secretion compared to control and were pooled for the next step. (B) Protein profile of eluted proteins from a Con A sepharose column under the linear gradient of α-D-methylmannoside (––––). *Value significantly lower than control (P < 0.05).
precipitation of >70% of the total proteins. The heat treated FF exerted a dose-dependent reduction of GnRH-induced LH secretion (Figure 1A) without affecting basal FSH secretion (Figure 1B), indicating it was substantially free of inhibin activity.

Chromatographic procedure
Consequently we used heparin-sepharose chromatography to separate the residual inhibin activity from GnSAF activity, as the latter is detected in the unbound fractions of the column whereas inhibin is retained in the column (Ling et al., 1985). The unbound fractions from heparin-sepharose column were pooled together, and after being concentrated to a final volume of 30 ml, they were further purified by Con A sepharose chromatography using a linear gradient of 0.00–0.25 mol/l α-D-methylmannoside as an eluent. GnSAF activity was eluted in the bound fractions (5–9) of Con A sepharose column (Figure 2). The active GnSAF fractions from the three Con A sepharose columns were pooled and lyophilized. The lyophilized material, after being dissolved in the appropriate solvent, was loaded directly onto a 4.6×250 mm internal diameter Vydac C4 reversed-phase HPLC column and developed with acetonitrile gradient in 0.1 mol/l TFA/H2O. GnSAF activity was eluted within the first protein peaks at a concentration of ~50% 0.1% TFA/CH3 CN (Figure 3). Fractions 15–19 from HPLC step exhibited maximum GnSAF activity and these were pooled and lyophilized.

Glycosylation studies
One third of the lyophilized material from the HPLC step was kept for native gel electrophoresis, while the other two thirds of the sample was used for glycosylation studies of the biological activity of GnSAF. Extended incubation of a highly purified GnSAF preparation with N-glycosidase F for 12, 24 or 36 h did not result in significant loss of its biological activity (Figure 4).

Native gel electrophoresis and microsequencing
One third of the lyophilized material with GnSAF activity from HPLC step was subjected to native gel electrophoresis on a rod gel (l = 10 cm, d = 0.5 cm). The rod gel after the electrophoresis was sliced into pieces of 0.25 cm and each slice was halved, using the one half for the detection of GnSAF activity in the bioassay and the other half for further analysis by SDS–PAGE. Native gel electrophoresis was a crucial step during GnSAF purification as the molecule retained its biological activity. GnSAF activity was detected in an area that corresponded to a length of 7.25–7.75 cm of the initial 10 cm rod gel (Figure 5). When the combined eluents from the gel pieces with GnSAF activity were analysed by SDS–PAGE under reducing conditions, one single band (at least 100 ng by silver staining) was observed with an apparent molecular weight of 12.5 kDa (Figure 6). Microsequence analysis of the 12.5 kDa protein revealed identity (almost 100% homology) to the C-terminal fragment of human serum albumin (HSA) (Figure 7).

Figure 3. Reverse-phase high-performance liquid chromatography (HPLC) purification. The fractions with GnSAF activity recovered from Con A sepharose columns were pooled, concentrated by ultrafiltration and finally lyophilized. The lyophilized sample was dissolved in 0.1% TFA in 7% CH3 CN/H2O (v/v). Fifty µl of this solution (200 µg of total protein) were injected on a Vydac C4 analytical reversed phase HPLC column (4.6×250 mm internal diameter). The chromatographic conditions were: solvent A: 0.1% TFA/H2O, solvent B: 0.1% TFA/CH3 CN. The gradient was 20–75% B in 40 min at a flow rate of 1 ml/min. The detection was monitored at 214 nm and the range was 0.16 absorbance units full scale (AUPS). Fractions of 1 ml were collected. Aliquots of individual fractions were tested for GnSAF activity (–●–) and inhibin activity (–○–) using the rat pituitary cell bioassay. Each point represents the mean ± SEM of triplicate observations. Major GnSAF activity was detected in fractions 15–19 [caused maximal significant reduction of gonadotrophin releasing hormone (GnRH)-induced luteinizing hormone (LH) secretion compared to control] which were pooled together for the next step. No inhibin activity was detected.
*Value significantly lower than control (P < 0.05).
As shown in Figure 8, the GnSAF active fractions obtained at each purification step evidently suppressed GnRH-induced LH secretion from the rat pituitary cells in a dose-dependent manner. Furthermore, these active fractions did not affect basal FSH secretion from the pituitary cells. The purification procedure from FF yielded a 12.5 kDa protein containing the activity of GnSAF. Details at various steps of the purification scheme are summarized in Table I. Although no dose-response curve was performed for the final purification step, an ED₅₀ value could be roughly evaluated. Based on the inhibition evident in fraction 7.5 cm of the native gel (Figure 5) and the staining intensity in Figure 6, and given that a total of three rod gels were used, an ED₅₀ value of ~30 ng could be estimated for the final purification step.

Figure 4. Effect of active GnSAF fractions (~2.5 µg) from high-performance liquid chromatography (HPLC) step before (i.e. GnSAF alone) and after being incubated with N-glycosidase F (for a total time of 12, 24, and 36 h at 37°C) on GnRH-induced luteinizing hormone (LH) secretion in vitro. Rat pituitary cells that were incubated with culture medium only were used as a control. N-glycosidase F (10 U) was added at 0, 12 and 24 h. The incubation buffer was 50 mmol/l sodium phosphate buffer (containing 10 mmol/l EDTA, 50 mmol/l HEPES, 0.035% α-mercaptoethanol and 0.5% NP-40), pH 7.5 and had no effect on GnRH-induced LH secretion at the concentration used in the assay. Each point represents the mean ± SEM of triplicate determinations in the bioassay. *Value significantly lower than control (P < 0.05).

Figure 5. Native gel electrophoresis. The GnSAF active fractions after reversed-phase high-performance liquid chromatography (HPLC) were pooled, concentrated using centrifugation under vacuum (Speed Vac®) and finally lyophilized. The lyophilized sample was then diluted in 50 µl sample buffer and separated by native polyacrylamide gel electrophoresis (PAGE) on a 7% rod polyacrylamide gel (running conditions: 2 mA, 1 W max power). One half of each 0.25 cm piece of the rod gel was used for the detection of GnSAF activity (~–) and inhibin activity (~–) in the rat pituitary bioassay. Each point represents the mean ± SEM of triplicate observations. GnSAF activity was detected in an area that corresponded to a length of 7.25–7.75 cm of the initial 10 cm rod gel. *Value significantly lower than control (P < 0.05).

Figure 6. Analytical sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE). The eluents from the gel pieces with GnSAF activity (Figure 4) were analysed by SDS–PAGE on a 15% slab polyacrylamide gel under reducing conditions. The GnSAF containing band (lane B) showed a single protein band (mol. wt. 12.5 kDa). Lane A: molecular weight standards: bovine albumin (66.0 kDa), egg albumin (45.0 kDa), glyceraldehyde-3-phosphate dehydrogenase (36.0 kDa), carbonic anhydrase (29.0 kDa), trypsinogen (24.0 kDa), trypsin inhibitor (20.1 kDa), α-lactalbumin (14.2 kDa).
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Figure 7. Partial amino acid sequence of the purified polypeptide with GnSAF activity from human follicular fluid. (A) Schematic of human serum albumin (HSA). Amino acid sequences of the peptide fragments resulting from a tryptic digest of the 12.5 kDa protein with GnSAF activity are noted below the areas of the HSA molecule with which homology was found. Unidentified amino acid residues are denoted by X. (B) Amino acid sequence of the C-terminal of HSA. The bold letters indicate the homologous areas with human GnSAF protein.

Figure 8. Dose-response curves for GnSAF purification. Increasing concentrations of steroid-extracted FF or GnSAF active fractions after heat treatment, heparine-sepharose, Con-A sepharose, and high performance liquid chromatography (HPLC-Vydac C4) were tested for GnSAF activity using an in-vitro rat pituitary bioassay. Each point represents the mean ± SEM of triplicate determinations in the bioassay. The intercept of 50% inhibition of gonadotrophin releasing hormone (GnRH)-induced luteinizing hormone (LH) secretion of each dose-response curve gives the ED50 value for GnSAF at each purification step.

Discussion

In the present study, we have isolated a candidate protein with GnSAF activity from human follicular fluid with an apparent molecular weight of 12.5 kDa that stained as a single band in a 15% SDS–polyacrylamide gel (silver staining). For the purification steps heparin-sepharose, Con-A sepharose, and reversed phase HPLC were used. Throughout the entire purification procedure GnSAF and inhibin activity were monitored in a quantitative in-vitro rat pituitary bioassay. Each point represents the mean ± SEM of triplicate determinations in the bioassay. The intercept of 50% inhibition of GnRH-induced LH secretion of each dose-response curve gives the ED50 value for GnSAF at each purification step.

GnSAF was found to have sequence homology to the 12.5 kDa C-terminal fragment of human serum albumin. If an N-terminus at amino acid 490 of HSA is assumed, the fragment with GnSAF activity would include the last loop of the nine loop-link-loop structures of HSA (He and Carter, 1992; Carter and Ho, 1994). HSA belongs to a multigene family of proteins that includes α-fetoprotein and vitamin D-binding protein. It is the most multifunctional transport protein known to date (Carter and Ho, 1994). The possibility of participation of the carboxy terminal fragment of HSA molecule to the regulation of LH secretion becomes a very interesting hypothesis. Of interest is that intact HSA molecule does not reduce GnRH-induced LH secretion (data not shown). Therefore, it could be assumed that the proteolytic cleavage of the molecule releases a specific regulatory activity. If the cleavage is not due to the purification conditions (something quite unlikely given the high stability of the albumin molecule), then it may become important to elucidate the role of specific proteases in follicular fluid under conditions of induced superovulation. However, the case of specific protein fragments exerting different biological activity from their intact proteins is not unknown. Endostatin and angioatin are two such examples. Endostatin (mol. wt: 20 kDa) is a C-terminal fragment of collagen XVII (O'Reilly et al., 1997), while angioatin (mol. wt: 38 kDa) was identified as an internal fragment of plasminogen (O'Reilly et al., 1994).

Both of these proteins are inhibitors of angiogenesis, currently used against cancer, while the molecules they derive from are not.

As demonstrated in this report, GnSAF is possibly a glycoprotein, based on the observation that GnSAF activity was eluted in the bound fractions of Con A sepharose chromatography. Deglycosylation at the extent accomplished under the described conditions had no effect on the biological activity of GnSAF (Figure 4).

The present study describes for the first time the isolation of GnSAF from human follicular fluid. The estimated ED50 value for the final purification step (~30 ng) strongly indicates a regulatory role on LH secretion for this peptide, which is of physiological importance. The estimated molecular weight of 12.5 kDa protein is similar to that initially suspected for human GnSAF (Fowler et al., 1992) and different from that reported for GnSAF/IF isolated from Sertoli cell-conditioned medium (Tio et al., 1994) and from bovine follicular fluid (Danforth...
and Cheng, 1995). GnSAF bioactivity in the 10–30 kDa size range has been demonstrated using crude serial ultrafiltration experiments (Fowler et al., 1992). However, FF GnSAF in that study was not purified to homogeneity. A 37 kDa protein with GnSAF/IF activity from 32 l rat Sertoli cell-conditioned medium has been isolated (Tio et al., 1994). Soon after this publication (Danforth and Cheng, 1995) the purification of a 69 kDa protein with GnSAF activity from bovine follicular fluid was reported. Both these proteins are monomeric but it is doubtful if they bear any common structural similarities, as different NH2-terminal sequences were detected. Compared to these putative GnSAF proteins, GnSAF isolated from FF shows properties similar to both of them. Both GnSAF obtained from human and from bovine follicular fluid GnSAF suppress GnRH-induced LH secretion without affecting basal FSH secretion. It is interesting that in both purification procedures GnSAF activity and inhibin activity are separated at initial stages of purification. Conversely, GnSAF isolated from Sertoli cell-conditioned medium demonstrates potent inhibin-like activity and causes reduction in both GnRH-induced LH secretion and basal FSH secretion. On the other hand, both human follicular fluid GnSAF and GnSAF isolated from rat Sertoli cell-conditioned medium proved to be resistant to treatment with acetonitrile, the organic solvent used in reverse phase HPLC. A highly purified preparation with GnSAF/IF activity from FF was described recently (Mroueh et al., 1996), and was compared with GnISF activity present in porcine follicular fluid. Human GnSAF caused reduction of GnRH-induced LH secretion and had no effect on basal FSH secretion. It was found that porcine GnSIF and human GnSAF have the same bioactivity in vitro and chromatographic characteristics. Antibodies raised against porcine GnSIF recognized two proteins with molecular weights of ~63 and ~59 kDa respectively (Mroueh et al., 1996).

The discrepancies mentioned above raise questions about the number of existing proteins that demonstrate GnSAF activity. There might be more than one protein involved in the regulation of LH secretion from pituitary, exerting suppression of GnRH-induced LH secretion.

The assumption of the existence of a non-steroidal ovarian factor which regulates the LH surge was introduced in order to explain the clinical observations of unsuccessful ovulation due to reduced mid-cycle LH surge in women participating in in-vitro fertilization programmes (Messinis and Templeton, 1989, 1990a,b, 1991; Messinis et al., 1991). On the other hand, the existing information on the modulation of LH surge is inadequate to fully explain the regulation of the precise timing and the exact amplitude of the LH surge, which is required for the follicular maturation and ovulation. However, recent evidence has further clarified the role of GnSAF in the control of the amplitude of the mid-cycle LH surge as a factor that antagonizes the sensitizing effect of oestradiol on the pituitary (Messinis et al., 1998). Nevertheless, the accumulating evidence on the regulation and existence of GnSAF/IF activity is very confusing. Although the production of GnSAF is considered to be stimulated by FSH in both rats and humans (Kopperna et al., 1991; Messinis et al., 1993), recent results suggest that in the rat ovarian cycle GnSAF is not regulated by FSH (Tio et al., 1998). Our present data also do not support the aspect that inhibin and GnSAF have mutual intrinsic bioactivities (Tio et al., 1994). Full sequencing of human GnSAF has not yet been reported, and this is also true for the other isolated proteins with GnSAF activity. The sequence of these peptides will show if novel proteins that regulate human ovulation exist. It will also clarify whether they bear any common structural similarities, answering questions on species differences, different proteins exerting the same in-vitro activity or different purification procedures. From a physiological point of view the isolation of GnSAF from FF would be of significance in the field of human fertility.

In conclusion, the present paper describes a purification procedure leading to electrophoretic homogeneity for the GnSAF factor from human follicular fluid and will enable further studies of the structure and physiology of this substance.

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References

<p>| Table I. Summary of gonadotrophin-surge attenuating factor (GnSAF) from human follicular fluid (FF) |</p>
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*ED50 was determined by the intercept of 50% inhibition of gonadotrophin releasing hormone (GnRH)-induced luteinizing hormone (LH) secretion of dose-response curves of GnSAF preparation at each purification step (Figure 8). ND = not determined.
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