SUMMARY

BIOCHEMICAL CHARACTERIZATION OF A POLYPEPTIDIC FACTOR WITH GnSAF BIOACTIVITY (GONADOTROPHIN SURGE ATTENUATING FACTOR)

DOCTORATE THESIS BY
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Gonadotrophin surge attenuating factor (GnSAF) is a non steroidal, as yet unidentified, ovarian factor that plays a key role in the control of LH secretion during the mid-cycle surge in the female. GnSAF acts on the anterior pituitary by reducing its responsiveness to gonadotrophin releasing hormone (GnRH) without affecting basal gonadotrophin secretion. GnSAF bioactivity has been identified in ovarian follicular fluid of many species including humans. In a previous study, for the isolation of GnSAF bioactivity from human follicular fluid, GnSAF was related to the C-terminal 95peptide of human serum albumin (HSA).

Interestingly, HSA, a 585-amino acid protein, displays binding capacity of several ligands, including long chain fatty acids, drugs and peptide hormones. HSA belongs to the albumin gene family together with alpha-fetoprotein (AFP), afamin (AFM) and vitamin D binding protein (DBP) that are similar in domain architecture and in several ligand binding sites. The molecule is composed of three structurally similar domains each of which consists of two subdomains, named IA, IB, IIA, IIB, IIIA, IIIB. Strikingly enough, the IIIB domain corresponds to the C-terminal 95peptide of HSA that was isolated from human follicular fluid as an active polypeptide.

In the present study we followed a new approach for the research of GnSAF. We chose the heterologous expression-secretion system of the methylotrophic yeast *Pichia pastoris* to produce recombinant HSA polypeptides and analyzed them for activity on rat pituitary primary cell cultures. We produced the C-terminal 95peptide of HSA (subdomain IIIB), in recombinant form, from culture supernatants of *P. pastoris*. We showed that supernatants containing the polypeptide possess significant

GnSAF bioactivity as they inhibit GnRH-induced LH secretion without affecting GnRH-induced FSH secretion and basal gonadotrophin levels. GnSAF activity of supernatants were blocked by anti-HSA antibody that immunoreacts with IIIB.

GnSAF activity of IIIB was found to be specific. Full length HSA and AFP, as well as domains I (1-197), II (189-385), III (381-585), are inactive. Moreover subdomain IB, structural counterpart of IIIB, is also inactive. Purified IIIB and domain III of HSA were tested for GnSAF activity in equivalent concentrations. Whereas IIIB presents significant bioactivity from 16-200nM, DIII is inactive from 40-800nM.

In addition, an engineered N-terminal deletion mutant of IIIB (509-585) displayed equivalent GnSAF bioactivity, showing that sequence 490-508 is dispensable for activity. In contrast, an engineered C-terminal deletion mutant (495-572) is completely inactive showing that the C-terminal a-helix of IIIB is crucial for activity.

We found by in silico analysis that an alternative spliced form of HSA mRNA, present in human liver cDNA library, compromises IIIB molecular weight and structure. It includes. codons 1-23 from exons 1-3 and, in the same open reading frame, codons 483-585 from exons 12-14 of HSA (1-23^483-585). The corresponding encoded polypeptide was expressed in *P. pastoris* system, subjected to GnSAF bioassay, and found to possess significant GnSAF activity. The recombinant polypeptide 464-585 of HSA, carrying 26 extra amino acids in its N-terminus, compared to IIIB, was also active.

Our findings show that subdomain IIIB of HSA is a stuctrurally independent polypeptide that displays specific GnSAF activity in the anterior pituitary. This activity is not derived from intact HSA or larger HSA domains. The above findings and the *P. pastoris* expression system will be useful for structure-function analysis of subdomain IIIB and for studying its action on the pituitary.

1: Tavoulari S, Frillingos S, Karatza P, Messinis IE, Seferiadis K. Related Articles, Links

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BACKGROUND: Gonadotrophin surge-attenuating factor (GnSAF) is an as yet unidentified ovarian factor that acts on the pituitary to attenuate the pre-ovulatory LH surge. In a previous study, GnSAF bioactivity was proposed to derive, at least in part, from a C-terminal domain (95peptide) of human serum albumin (HSA). METHODS AND RESULTS: We employ here the expression-secretion system of Pichia pastoris to produce and assay selected recombinant polypeptides of HSA for GnSAF activity. We show that the C-terminal 95peptide of HSA (residues 490-585; subdomain IIIB) can be expressed from P.pastoris in secreted form and supernatants from clones expressing this polypeptide reduce the GnRH-induced LH secretion of primary rat pituitary cultures by 50-82%. When expressed in the same system, HSA domain III (residues 381-585) or full-length HSA (residues 1-585) are inactive. The bioactive subdomain IIIB is also separable from either domain III or full-length HSA on Blue Sepharose chromatography. CONCLUSIONS: Taken together, the findings highlight the putative importance of HSA subdomain IIIB as a GnSAF-bioactive entity and introduce a unique experimental tool to engineer this molecule for structure-function analysis.

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