Inhibition of Iodine-125-Labeled Human Follitropin Binding to Testicular Receptor by Epidermal Growth Factor and Synthetic Peptides†

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ABSTRACT: Two tetrapeptide sequence homologies between mouse epidermal growth factor precursor (mEGFP) and human follitropin (FSH) were revealed by a computer program that identifies identical residues among polypeptide sequences. The two tetrapeptides, Lys-Thr-Cys-Thr (KTCT) and Thr-Arg-Asp-Leu (TRDL), are present in the hormone-specific β subunit of FSH from all species studied. These tetrapeptides are not present in the α subunit, which is common to all pituitary glycoprotein hormones. Both tetrapeptides are also found in mEGFP, and one tetrapeptide, TRDL, is located within the 53-residue form of mEGF purified from mouse submaxillary glands. Computer-generated hydropathy profiles predicted that both tetrapeptides are located in hydrophilic portions of the FSH β subunit and that TRDL is in a hydrophilic portion of commercially available mEGF. Therefore, the tetrapeptides might be accessible to receptor binding sites for FSH. We report that mEGF inhibits binding of ^{125}I-labeled human FSH to receptors in testis by 50% (I_{50}) at a concentration of 1.8×10^{-5} M. No binding inhibition was observed by GnRH or arginine-vasopressin at 10^{-4} M, neither of which contain the tetrapeptide sequences. FSH β subunit, which contains both tetrapeptides, also inhibited binding ($I_{50} = 9 \times 10^{-8}$ M) of ^{125}I-labeled human FSH to testis receptor. Thus, it appears that FSH β subunit and mEGF are capable of inhibiting binding of FSH to testicular FSH receptors, presumably through interactions that include the homologous tetrapeptides. This presumption was supported by the observation that the synthetic tetrapeptides (KTCT or TRDL) were also active in inhibiting binding of ^{125}I-labeled human FSH to testis receptor.

Local regulators of gonadotropin action have been hypothesized to account for follicular "selection" in the absence of detectable changes in hormone receptor concentrations, hormone levels in serum, or local blood flow (Sluss et al., 1983). These regulators are presumed to function as paracrine or autocrine agents in modulating the response of individual follicles to pituitary gonadotropins which are necesssary for folliculogenesis. Such agents might determine which follicles continue development to ovulation and which undergo atresia. To date, no such regulators have been demonstrated unequivocally. However, follicular fluid has been shown to have inhibitory effects on various gonadotropin-dependent aspects of ovarian (Channing et al., 1982), as well as testicular (Jagiello & Vogel, 1981), tissue. Available data suggest that putative regulators may include nonsteroidal, probably peptide, factors (Channing et al., 1982; Sluss & Reichert, 1984). Further support for the notion that nonsteroidal paracrine agents play a role in folliculogenesis is provided by reports of factors that modulate granulosa cell function in vitro (Hsueh et al., 1984). Numerous substances that modulate granulosa cell function have been described (Hsueh et al., 1984; Erickson, 1983), as have specific receptors for catecholamines (Adashi & Hsueh, 1981), GnRH (Hsueh & Erickson, 1979), insulin (May et al., 1980), and mouse epidermal growth factor (Vlodavsky et al., 1978). Epidermal growth factor (EGF) receptors have been reported in Sertoli (Morris & Mather, 1984), Leydig (Ascoli, 1981), granulosa (Vlodavsky et al., 1978), and luteal (Gospodarowicz et al., 1978) cells.

Our efforts to identify and evaluate regulators of follitropin (FSH) action have included studies of inhibitors of ^{125}I human FSH binding to receptors in testicular (Reichert & Abou-Issa, 1977), as well as ovarian tissue (Darga & Reichert, 1978). We have previously reported partial purification of factors that inhibit FSH binding to receptor (Sluss & Reichert, 1984). Some inhibitors of FSH would be expected to function through binding to sites on the receptor for FSH normally in contact with FSH. We reasoned that such inhibitors would show sequence homology with FSH.

The studies reported in this paper were intended to determine any sequence homology between FSH and factors that reportedly inhibit FSH action in granulosa (Mondschein & Schomberg, 1981) and Sertoli (Mather, 1982) cells or that inhibit binding of ^{125}I human FSH to testicular receptors. By utilization of a computer program (Krystek et al., 1985a,b) that identifies identical residues among polypeptide sequences, two common tetrapeptide sequences were found in mEGFP and FSH-β but not FSH-α. These tetrapeptides were also not observed in polypeptides that were examined because of their suspected effects on gonadal cell function. Since these sequences represented statistically significant homologies between mEGF and FSH, we tested mEGF for an "FSH-like activity", the ability to inhibit ^{125}I human FSH binding to testis receptor, in order to determine if the observed homology was meaningful. We substantiated that both mEGF and FSH-β (which contain homologous sequences) but not GnRH or arginine-vasopressin (which do not contain the tetrapeptides) were capable of inhibiting binding. We also synthesized the homologous sequences and found them to be active in inhibiting ^{125}I human FSH binding to testis receptor in vitro, further supporting the notion that the observed activity of mEGF and FSH-β was due to the peptide sequence homologies with intact FSH.

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EXPERIMENTAL PROCEDURES

Materials. Epidermal growth factor was obtained from Sigma Chemical Co. (St. Louis, MO; lot 44F8895) as a lyophilized powder and was dissolved at approximately 0.3 mg/mL in assay buffer (see Methods) prior to testing for inhibition of ^{125}I human FSH binding. Partially purified human FSH was used to determine nonspecific binding.

Highly purified (4000 IU/mg) human FSH (LER-1781) was radioiodinated by a lactoperoxidase procedure (Andersen & Reichert, 1982) modified as described in detail previously (Sluss et al., 1983; Sluss & Reichert, 1984). Specific activity of ^{125}I human FSH, as determined by the self-displacement method (Catt et al., 1974; Branca et al., 1985), was 24–36 μCi/μg, and maximum bindability was 26–39% as determined by binding to excess receptor (Branca et al., 1985).

Membrane-enriched fractions of calf testes homogenate were used as the receptor source for radioligand receptor assays. Calf testes (frozen, from Utica Veal Co., Utica, NY) were used to prepare a particulate fraction (7000-g pellet) which was washed and concentrated as described elsewhere (Sluss & Reichert, 1984; Branca et al., 1985). The apparent affinity constant (K_D) was 2.5×10^{-11} M, and the number of receptors was 1.63×10^{-11} M/mg wet weight of particulate fraction.

Sequences of FSH were taken from Pierce and Parsons (1981), while the sequence of EGF was from Savage et al. (1972). Recently, the sequence of the messenger RNA for the precursor of EGF was reported (Gray et al., 1983), and the sequence of amino acids encoded by this messenger was used to test for further homologies with the FSH sequence. Homologies and hydropathy comparisons were analyzed by using previously reported programs (Krystek et al., 1985a,b).

FSH from human (LER-1781) and bovine (LER-1596) pituitaries as well as the β subunit of human FSH were prepared in our laboratory (Reichert & Ward, 1974). GnRH (lot 19-192-AL) was obtained from NIAMDD. Arg-vasopressin, MSH, ACTH, angiotensin I, and Met-enkephalin were obtained from Sigma Chemical Co. (St. Louis, MO). Molecular weights of 33 000, 16 000, 6041, 1182, and 1083 are assumed for FSH, FSH β subunit, EGF, GnRH, and Arg-vasopressin, respectively.

Methods. The radioligand receptor assay used to study FSH–receptor interactions has been described in detail (Sluss et al., 1983; Sluss & Reichert, 1984). Briefly, all assay components were mixed at the beginning of a 16-h incubation (20 °C) to allow the binding reaction to reach steady-state conditions. Total assay volume was 500 μL and contained 3 mg (wet weight, 4.9×10^{-11} M binding sites) of calf testes membranes, approximately 200 000 cpm of ^{125}I human FSH, and either sample to be tested or unlabeled bovine FSH. Assay buffer was 0.05 M *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES), 5 mM $MgCl_2$, 0.1 M sucrose, and 0.1% ovalbumin, adjusted to pH 7.5 with KOH (at 25 °C). It is to be noted that NaOH should not be used for such adjustments due to effects of Na^+ on the FSH–receptor interaction (Andersen & Reichert, 1984). Separation of bound from free hormone was accomplished by centrifugation at 30000*g* for 15 min at 4 °C.

Specific binding to receptor is defined as total binding minus nonspecific binding. Nonspecific binding (^{125}I human FSH bound in the presence of a 300-fold excess of unlabeled FSH) and total binding (^{125}I human FSH bound in the absence of unlabeled ligand) were measured in each assay. Binding data were analyzed by a four-parameter logistic curve-fitting program (Rodbard & Hutt, 1974) adapted for use on an IBM personal computer. Weighting coefficients used were A_0 = 1 and A_1 = 0 to obtain curve fitting to unweighted data.

Homology between human FSH and EGF (or other polypeptides) was monitored by a simple program (Krystek et al., 1985b) that identifies identical residues in two polypeptide sequences. The program does not look for conservative substitutions as defined by Dayhoff (Dayhoff, 1978), so that the homology reported here is the minimal amount that exists between these two polypeptides. The program generates a dot matrix (see Figure 1), with each dot representing identical residues, and two or more consecutive identities are emphasized in the matrix with a large dot. A certain number of identical residues is expected by random chance. This number was estimated by assigning a probability of 1/20 for each amino acid. The expected frequency of identical residues can then be defined as $(1/20)^n$ times the number of possible comparisons, where n = the number of amino acids in the homologous peptide (i.e., three for a tripeptide, four for a tetrapeptide, etc.) and the number of possible comparisons is the product of the number of amino acids in each sequence being compared. For example, for an identical tripeptide in FSH-β (118 residues) and EGF (53 residues), the expected frequency is 118×53 (the number of comparisons) $\times (1/20)^3$ (for a tripeptide) or 0.8.

The method of Kyte and Doolittle (Kyte & Doolittle, 1982; Krystek et al., 1985a) was used to generate hydropathy profiles for the β subunit of FSH and for epidermal growth factor. This method calculates the average hydrophobicity or hydrophilicity of overlapping heptapeptides throughout a polypeptide and provides an estimate of the hydropathic nature of proteins.

Solid-phase synthesis (Merrifield, 1963; Stewart & Young, 1984) of each tetrapeptide was performed in a Biosearch Sam Two automated peptide synthesizer, starting with the appropriate protected C-terminal L-amino acid resin (Peninsula Laboratories, Inc., Belmont, CA). The *tert*-butyloxycarbonyl (Boc)-benzyl protection scheme was employed together with a double-coupling protocol utilizing dicyclohexylcarbodiimide for incorporation of each additional L-amino acid derivative (Peninsula Laboratories, Inc.). Fifty percent trifluoroacetic acid (TFA) in CH_2Cl_2 was used for removal of the Boc group and 5% diisopropylethylamine (DIEA) in CH_2Cl_2 for neutralization. The peptides were isolated after HF-mediated deprotection and cleavage by extraction into acetic acid followed by lyophilization. Peptides 1 and 3 were oxidized with $K_3Fe(CN)_6$ (Hope et al., 1962) prior to purification.

peptide 1	H-Lys-Thr-Cys-Thr-OH
peptide 2	H-Thr-Arg-Asp-Leu-OH
peptide 3	H-Cys-Ala-Gly-Tyr-OH

Purification of peptides 1 and 3 was by reverse-phase high-performance liquid chromatography (HPLC) on a 10u-C18 column (7.8 × 250 mm, Creative Biomolecules, Inc., Hopkinton, MA) using water–acetonitrile mixtures containing 0.05% TFA (Bennet et al., 1979). Peptide 2 was not retained by the column under these conditions and was purified by gel filtration HPLC (LKB, TSK30005W, 7.5 × 600 mm). The homogeneity of the peptides was verified by thin-layer and high-performance liquid chromatography and their composition confirmed by amino acid analysis.

RESULTS

Figure 1 is a dot matrix analysis that would show any sequence common to EGF and FSH-β. Identities are represented by a small dot, and where there are two or more consecutive identical residues, a correspondingly larger dot is

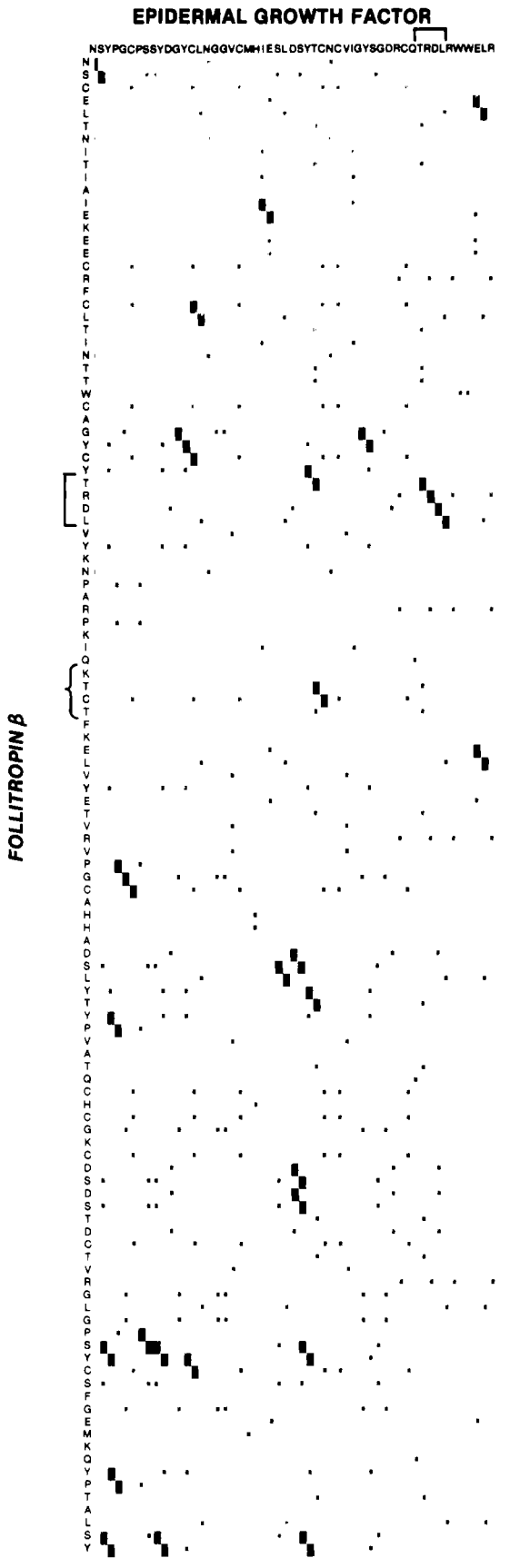

FIGURE 1: Computer-generated dot matrix representation of the homology between EGF (x axis) and FSH-β (y axis). Small dots represent identical residues, while larger dots represent two or more consecutive identities (residues found in both molecules).

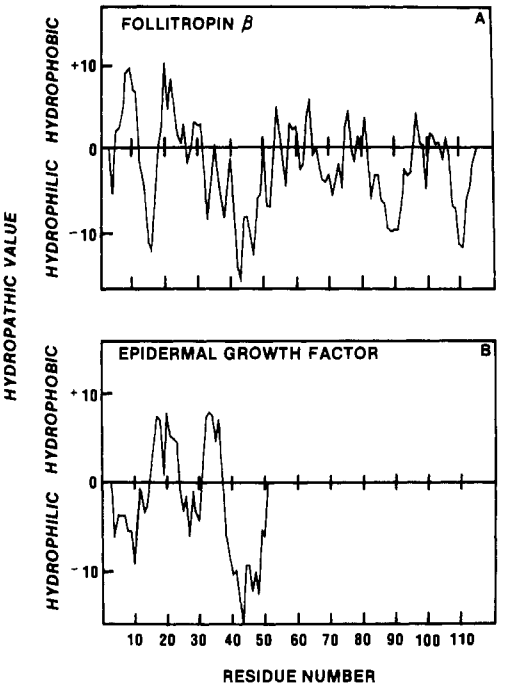

FIGURE 2: Computer-generated hydropathy profiles of EGF and human FSH-β. Kyte and Doolittle (Krystek et al., 1985) indices were used for this analysis. Values above the zero line are hydrophobic, while values below are hydrophilic. The tetrapeptide TRDL is at position 34–37 of human FSH-β and at position 44–47 of EGF.

displayed. Clearly, there are no major stretches of homology such as are apparent in highly related molecules (Krystek et al., 1985a). There are, however, 23 instances in which two identical residues are aligned which is only slightly more than the number expected (15.6) from our calculation of expected frequency. There are two instances in which tripeptide identities are observed: Gly-Tyr-Cys (GYC) at positions 12–14 of EGF or 30–32 of FSH-β and all other glycoprotein hormone β subunits and Pro-Gly-Cys (PGC) at positions 4–6 of EGF or 64–66 of FSH-β and all other glycoprotein hormone β subunits. The calculated frequency of triplets of identity occurring by chance (i.e., in unrelated molecules of this length) was 0.8. Finally, there is one tetrapeptide that is common to both proteins, the sequence Thr-Arg-Asp-Leu (TRDL) at positions 44–47 of mEGF and 34–37 of human FSH-β. This four-residue peptide is not observed in any other glycoprotein hormone β subunit. The expected frequency of a common tetrapeptide in unrelated molecules of this length is 0.03. The TRDL sequence was not found in FSH-α, GnRH, Arg-vasopressin, or several other hormonal polypeptides (e.g., lutropin, MSH, ACTH, angiotensin I, or enkephalin).

The primary structure of the gene product containing mEGF was recently published (Gray et al., 1983). This 1168-residue sequence was compared to the α and β subunits of FSH. While each subunit contained some triplets of identity, only FSH-β contained identical tetrapeptides. Two such tetrapeptides were found, including the TRDL peptide in the actual EGF molecule. The other was Lys-Thr-Cys-Thr (KTCT) which is position 49–52 in FSH-β and 440–443 in the EGF precursor. The expected frequency of identical tetrapeptides in molecules of this size is 0.07. Other observations on sequence include the existence of Cys-Ala-Gly-Tyr (CAGY) at position 28–31 of FSH-β (vide infra).

Figure 2 is a representation of the hydrophobic and hydrophilic nature of mEGF and FSH-β as analyzed by the method of Kyte and Doolittle (Kyte & Doolittle, 1982; Krystek et al., 1985a). The TRDL tetrapeptide is in a hydrophilic

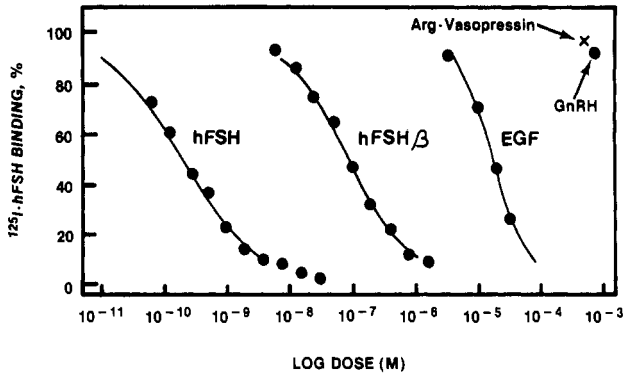

FIGURE 3: Inhibition of ^{125}I human FSH binding to calf testis membranes. Dose–response curves were generated as described under Experimental Procedures by fitting all data points (unweighted) shown to a four-parameter logistic curve. Computer-generated curves (solid lines) between 90 and 10% of initial specific binding in the absence of inhibitor (y axis) are shown for each inhibitory substance. Calculated half-maximal inhibitory doses (I_{50} ± SE based on all observations) of human FSH, human FSH-β, and EGF were 3.28 ± 0.22 ng ($2 \times 10^{-10} \pm 1 \times 10^{-11}$ M), 716 ± 40 ng ($9 \times 10^{-8} \pm 5 \times 10^{-9}$ M), and 54 ± 3 μg ($1.8 \times 10^{-5} \pm 1 \times 10^{-6}$ M), respectively. Only the highest dose tested is indicated for the peptides (GnRH and Arg-vasopressin) which did not significantly inhibit ^{125}I human FSH binding.

portion of each molecule and is therefore predicted to be accessible to solvent (or receptor) rather than buried in the hydrophobic interior of the polypeptides. The tetrapeptide is in a substantially more hydrophilic region in EGF than in FSH-β and may be even more accessible in EGF than in FSH-β. In both proteins TRDL appears to lie in regions of β structure. This conclusion is based on the sequence analysis (not shown) using Chou–Fasman calculations (Chou & Fasmen, 1978) and on extensive data for β subunits of the glycoproteins being primarily β structure with virtually no α helix (Pierce & Parsons, 1981). Since TRDL is in hydrophilic regions of β structure which should present alternate side-chain residues in sequence on the protein surface, free TRDL tetrapeptide should be a reasonable analogue for the corresponding sites of mEGF and FSH-β.

Inhibition of ^{125}I human FSH binding to calf testis receptor by various peptides is shown in Figure 3. In the absence of unlabeled ligand, 10% of total labeled hormone added to the assay was specifically bound. This reflects a 30% occupany of available receptor. Under these conditions, the half-maximal inhibitory dose (I_{50}) is influenced by both the apparent K_D and the number of receptor sites (Rodbard, 1973). Human FSH, β subunit of human FSH, and mEGF, but not GnRH or Arg-vasopressin, inhibited the binding of ^{125}I human FSH to calf testis receptor in a dose-dependent manner. No inhibition of binding was observed at high concentrations of GnRH (up to 6.8×10^{-4} M) or of Arg-vasopressin (up to 5.5×10^{-4} M). The dose of mEGF required for inhibition in the radioligand-receptor assay is well below that expected for simply solute effects (Andersen & Reichert, 1982). Thus, it appeared possible that the activity of mEGF was due to interactions with the receptor for FSH, possibly by virtue of the shared tetrapeptide as suggested by our interpretation of Figure 2 (vide supra). This possibility was tested by synthesis of tetrapeptides which, as fragments of mEGF and/or FSH-β, might account for binding inhibition of the parent ligands. Synthetic TRDL (the tetrapeptide common to both mEGF and FSH-β), KTCT (the tetrapeptide common to the EGF precursor and FSH-β), and CAGY (Cys-Ala-Gly-Tyr, a peptide sequence found in a predicted hydrophobic region of FSH-β but not present in EGF or its precursor) were tested for activity in the radioligand-receptor assay. Lyophilized synthetic peptides were reconstituted with assay buffer (which contains 0.1% ovalbumin) and tested under standard assay conditions (16 h at 20 °C). Results of these assays are illustrated in Table I. As indicated, either KTCT or TRDL inhibited binding of ^{125}I human FSH to receptor. Significant (17–68%) inhibition of ^{125}I human FSH binding was observed (Table I) by KTCT (M_r 452) at final concentrations between 4.4 (1 mg/tube) and 8.8 (2 mg/tube) mM. TRDL (M_r 503) was approximately half as potent, requiring final concentrations of 8 (2 mg/tube) to 16 mM (4 mg/tube) to inhibit binding to the same degree as KTCT. Synthetic CAGY, prepared in a fashion similar to that used to synthesize KTCT and TRDL, was utilized as a control in these assays since it is found in FSH-β (but not EGF or its precursor). However, since it is located in a region of the protein which is predicted to be hydrophobic, CAGY would not be expected to interact with solvent or other molecules (e.g., receptors for FSH) or to account for binding inhibition by EGF. CAGY (M_r 413) was tested in four assays at final concentrations between 4.8 (1 mg/tube) and 19 (4 mg/tube) mM. No significant (5.5 ± 3.2%) inhibition of binding was observed for CAGY over this dose range.

Table I: Inhibition of ^{125}I Human Follitropin Binding by Synthetic Tetrapeptides

peptide	dose (mg/tube)	% BI ($\bar{x}$ ± SE)[a]	no. of assays
TRDL	2.0 or 2.4	27.0 ± 0.9	3
	4	62.7 ± 1.6	3
KTCT	0.8 or 1.0	17.2 ± 4.3	5
	2.0	68.5 ± 2.8	2

[a] Peptides were tested in each assay in duplicate. Means of assay duplicates were used to obtain means and SE as reported. Peptides were tested in a total of five assays utilizing three batches of calf testis membranes and four batches of radioligand. Typical assay conditions: 16 h/20 °C; total cpm added = 200 000; specific binding = 12 000 cpm = 0% inhibition; nonspecific binding = 3500 cpm = 100% inhibition; I_{50} follitropin = 4 ng.

Discussion

The 53-residue sequence of mEGF purified from mouse submaxillary gland (Savage et al., 1972) agrees with the "transcribed" sequence from a mouse cDNA clone (Gray et al., 1983). The cDNA sequence analysis suggests that mEGF is synthesized as a large (M_r 138 000) protein precursor. This precursor molecule contains seven "EGF-like" sequences (Merrifield, 1963) in addition to EGF as purified from mouse submaxillary glands. EGF can modulate gonadal cell function in vitro (Knecht & Ranta, 1982; Mondschein & Schomberg, 1984), and receptors for EGF have been reported in gonadal tissues (Gospodarowicz et al., 1978; Vlodavsky et al., 1978; Ascoli, 1981; Morris & Mather, 1984). However, there is no direct evidence for a physiological role of EGF (purified from submaxillary glands) in gonadal function in vivo at this time. One possibility is that the EGF precursor is translated in gonadal tissue and EGF or EGF-like peptides play a role as local regulators of gonadotropin action.

Our analysis demonstrates that mEGF contains three sequence homologies with human FSH-β, the hormone-specific subunit of FSH. These homologies are comprised of two tripeptides and one tetrapeptide. This degree of homology cannot alone support the notion that EGF is "gonadotropin-like" or vice versa. Further, these three sequence homologies are not "in line" [i.e., they are not on a principle diagonal as defined by Needleman & Wunsch (1970)] and thus do not represent interrupted stretches of a larger homology. Each tripeptide, GYC and PGC, is found in all glycoprotein hormone β subunits, possibly by virtue of their forming the highly

conserved disulfide bonds. They are associated with hydrophobic portions of the molecules, are probably not accessible to solvent, and therefore are unlikely to bind to the hormone receptor directly. Furthermore, one tripeptide, GYC, is part of the sequence CAGYC found in all glycoprotein hormone β subunits (Pierce & Parsons, 1981) and the so-called CAGY peptides (Kurosky et al., 1977; Jagiello & Mesa-Tejada, 1979). Assuming they are not due to random chance alone, the tripeptides may be related less to polypeptide function (e.g., binding) than to maintaining essential structural integrity.

We did, however, view the tetrapeptide homology between mEGF and FSH-β to be of significance. EGF shares the TRDL tetrapeptide with FSH-β, but not with α subunit and not with lutropin or the other gonadotropin β subunits. This analysis also revealed another tetrapeptide, KTCT, found in both the EGF precursor (but not in EGF per se) and the β subunit of FSH. KTCT is found in all known FSH-β subunits but is not found in any other subunits of the glycoprotein hormones [sequences from Pierce & Parsons (1981)]. Furthermore, KTCT and TRDL are both found (Figure 2) in hydrophilic portions of FSH-β (residues 49–52 and 34–37, respectively) which are probably folded in β-pleated sheets, while TRDL is found in a region of substantial hydrophilicity in EGF (residues 44–47). Thus, these tetrapeptides were predicted to be accessible to solvent and therefore might be able to interact with other molecules.

On the basis of these considerations we anticipated that if the tetrapeptide TRDL was involved with the "FSH-like" activity of EGF as well as FSH action in gonadal tissue, then EGF and FSH-β should inhibit the binding of (intact) FSH to receptor. Our results (Figure 3) demonstrate that mEGF can inhibit the in vitro binding of ^{125}I human FSH to calf testis membrane receptors, as can FSH-β. Larger doses of FSH-β and EGF are required to achieve binding inhibition comparable to that obtained with intact FSH. The conformation of intact FSH is likely to be different from that of the dissociated β subunit of FSH. Since binding affinities are related to conformational factors, such differences may find expression in the varying doses of peptides required to achieve comparable binding inhibition. Furthermore, we have not as yet examined the kinetics of inhibition or rates of degradation of the various ligands in this system, each of which might also influence apparent potency. It does not appear that inhibition by mEGF was the result of nonspecific effects on the in vitro binding system, since doses of other polypeptides (Arg-vasopressin and GnRH) 10-fold higher than that of mEGF did not inhibit binding.

Binding inhibition by these relatively high doses of FSH-β and mEGF could conceivably be attributed to contamination of the preparations by intact FSH. For example, 1% or 0.02% contamination of FSH-β and mEGF, respectively, would account for the observed I_{50} values of these proteins. However, FSH-β contains less than 0.1% intact FSH by bioassay (unpublished results), and mEGF is purified from submaxillary glands so that contamination by intact FSH is unlikely to account for the observed inhibition of binding. The relative potencies of FSH-β (containing KTCT and TRDL) and mEGF (containing only TRDL) and the observed activity of both synthetic peptides (no possible contamination by intact FSH) support the suggestion that FSH-β and mEGF inhibit binding by virtue of the homologous KTCT and TRDL tetrapeptides (vide infra).

The dose of mEGF required to inhibit FSH binding is much larger than that required to produce mEGF effects on gonadal tissues, which are due to interactions with receptors for EGF and not to interactions with the receptor for FSH. Nevertheless, our binding data support the notion that the common tetrapeptide TRDL may be involved with FSH receptor–FSH and FSH receptor–EGF interactions. Observations that KTCT is common to FSH β subunits and the mEGF precursor suggest that this tetrapeptide may also be involved in FSH–receptor interaction.

These suggestions were examined by direct testing of the synthetic tetrapeptides in the radioligand assay (Table I). We demonstrated that TRDL and KTCT were active in inhibiting ^{125}I human FSH binding to receptor. While inhibition of binding in vitro, particularly at high doses of inhibitor, is not definitive proof of receptor–ligand interactions, these data are interpreted as evidence of the inherent inhibitory activity of KTCT and TRDL for two reasons. First, CAGY represents both a procedural (synthesized in the same manner as KTCT and TRDL) and a theoretical (predicted to be in a hydrophobic region of FSH-β and not present in EGF or its precursor) control and was not active over the dose range (4.8–19 mM) at which inhibition was observed for KTCT or TRDL. Second, solute effects on the radioligand-receptor assay employed have been well documented (Andersen & Reichert, 1982) and a correlation between the *B* coefficient of viscosity and the ability of salts to inhibit binding reported (Andersen & Reichert, 1984). Effective doses of KTCT or TRDL are approximately 10-fold lower than the most potent (I_{50} = 100 mM) ion Na^+ (as NaCl) identified previously (Andersen & Reichert, 1982).

Our observation that high doses (millimolar) of KTCT or TRDL are required for binding inhibition can be explained if it is assumed that each tetrapeptide interacts with an epitope of the FSH receptor binding site, doing so with a relatively small contribution to the total free energy change observed for binding of intact FSH. Furthermore, it is often observed that small peptides representing active sequences of larger proteins are less potent than the parent polypeptide, presumably a reflection of the structural limitations imposed on small peptides by the surrounding residues. For example, the portion of EGF claimed to interact with receptors for EGF showed greatly deceased potency ($1/10^4$) when tested as a synthetic tetrapeptide (Komoriya et al., 1984). It should be recognized that while the doses of synthetic peptide required to inhibit FSH binding are high (mM) in a physiological sense, they are nonetheless significant in terms of molecular interactions and thermodynamics of binding. Furthermore, as with EGF, it will be necessary to determine if peptide degradation is occurring and to establish steady-state conditions for each ligand before accurate quantitation of effective doses of synthetic peptides is possible.

The studies reported in this paper identify two tetrapeptide regions of FSH-β which appear to inhibit FSH binding to receptor. One, TRDL, is shared with mEGF, and the other, KTCT, is shared with the mEGF precursor protein. FSH-β, mEGF, KTCT, and TRDL inhibit ^{125}I human FSH binding to receptor in vitro (ordered in decreasing potency). These observations suggest the testable hypothesis that ^{125}I human FSH binding to receptor was inhibited by interactions of the various ligands with FSH receptor sites recognizing TRDL and/or KTCT. These observations also suggest that small peptides containing these sequences are potential pharmacologic modulators of FSH action in vivo.

Registry No. EGF, 62229-50-9; FSH, 9002-68-0; KTCT, 100859-26-5; TRDL, 100859-27-6.

REFERENCES

Adashi, E. Y., & Hsueh, A. J. W. (1981) *Endocrinology (Baltimore) 108*, 2170–2178.

Andersen, T. T., & Reichert, L. E., Jr. (1982) *J. Biol. Chem. 257*, 11551–11557.

Andersen, T. T., & Reichert, L. E., Jr. (1984) *Mol. Cell. Endocrinol. 35*, 41–46.

Ascoli, M. (1981) *J. Biol. Chem. 256*, 179–183.

Bennet, H. P. J., Browne, C. A., Goltzman, D. and Solomon, S. (1979) *Pept. Struct. Biol. Funct. Proc. Am. Pept. Symp., 6th* 121–124.

Branca, A. A., Sluss, P. M., Smith, R. A., & Reichert, L. E., Jr. (1985) *J. Biol. Chem. 260*, 9988–9993.

Catt, K. J., Tsuruhara, T., Mendelson, C., Ketelslegers, J. M., & Dufau, M. L. (1974) in *Hormone Binding and Target Cell Activation of the Testis* (Dufau, M. L., & Means, A. R., Eds.) pp 1–30, Plenum, New York.

Channing, C. P., Anderson, L. D., Hoover, D. J., Kolena, J., Osteen, K. G., Pomerante, S. H., & Tanabee, K. (1982) *Recent Prog. Horm. Res. 28*, 331–408.

Chou, P. Y., & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol. 47*, 45–148.

Darga, N. C., & Reichert, L. E., Jr. (1978) *Biol. Reprod. 19*, 235–241.

Dayhoff, M. O. (1978) *Atlas of Protein Sequences and Structures*, Vol. 5, National Biomedical Research Foundation, Washington, DC.

Erickson, G. F. (1983) *Mol. Cell. Endocrinol. 29*, 21–49.

Gospodarowicz, D., Vlodavsky, I., Biakecki, H., & Brown, K. D. (1978) in *Novel Aspects of Reproductive Physiology* (Spilman, C. H., & Wilks, J. W., Eds.) pp 107–108, Spectrum, New York.

Gray, A., Dull, T. J., & Ullrich, A. (1983) *Nature (London) 303*, 722–725.

Hope, D. B., Murti, V. V. S., & duVigneaud, V. (1962) *J. Biol. Chem. 237*, 1563–1566.

Hsueh, A. J. W., & Erickson, G. F. (1979) *Science (Washington, D.C.) 204*, 8854.

Hsueh, A. J. W., Adashi, E. Y., Jones, P. B. C., & Welsh, T. H., Jr. (1984) *Endocr. Rev. 5*, 76–127.

Jagiello, G., & Mesa-Tejada, R. (1979) *Endocrinology (Baltimore) 104*, 302–306.

Jagiello, G., & Vogel, H. J. (1981) *Bioregulators of Reproduction*, Academic, New York.

Knecht, M., & Ranta, T. (1982) *Endocrinology (Baltimore) 110* (Suppl.), 290 (Abstract 841).

Komoriya, A., Hortsch, M., Meyers, C., Smith, M., Kanepy, H., & Schlessinger, J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1351–1355.

Krystek, S. R., Reichert, L. E., Jr., & Andersen, T. T. (1985a) *Endocrinology (Baltimore) 117*, 1110–1124.

Krystek, S. R., Dias, J. A., Reichert, L. E., Jr., & Andersen, T. T. (1985b) *Endocrinology (Baltimore) 117*, 1125–1131.

Kurosky, A., Markei, D. E., Peterson, J. W., & Fitch, W. M. (1977) *Science (Washington, D.C.) 195*, 299–301.

Kyte, J., & Doolittle, R. F. (1982) *J. Mol. Biol. 157*, 105–132.

Mather, J. P. (1982) *Ann. N.Y. Acad. Sci. 383*, 44–68.

May, J. V., McCarty, K., Jr., Reichert, L. E., Jr., & Schomberg, D. W. (1980) *Endocrinology (Baltimore) 107*, 1041–1044.

Merrifield, R. B. (1963) *J. Am. Chem. Soc. 85*, 2149–2154.

Mondschein, J. S., & Schomberg, D. W. (1981) *Science (Washington, D.C.) 211*, 1179–1180.

Mondschein, J. S., & Schomberg, D. W. (1984) in *Regulation of Target Cell Responsiveness* (McKerns, K. W., Aakvaag, A., & Hansson, V., Eds.) Vol. 1, pp 463–485, Plenum, New York.

Morris, P. L., & Mather, J. P. (1984) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 43*, 5422.

Needleman, S. B., & Wunsch, C. D. (1970) *J. Mol. Biol. 48*, 443–453.

Pierce, J. G., & Parsons, T. F. (1981) *Annu. Rev. Biochem. 50*, 465–495.

Reichert, L. E., Jr., & Ward, D. N. (1974) *Endocrinology (Philadelphia) 94*, 655–664.

Reichert, L. E., Jr., & Abou-Issa, H. (1977) *Biol. Reprod. 17*, 614–621.

Rodbard, D. (1973) *Adv. Exp. Med. Biol. 36*, 289–326.

Rodbard, D., & Hutt, D. M. (1974) *Symp. RIA Relat. Proc. Med.*, 1–165.

Savage, C. R., Jr., Inagami, T., & Cohen, S. (1972) *J. Biol. Chem. 247*, 7612–7621.

Sluss, P. M., & Reichert, L. E., Jr. (1984) *Biol. Reprod. 30*, 1091–1104.

Sluss, P. M., Fletcher, P. W., & Reichert, L. E., Jr. (1983) *Biol. Reprod. 29*, 1105–1113.

Stewart, J. M., & Young, J. D. (1984) in *Solid Phase Peptide Synthesis*, 2nd ed., pp 42–68, Pierce Chemical, Rockford, IL.

Vlodavsky, F., Brown, K. D., & Gospodarowicz, D. (1978) *J. Biol. Chem. 253*, 3744–3750.