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Identification of a Receptor Binding Region on the β Subunit of Human Follicle-Stimulating Hormone†

Alan L. Schneyer,*,‡ Patrick M. Sluss,‡,§ James S. Huston,‖ Richard J. Ridge,‖ and Leo E. Reichert, Jr.‡

Department of Biochemistry, Albany Medical College, Albany, New York 12208, and Creative Biomolecules, 35 South Street, Hopkinton, Massachusetts 01748

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ABSTRACT: Mouse epidermal growth factor (mEGF) and the β subunit of human follicle-stimulating hormone (hFSH) (hFSH-β) have been shown to inhibit binding of intact hFSH to its testes membrane receptor in vitro. Both hFSH-β and mEGF contain the tetrapeptide sequence Thr-Arg-Asp-Leu (TRDL). Previous results demonstrated that synthetic TRDL inhibited binding of intact hFSH to receptor. We therefore investigated the possibility that TRDL was located on an exposed region of FSH-β using a polyclonal antiserum to hFSH [NHPP anti-hFSH batch 4 (AB4)] which recognized determinants on intact hFSH and its β subunit, but not the α subunit. Pituitary FSH preparations from several mammalian species produced parallel inhibition curves in a heterologous [AB4 and ^{125}I-labeled ovine FSH (^{125}I-oFSH)] radioimmunoassay with relative potencies similar to those observed for the same preparations assayed by radioligand receptor assay. This antiserum also competitively inhibited ^{125}I-FSH binding to receptor. Thus, AB4 appeared to recognize antigenic determinants that are highly conserved and located at or near regions involved with hormone recognition of receptor for FSH. Synthetic TRDL inhibited 50% of ^{125}I-hFSH binding to antiserum at a concentration of 1.36 mg/tube (9×10^{-3} M). Other tetrapeptides (Thr-Pro-Arg-Lys and Lys-Thr-Cys-Thr) had no inhibitory activity at comparable concentrations. A mixture of the free amino acids T, R, D, and L inhibited radioligand binding only at significantly higher concentrations than TRDL. The presence of TRDL in a receptor contact region of FSH was further suggested by its ability to inhibit the in vitro biological response to oFSH by cultured Sertoli cells at noncytotoxic levels. The reduced potency of TRDL relative to FSH in all three assay systems suggests that it represents only a portion of a larger determinant on the intact hormone and its β subunit. These results support a model of hormone–receptor interaction involving multiple, discrete contact points (determinants), some of which influence binding while others may be involved with initiation of a cellular response. Our results further suggest that TRDL is a constituent of one FSH receptor binding region.

Although our knowledge of receptor recognition sites for glycoprotein hormones is still imprecise, their existence is usually inferred from structure–function studies of hormone–receptor interactions (Ward, 1978; Pierce & Parsons, 1981). Receptor recognition of individual hormones in serum occurs through formation of high-affinity hormone–receptor complexes. The interactions between hormone and receptor are thought to be characterized by the same general features as other protein–protein interactions that are well understood from studies such as X-ray crystallography. Electrostatic, van der Waals, and hydrophobic interactions are responsible for noncovalent association at protein–protein interfaces. Such contact surfaces can comprise structural features that include a sequence of adjacent amino acids in the polypeptide as well as residues that come into close proximity in the folded structure. Such sequences of adjacent amino acids have been extremely useful probes of antigenic structure (Geysen et al., 1987) and, more recently, of hormone contact surfaces that are important for interactions with their receptors (Keutman et al., 1987).

While investigating the nature of follicle-stimulating hormone (FSH)[1] binding to gonadal receptors, we observed that

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* Address correspondence to this author at the Reproductive Endocrine Unit, Massachusetts General Hospital, Boston, MA 02114.
‡ Albany Medical College.
§ Present address: Department of Urology, Box 656, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642.
‖ Creative Biomolecules.

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mouse epidermal growth factor (mEGF) and the β subunit of human FSH (hFSH-β) inhibited binding of intact human FSH (hFSH) to receptors on calf testes membranes in vitro (Sluss et al., 1986). Comparison of the constituent amino acid sequences identified a unique tetrapeptide common to hydrophilic regions of both mEGF and FSH-β having the sequence Thr-Arg-Asp-Leu (TRDL) (Sluss et al., 1986; Krystek et al., 1985). In addition, synthetic TRDL inhibited binding of intact FSH to its receptor (Sluss et al., 1986). These observations suggest that both FSH-β and mEGF interact with the FSH receptor through contacts that include the exposed TRDL sequence. Thus, TRDL appears to represent a portion of the receptor binding region of FSH (Sluss et al., 1986), and the present research sought to further characterize this identity based on the possibility that TRDL might be detectable as an antigenic determinant.

We have now investigated the cross-reactivity of TRDL with a polyclonal antiserum to intact hFSH that recognizes determinants on hFSH-β and a variety of mammalian FSH preparations, and which competitively inhibits binding of intact hFSH to membrane receptors. Synthetic TRDL inhibited hFSH binding to this antiserum and was an antagonist of FSH bioactivity in vitro. Thus, TRDL appears to represent part of an antigenic site on the surface of FSH-β that interacts with its receptor.

Experimental Procedures

Preparation of Radioligand. Radiolabeled hFSH suitable for both RIA and RRA was prepared as described previously (Schneyer et al., 1986). This method involves lactoperoxidase-catalyzed iodination of highly purified human FSH (LER 1781; 4000 IU/mg). Polyacrylamide gel electrophoresis (PAGE) was utilized to purify radioligands following radioiodination (Schneyer et al., 1986). The resulting radioligands were at least 35% bindable to excess FSH receptors and 90% bindable to excess anti-hFSH antibodies.

Radioligand Receptor Assay. Preparation of calf testes membranes containing FSH receptors and procedures for RRA of hFSH have been previously described (Schneyer et al., 1986). Briefly, 3 mg wet weight of receptor preparation, 2.5 ng of radioligand, and sample or buffer (to a total volume of 0.5 mL) were incubated for 18 h at 20 °C, after which bound hormone was recovered by centrifugation (30000g) for 15 min, rinsed with buffer, and recentrifuged. Nonspecific binding (NSB) was defined as radioligand bound in the presence of a 300-fold excess of unlabeled hFSH.

Radioimmunoassay (RIA). Anti-hFSH-4 (AB4) was used throughout this study and was provided by the National Hormone and Pituitary Program (NHPP). As previously described (Schneyer et al., 1986), homologous hFSH RIAs utilized this antiserum at a final dilution of 1:120 000, along with 0.5 ng of radioligand (40 000 cpm) and sample or buffer in an incubation volume of 0.3 mL. After incubation at 4 °C for 24 h, sheep anti-rabbit γ-globulin antiserum was added at 1:20 dilution for an additional 24 h at 4 °C, after which the tubes were centrifuged at 7000g for 30 min to separate bound from free hormone. NSB was defined as radioligand precipitated when nonimmune rabbit serum was substituted for the primary antiserum.

Pituitary FSH preparations from species other than human were assayed by using a heterologous system in which AB4 was used at a final dilution of 1:75 000 and radioiodinated oFSH (LER 1976; 60 × S1 units/mg) prepared as described for hFSH. Precision, sensitivity, and specificity were similar to those obtained in the homolgous RIA (Schneyer et al., 1986).

RIA of hFSH Subunits. Antisera to both α and β hFSH subunits were obtained from NHPP. Anti-α was used at a 1:500 000 final dilution while anti-β was used at 1:60 000. Purified hFSH subunits (as distributed by NHPP) were iodinated by using a Chloramine-T procedure (Greenwood et al., 1963), followed by PAGE purification as described for intact hFSH. Other assay conditions were identical with those described for the intact FSH RIA. While the eluents from 3-mm PAGE gel slices were tested for specific binding to each antiserum, only fractions within the incorporation peak exhibiting maximal specific binding were used as radioligand in RIAs. In our assay, anti-α bound less than 1.0% of the ^{125}I-FSH-β peak fraction while anti-β bound less than 0.1% of the radioiodinated α-peak fraction.

In Vitro Bioassay. Preparation and culture procedures for assays utilizing immature rat Sertoli cell cultures were as described previously (Fletcher & Reichert, 1984; Sluss et al., 1987). In this assay, oFSH (LER 1996; 7 × S1 units/mg by in vivo bioassay) stimulates conversion of androstenedione (10^{-5} M) to estradiol. Estradiol was then quantitated by RIA after ether extraction, as a measure of FSH bioactivity. Inhibition of FSH-stimulated estradiol production by increasing doses of synthetic peptides was determined at 50 ng of FSH/mL, while the half-maximal response was obtained at 10 ng of FSH/mL.

Cytotoxicity of synthetic peptides was determined by analysis of UV absorption (260 nm) of sodium hydroxide solubilized cells remaining adhered to culture plates (dead cells do not adhere) following treatments, and by the ability of cell cultures to respond to restimulation (24 h) with 50 ng of FSH/mL after a 24-h wash with culture medium.

Materials. FSH preparations tested for potency by RIA and RRA were hFSH (LER 1781; 4000 IU/mg), oFSH (LER 1976; 60 × S1 units/mg), oFSH (NIH-S8), rFSH (NIH-R1), bFSH (NIH-B1), and dolphin FSH prepared in this laboratory (ALS-101; 0.5 × S1 unit/mg). TRDL and KTCT were prepared (Sluss et al., 1986) at Creative Biomolecules (Hopkinton, MA). TPRK (Kentsin) and free amino acids were obtained from Sigma (St. Louis, MO).

Data Analysis. RIA and RRA results, as well as competitive inhibition data, were analyzed by using NIHRIA and LIGAND computer programs (Rodbard & Hutt, 1974).

Results

Hormonal specificity of AB4 in a homologous RIA has been reported previously (Schneyer et al., 1986). When applied to a heterologous RIA in which PAGE-purified oFSH was the radioligand, parallel inhibition curves were observed with a variety of mammalian FSH preparations, including human, ovine, rat, bovine, and dolphin ($N = 6$; $p < 0.05$). When assayed by RRA, these preparations again produced parallel inhibition curves. The potencies of these preparations relative to highly purified human FSH (LER 1781) are listed in Table I. Recognition of heterologous FSH preparations by AB4 and the similarity of relative potencies by RIA to those de-

[1] Abbreviations: mEGF, mouse epidermal growth factor; TRDL, Thr-Arg-Asp-Leu; hFSH-β, β subunit of human FSH; hFSH-α, α subunit of human FSH; FSH, follicle-stimulating hormone; RIA, radioimmunoassay; RRA, radioligand receptor assay; TPRK, Thr-Pro-Arg-Lys; KTCT, Lys-Thr-Cys-Thr; PAGE, polyacrylamide gel electrophoresis; NHPP, National Hormone and Pituitary Program; K_A, apparent affinity constant of association; R_t, receptor number; ED_{50}, 50% inhibition of radioligand binding; AB4, NHPP anti-hFSH batch 4; oFSH, ovine FSH; bFSH, bovine FSH; rFSH, rabbit FSH; NSB, nonspecific binding; LH, luteinizing hormone; hCG, human chorionic gonadotropin.

Table I: Relative Potencies of Pituitary Preparations from a Variety of Mammalian Species As Determined by RIA and RRA[a]

species	prepn	RIA ED_{50} (ng) ± SE	RIA rel potency	RRA ED_{50} (ng) ± SE	RRA rel potency
human	LER 1781	3.2 ± 0.17	1	6.4 ± 0.80	1
ovine	LER 1976	3.6 ± 0.24	0.89	7.0 ± 0.40	0.91
ovine	NIH-S8	129 ± 8.30	0.02	106 ± 9.10	0.06
rat	NIH-R1	168 ± 0.12	0.02	666 ± 60.0	0.01
bovine	NIH-B1	183 ± 7.10	0.02	261 ± 24.0	0.02
dolphin	ALS-101	197 ± 18.0	0.02	500 ± 24.0	0.01

[a] NPA-anti-hFSH-4 and PAGE-purified ^{125}I-oFSH (LER 1976; 60 × S1 units/mg) were utilized for the RIA. The dose at which 50% of the radioligand is inhibited (ED_{50}) from binding to antiserum or receptor for each FSH preparation is listed as nanograms per tube ± the standard error (SE). Human (LER-1781) and ovine (LER-1976) FSH preparations are highly purified. Low relative potency of other preparations is consistent with their early stage of purification.

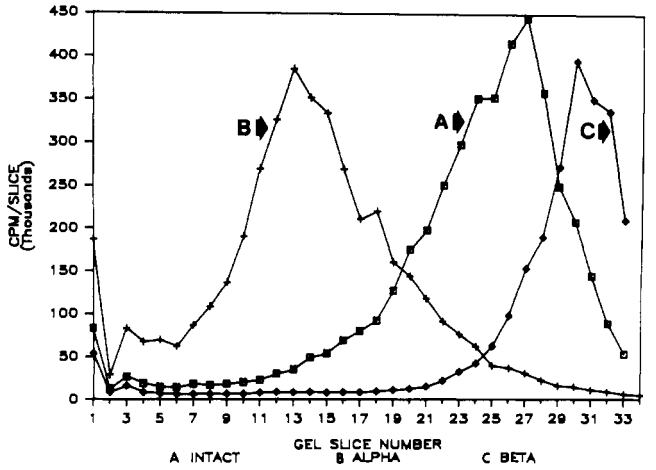

FIGURE 1: Differential electrophoretic mobility of intact radiolabeled FSH and its subunits using polyacrylamide gel electrophoresis (PAGE). Radioactivity profiles of 3-mm gel slices from intact hFSH (A) and its α (B) and β (C) subunits after purification by PAGE. Each preparation was electrophoresed on separate gels under identical conditions, demonstrating that iodinated subunits can be separated from intact radiolabeled FSH by this method. The identity of each peak was confirmed by RIA using antisera to intact hFSH (anti-intact) or its α (anti-α) and β (anti-β) subunits as described in Figure 2. The radioactive peak for hFSH-α was at fraction 13 and was substantially free from intact hormone as demonstrated by SDS–PAGE autoradiography (data not shown). The peak of radioactivity for the FSH-β gel was located in fractions 30–32. Radioiodination of intact hFSH produces iodinated subunits as well as intact hormone and can be separated by its intermediate electrophoretic mobility (radioactive peak at fraction 27).

termined by RRA indicate that this antiserum recognizes highly conserved regions of FSH.

The antigenic sites recognized by AB4 were localized to FSH-β by comparing binding of iodinated hFSH or β subunits after electrophoretic purification. The radioactivity profiles shown in Figure 1 demonstrate separation of radioiodinated intact hFSH (A) from its α (B) and β (C) subunits by virtue of their electrophoretic mobility. Identity of the radioligand within each radioactive peak was confirmed by investigating the specific binding of each peak to anti-intact hFSH (A), NPA anti-α hFSH (B), or anti-β hFSH (C), as shown in Figure 2. Anti-β and AB4 (anti-intact) bound hormone present in the intact hormone and β-subunit regions of the gels (fractions 25 and 30, respectively), but neither antiserum bound radiolabeled hormone present in the α-region (fraction 11), demonstrating that AB4 only recognizes determinants

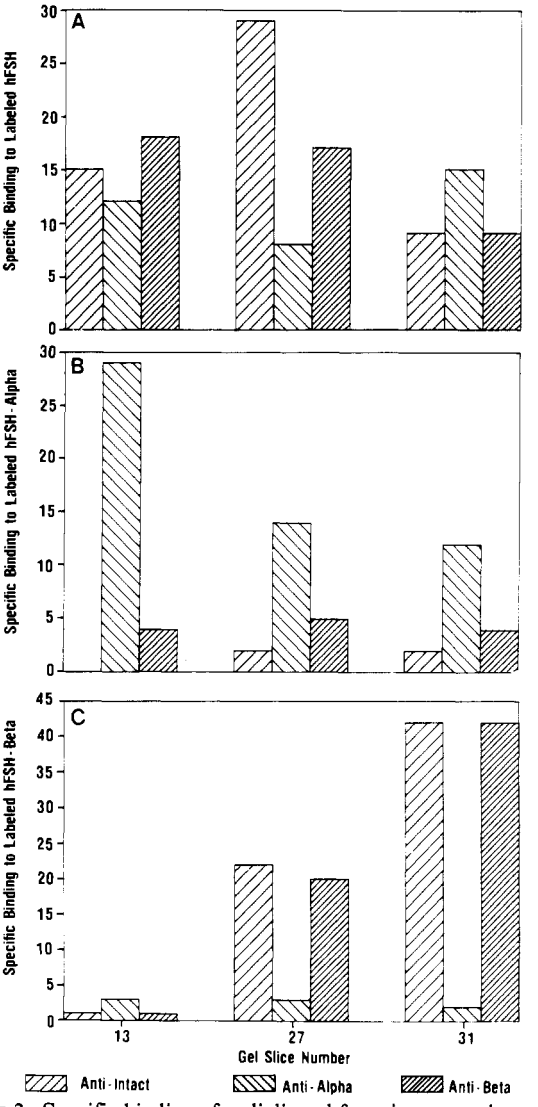

FIGURE 2: Specific binding of radioligand from incorporation peaks to anti-hFSH (anti-intact; NPA anti-hFSH-4), anti-α (NPA anti-hFSH-α), or anti-β (NPA anti-hFSH-β). (A) RIA of fractions (3-mm slices) from radioiodinated intact hFSH after PAGE. Fraction 27 contained the greatest specific binding to anti-hFSH and was relatively free from subunits (determined from SDS–PAGE autoradiography). Specific binding to anti-α and anti-β antisera indicated that these antisera recognize determinants on the intact molecule, in addition to those unique to each subunit. (B) RIA of fractions (3-mm gel slices) from radioiodinated FSH-α after PAGE. While the incorporation peak at fraction 13 contains mostly α subunit, specific binding to anti-β antiserum at this fraction as well as at fraction 27 and 31 indicates that some β subunit is also present at the latter fractions. Anti-intact hFSH did not bind any radioligand from fraction 13, demonstrating its specificity for determinants on the β subunit of FSH, and confirming the lack of intact hormone in this fraction. Some intact hFSH may be present at fractions 27 and 31, since both anti-intact and anti-β FSH bind radioligand at these fractions. (C) RIA of fractions (3-mm gel slices) from radioiodinated FSH-β after PAGE. Fraction 31 consists almost exclusively of iodinated β subunit and is bound equally by both anti-intact and anti-β antisera. No intact hormone was observed in this peak after SDS–PAGE autoradiography. Minimal binding to anti-α antiserum at each fraction suggests either that small amounts of α subunit are contained in this β-subunit preparation or that anti-α antiserum cross-reacts minimally to β subunit.

located on FSH-β and exposed on the intact hormone.

In order to examine the impact of antibody binding to this region of hFSH on receptor recognition by hormone, the ability of AB4 to inhibit ^{125}I-FSH binding to receptor was determined. Binding isotherms were generated by addition of increasing concentrations of ^{125}I-hFSH at each concentration of AB4 tested. The results were analyzed by using a nonlinear

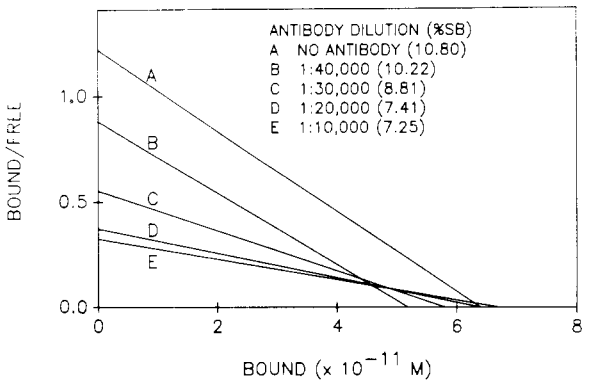

FIGURE 3: Competitive inhibition of ^{125}I-hFSH binding to receptor by anti-hFSH antiserum. Concentrations of ^{125}I-hFSH ranging from 0.5 to 10 ng were used to generate binding isotherms at a receptor concentration of (6.4 ± 0.66) × 10^{-11} M. Binding data were analyzed by nonlinear curve-fitting computer programs to determine the apparent affinity constant (K_A) and the total number of receptors (R_t) from Scatchard plots (as shown in figure). Binding isotherms were generated at antiserum concentrations of 0, 1:40 000, 1:30 000, 1:20 000, and 1:10 000 final dilutions. Calculated K_A (×10^9 M ±SE) was reduced (19.00 ± 4.3, 17.01 ± 3.5, 9.5 ± 1.9, 5.8 ± 1.6, and 4.8 ± 2.6, respectively) while R_t (×10^{-11} M ±SE) was unaffected (6.40 ± 0.66, 5.19 ± 1.2, 5.81 ± 0.6, 6.40 ± 1.1, and 6.70 ± 2.6, respectively) by the antiserum concentration tested. The observed decrease in apparent affinity constant in the absence of significant effects on receptor number suggests competitive inhibition of radioligand binding to receptor by the antiserum.

curve-fitting computer program (LIGAND) and are graphically summarized in Figure 3. Linear Scatchard plots indicating a single class of high-affinity binding sites were obtained for each binding isotherm. Increasing concentrations of AB4 decreased specific radioligand binding to receptor but had no significant effect on the calculated total number of receptors. In addition, the apparent (steady-state) affinity constant of association (K_A) was positively correlated (0.992, $P < 0.01$) with decreased specific binding due to increasing antibody concentration. These data indicate that AB4 competitively inhibits ^{125}I-hFSH binding to receptor and suggest that some antibody binding sites are at or near a region of FSH-β that interacts with the FSH receptor.

Synthetic TRDL and hFSH-β also inhibited binding of radiolabeled hFSH to AB4 in a concentration-dependent manner, although the amount of TRDL necessary for 50% inhibition (ED_{50}) was 1.36 mg, as compared to 1.24 ng for hFSH and 1.23 ng for FSH-β (Figure 4). The slopes of these inhibition curves were not parallel, indicating that some of the antibodies within this antiserum recognize additional or more inclusive domains. However, neither TPRK, which contains two of the four amino acids in TRDL, nor KTCT inhibited hFSH binding at doses (1.5 mg) comparable to those at which TRDL inhibited 50% of the intact hFSH binding. In addition, a mixture of the four free amino acids composing TRDL inhibited radioligand binding only at doses greater (>2.5 mg/tube) than the ED_{50} for intact TRDL, and the resulting inhibition curve was not parallel to TRDL (Figure 4).

In vitro biological activity of TRDL was investigated by using cultured rat Sertoli cells. Inhibition of FSH-stimulated (50 ng/mL) conversion of androstenedione to estradiol in these cultures was observed at TRDL concentrations of 100 μg/mL and above (Figure 5, open bars). Cytotoxicity of TRDL at doses used in this study was investigated as a possible cause of inhibition of FSH-stimulated estradiol secretion. Basal levels of estradiol production, however, were not affected by the presence of TRDL (Figure 5, solid bars). No changes in gross morphology or in the absorbance (260 nm) of sodium hydroxide solubilized cells (after test period) were observed

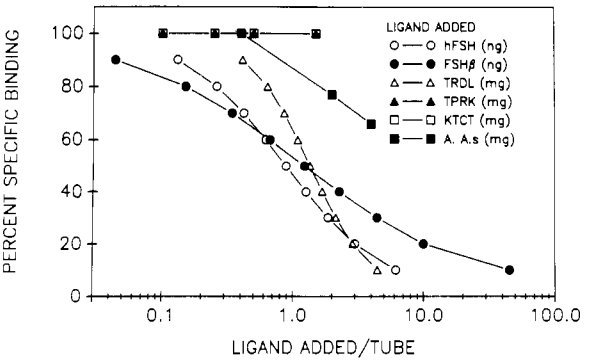

FIGURE 4: Radioimmunoassay of hFSH, β subunit of hFSH (hFSH-β), synthetic peptides TRDL, TPRK, and KTCT, and individual amino acids comprising TRDL (A.A.s) using NPA-anti-hFSH-4 and PAGE-purified intact hFSH as radioligand. Slopes for the resulting inhibition curves were −1.03 ± 0.07, −0.66 ± 0.05, and −1.84 ± 0.13 while the ED_{50}'s were 1.24 ± 0.11 ng 1.23 ± 0.15 ng, and 1.36 ± 0.17 mg for hFSH, hFSH-β, and TRDL, respectively. At concentrations up to 1.5 mg, TPRK and KTCT did not inhibit ^{125}I-hFSH binding despite having amino acids in common with TRDL. Note that the abscissa is calibrated in nanograms for FSH and FSH-β and in milligrams for peptides and amino acids.

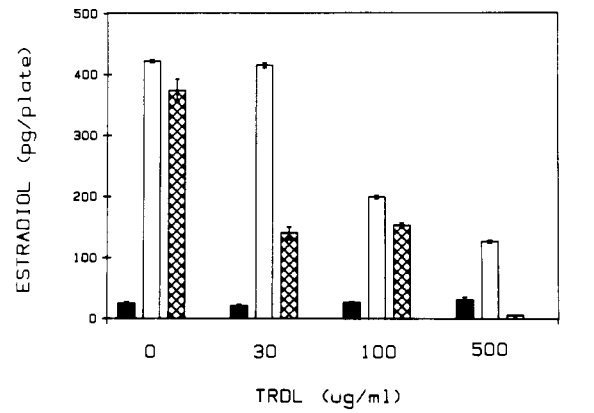

FIGURE 5: In vitro biological effects of TRDL on Sertoli cell cultures. FSH-stimulated (50 ng/mL) estradiol production (open bars) is significantly ($p < 0.01$) reduced in the presence of 100 and 500 μg of TRDL. However, basal estradiol production (black bars) is unaffected by the presence of up to 500 μg of TRDL. After cultures were incubated with fresh medium for 24 h, cells were restimulated with fresh medium containing 50 ng/mL FSH (cross-hatched bars). FSH-stimulated estradiol production was reduced after exposure to TRDL, but it remained significantly ($p < 0.05$) greater than basal at both 30 and 100 μg/mL TRDL.

in cells treated with doses of TRDL under 500 μg/mL. Since dead cells do not remain adhered to the culture plates, the absorbance at 260 nm is an indirect measure of viable cells remaining on plates after treatment. However, as shown in Figure 5 (cross-hatched bars), FSH-stimulated estradiol secretion was completely inhibited by previous exposure to 500 μg/mL TRDL. In contrast, cells treated with 30–100 μg of TRDL were able to respond to a subsequent FSH restimulation. Thus, inhibition by lower doses of TRDL appears to be reversible, indicating the absence of nonspecific disruption of cellular processes affecting androstenedione conversion to estradiol. TRDL inhibition of in vitro FSH biological activity lends further support to the interpretation that this sequence may be within a region of FSH-β that contributes to receptor recognition and binding.

DISCUSSION

Antigenic determinants exposed on both hFSH-β and intact hFSH, as well as on FSH preparations from all mammalian species tested, were recognized by AB4, suggesting that this

antiserum binds to highly conserved antigenic determinants of FSH. Our results demonstrating competitive inhibition of radiolabeled FSH binding to receptor by AB4 indicate that these conserved determinants may also be receptor recognition regions. Evolutionary conservation of receptor contact regions is further supported by studies demonstrating receptor recognition by FSH preparations from diverse species (Licht et al., 1977).

Synthetic TRDL inhibited binding of PAGE-purified ^{125}I-hFSH to AB4, as did intact FSH and its β subunit. Greater than 80% inhibition was observed at the maximum TRDL dose tested. This observation suggests that a major antigenic epitope on the β subunit of FSH includes the TRDL sequence. The low binding affinity shown by AB4 for TRDL compared to both FSH and its β subunit, as well as nonparallel inhibition curves observed for these parameters (Figure 4), may derive from a flexible conformation in the synthetic tetrapeptide as compared to a more rigid TRDL portion of the antigenic determinant on FSH (Westof et al., 1984; Barlow et al., 1986). In previous immunological studies, high-affinity antisera to a variety of intact molecules were used to demonstrate reduced apparent affinity for synthetic fragments of the immunogens (Westhof et al., 1984; Berzofsky, 1985; Berzofsky & Berkower, 1976; Komoriya et al., 1984). The magnitude of these reductions in binding affinity was similar to that observed for the interaction between AB4 and TRDL.

The specificity of the FSH antiserum for synthetic TRDL was assessed through binding measurements of two tetrapeptides, TPRK and KTCT. Neither of these peptides inhibited radioligand binding to antiserum at doses similar to those used for TRDL. Since both TRDL and KTCT inhibited binding of hFSH to receptor in our previous studies (Sluss et al., 1986), the present results suggest that KTCT is not part of an antigenic epitope recognized by AB4 and that several independent sites may be involved in hormone–receptor binding and activation. Individual amino acids comprising TRDL inhibited binding only at doses exceeding the ED_{50} for intact TRDL. In addition, the slope of the inhibition curve so generated was not parallel to the peptide inhibition curve. The inhibition of ^{125}I-hFSH binding by free amino acids may represent "non-specific" ionic or charge effects on antibody binding as suggested by the relatively flat dose–response curve.

Results of the present studies, and those from previous immunochemical analyses (Sachs et al., 1972; Atassi, 1975; Berzofsky & Berkower, 1976; Sairam et al., 1981; Lerner, 1982; Todd et al., 1982; Niman et al., 1983; Westof et al., 1984), receptor binding studies (Sluss et al., 1986; Schneyer et al., 1986; Krstenansky et al., 1986; Sairam & Bhargavi, 1985), and chemical modification experiments (Ward, 1978; Pierce & Parsons, 1981), suggest a model of hormone–receptor interaction involving multiple contact points or determinants, some of which confer specificity and selectivity on the binding reaction, while others act to stimulate postbinding responses. In this model, interaction of hormone with membrane receptors can be viewed as analogous to hormone interaction with antibodies in polyclonal antisera raised to native hormone, in that both interactions (antigen–antibody or hormone–receptor) involve multiple determinants. Since polyclonal antisera are composed of heterogeneous populations of antibodies recognizing specific determinants on the molecule used for immunization, the measured affinity for a particular antigen represents an average for all of the constituent antibodies that bind the immunogen (Berzofsky, 1985; Berzofsky & Berkower, 1976). Sites on both the α and β subunits of pituitary glycoprotein hormones can be included in this model (Ward, 1978; Pierce & Parsons, 1981; Grasso & Crisp, 1985; Reichert et al., 1973). For example, a receptor binding region in hCG-β and hLH-β was recently demonstrated by using synthetic peptides (Keutman et al., 1987). Half-maximal inhibition of intact radioligand by peptides comprised of amino acids 38–57 was observed at doses approximately 10-fold lower than that observed in this study for TRDL. This may reflect increased conformational stability of the 20 amino acid hCG-β peptide (Keutman et al., 1987), as compared to TRDL, or indicate that these peptides include more of the receptor recognition region on intact hCG. Shorter segments of this region (45–57) exhibited a 1000-fold reduction in affinity relative to the entire 38–57 peptide. The deleted portion contained a TRVL sequence (42–45) analogous to the TRDL sequence of FSH-β (34–37). Another peptide from hCG-β inhibited binding of intact hCG to receptor at similar doses to the 38–57 peptide but contained amino acids 93–101. This sequence is based on the "determinant loop" of LH-β proposed earlier as a receptor contact region (Ward, 1978). Thus, LH-β seems to contain at least two receptor contact points that each inhibit binding of intact hormone at similar doses, but with combined affinities approximating the affinity for the intact hormone.

Expanding the implications of this model, FSH binding inhibitors isolated from gonadal tissues (Sluss et al., 1987; Sluss & Reichert, 1984) may modulate FSH action by mimicking at least a portion of the determinants on FSH necessary for receptor binding and/or subsequent biological response, thereby effectively inhibiting its action. Thus, if TRDL contributes to one of several such sites, its interaction with the receptor would involve a reduced free energy of binding, with a correspondingly lower affinity for receptor compared with the intact hormone. Lower receptor affinities were observed for synthetic peptides derived from hormones such as mEGF (Heath & Merrifield, 1986) and glucagon (Krestenansky et al., 1986).

When combined with evidence from previous studies, our results support a model of hormone–receptor interaction composed of multiple, possibly independent, contact points that together provide interactions of relatively high affinity and specificity. Individual receptor subsites would be expected to recognize corresponding contact subsites on the hormone. The sum of these interactions produces the measurable binding between hormone and receptor. If TRDL is part of one receptor binding determinant, synthesis of larger peptides could allow identification of additional epitopes on the native hormone (Berzofsky, 1985; Lerner, 1982; Todd et al., 1982) and result in synthetic peptides with higher affinities than TRDL for the FSH receptor. It is conceivable that such receptor binding peptides could potentially be designed to either promote a biological response and thus stimulate fertility or inhibit the biological response and thus act as a contraceptive.

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Reductive Methylation of Lysine Residues in Acidic Fibroblast Growth Factor: Effect on Mitogenic Activity and Heparin Affinity†

J. Wade Harper and Roy R. Lobb*

Center for Biochemical and Biophysical Sciences and Medicine and Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115

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ABSTRACT: Reductive methylation of bovine brain derived acidic fibroblast growth factor (aFGF) with formaldehyde and sodium cyanoborohydride reduces its capacity to stimulate mitogenesis in Balb/C 3T3 cells, and this correlates with the modification of less than 3 of its 12 lysine residues. Fractionation of methylated aFGF on immobilized heparin shows that the affinity of the modified mitogen for heparin is also decreased substantially. The capacity of methylated mitogen of low heparin affinity (LA-aFGF) to stimulate mitogenesis is also reduced, and this correlates with a reduced affinity for its cell surface receptor. Structural characterization of LA-aFGF using peptide mapping and sequencing procedures demonstrates that Lys-118 is the primary site of modification. The results indicate that in aFGF, Lys-118 plays an important role in heparin binding and suggest that this residue and its local environment are involved in the interaction of aFGF with both heparin and its cell surface receptor.

Bovine brain derived acidic fibroblast growth factor (aFGF),[1] a class 1 heparin-binding growth factor (Lobb et al., 1986a,b), induces mitogenesis in a variety of mesoderm- and neuroectoderm-derived cells in vitro and is angiogenic in vivo (Thomas et al., 1984, 1985; Gospodarowicz et al., 1986; Lobb et al., 1985, 1986a; Thomas & Gimenez-Gallego, 1986). The primary structure of bovine aFGF has been determined (Esch et al., 1985a; Gimenez-Gallego et al., 1985; Strydom et al.,

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* Address correspondence to this author at the Center for Biochemical and Biophysical Sciences and Medicine, Seeley G. Mudd Bldg., 250 Longwood Ave., Boston, MA 02115.

[1] Abbreviations: aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; HBGF, heparin-binding growth factor; LA-aFGF, methylated aFGF of low heparin affinity; HPLC, high-performance liquid chromatography; PTC, phenylthiocarbamoyl; PTH, phenylthiohydantoin; TFA, trifluoroacetic acid; MeLys, ϵ-methyllysine; Me_2Lys, ϵ-dimethyllysine; BSA, bovine serum albumin; DME, Dulbecco's modified Eagle's medium; PBS, Dulbecco's calcium- and magnesium-free phosphate-buffered saline; Tris, tris(hydroxymethyl)aminomethane.

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