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SPECIFIC BINDING OF LUTEINIZING HORMONE RELEASING HORMONE TO HUMAN LUTEAL TISSUE

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Homogenates of human luteal tissue bound radioiodinated luteinizing hormone releasing hormone agonist. Specific binding was both time- and temperature-dependent. Native LHRH and two LHRH agonists competed for binding, whereas TRH, somatostatin and oxytocin did not, indicating that the binding sites were specific. The apparent Ka values were 2 x $10^7 M^{-1}$ for both LHRH agonists and $10^6 M^{-1}$ for native LHRH. This is the first demonstration of specific binding of LHRH to human ovarian tissue. No binding could be detected to ovarian tissue from postmenopausal women.

The hypothalamic decapeptide luteinizing hormone releasing hormone (LHRH) and its analogues have been shown to exert direct actions on the gonads in a variety of species including rat (1), pig (2), rabbit (3), cow (4) and chicken (5). These extra-pituitary actions are likely to be mediated via specific receptors: such receptors have been demonstrated on rat follicular and luteal tissue (2) and on both rat (6) and porcine (7) Leydig cells. However, the relevance of these observations to the human remains uncertain. Since LHRH and its agonists analogues are used clinically to modify reproductive function (8) it is clearly of primary importance to determine whether LHRH is capable of acting at sites other than the pituitary in the human. We report here the detection of specific binding sites for LHRH on human corpora lutea using radiolabelled I^{125}-LHRH agonist as ligand. Our findings indicate that human ovarian function may be directly modified by LHRH and related peptides.

Abbreviations:- LHRH = luteinizing hormone releasing hormone
TRH = thyrotropin releasing hormone, BSA = bovine serum albumin
NSB = non specific binding, Bo = specific binding, TC = total counts
IgG = immunoglobulin, PBS = phosphate buffered saline, PEG = polyethylene glycol

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MATERIALS AND METHODS

Source of tissue: Human luteal tissue was obtained from women undergoing laparotomy during the luteal phase of the cycle (the informed consent of the patient was obtained for this procedure). The tissue was transported immediately to the laboratory where it was stored at -40 or -70°C or used immediately for the binding assay.

Hormones (D-Ser-t-bu^{6}, des Gly NH^{10})LHRH ethylamide (LHRH agonist, Hoechst UK), TRH and somatostatin were provided by the courtesy of Dr. J Sandow (Hoechst A.G.) 6-D-2-naphthyl-alanine-LHRH (D-Nal (2)^{6} LHRH) was provided by Dr. R. Vickery (Syntex). Oxytocin was a gift from Sandoz labs.

Preparation of ^{125}I-LHRH agonist
LHRH agonist (LHRH ethylamide) was labelled with ^{125}I using lactoperoxidase as described previously (6). The specific activity of the label was 909 μCi/mg as assessed by self displacement of binding in isolated rat Leydig cells.

Binding of ^{125}I-LHRH agonist
Ovarian tissue was homogenized in 10mM Tris HCl (0.3M sucrose, 1mM EDTA pH 7.4) using either a Polytron homogenizer or a glass pestle hand homogenizer. Aliquots of homogenate were then used for the binding assay. Incubations were carried out in triplicate in the presence of ^{125}I-LHRH agonist (120,000 cpm) and the non specific binding was determined by incubation in the presence of 10^{-5}M unlabelled hormone. Incubations were carried out for various times (Fig.2) and routinely for either 2h at 4°C (Table 1) or 1 hr at 22°C (Fig 1 & 3). Termination of the reaction was achieved by chilling the tubes on ice before separation of the bound ligand one of 2 methods; (i) by dilution of the incubate with 2ml ice cold phosphate-buffered saline (PBS) followed by filtration (with 3 x 2ml washes with PBS) under vacuum through Whatman GF/C filters, which had been presoaked overnight in 2% BSA to lower non-specific binding, (Fig 1, Table 1) or (ii) by the addition of 0.5ml of 0.5% Bovine immunoglobulin (IgG) and 1 ml 25% polyethylene glycol (PEG) (Fig.2). After vortexing thoroughly the tubes were centrifuged at 2000 rpm at 4°C for 15 mins. Radioactivity retained by the filters or in the pellet was measured in a gamma counter.

RESULTS

Ultrafiltration using membrane filters was compared with PEG precipitation, a method used previously for the recovery of detergent-solubilized gonadal hCG receptors (10). Although both methods enabled specific LHRH binding to be detected, the standard filtration method for LHRH using Whatman GF/C filters was found to be less sensitive than PEG precipitation (Fig.1).

Specific binding of LHRH agonist to human luteal tissue was dependent on both the temperature and duration of incubation (Fig 2). This pattern of binding is similar to that reported for rat testicular tissue (9), namely rapid binding followed by a sharp decline at 37°C and a slower rise to maximal levels at 20 and 4°C. These binding characteristics presumably reflect changes in stability of the ligand and/or receptor at these temperatures.

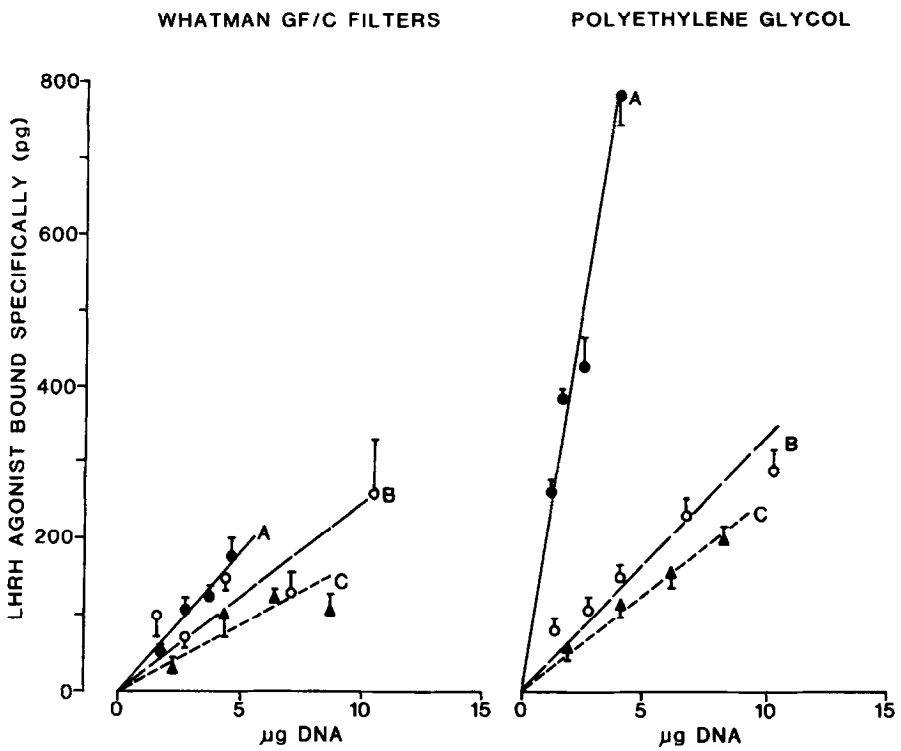

Fig.1 Comparison of recovery of bound hormone after incubation with homogenates from 3 corpora lutea (a, b and c). The stage of the luteal phase was determined by histology of both luteal and endometrial tissue and by peripheral steroid hormone measurement as described previously (21). Corpora lutea (a) and (b) were from the mid-luteal phase and (c) from the early luteal phase. Results are expressed as pg bound (mean ± range) at different concentrations of DNA (measured by method of Burton (22).

The specificity and affinity of the binding sites for LHRH was examined by testing the ability of unlabelled hormones to compete for binding of iodinated LHRH agonist. The apparent affinity constants (Ka) calculated from displacement curves with LHRH agonist as tracer were found to be 3 x $10^7 M^{-1}$ in 3 separate experiments using 3 different homogenates of human corpus luteum (Fig.3). This value was also obtained for a second LHRH agonist ((D-Nal(2)^6) LHRH), whereas native LHRH gave an apparent Ka of $10^6 M^{-1}$ indicating that the affinity of the binding site was slightly higher for LHRH agonists than the native hormone. Binding was specific for LHRH and LHRH agonists: neither oxytocin, somatostatin nor TRH competed for binding at concentrations up to 10000ng (Fig 3).

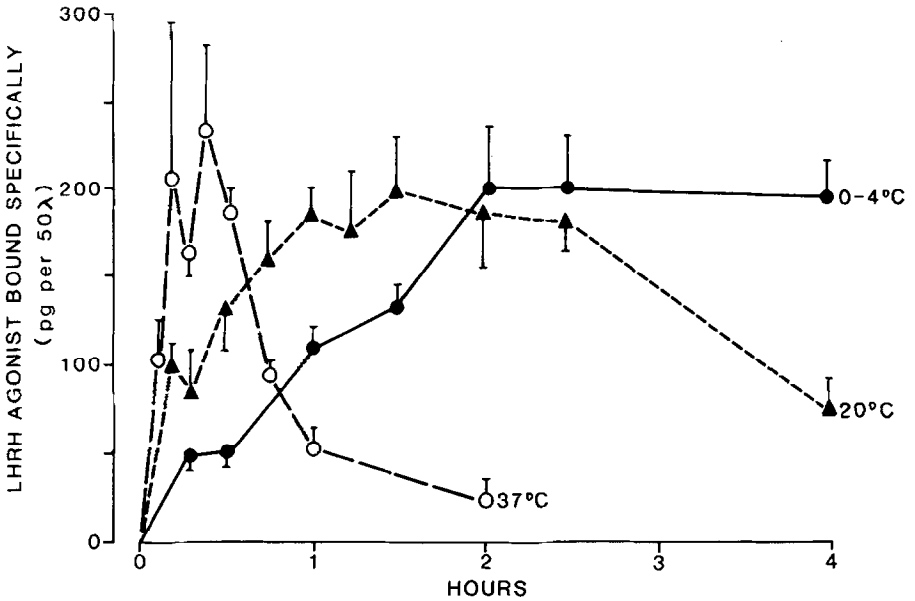

Fig.2 Specific binding of ^{125}I LHRH agonist (D-Ser-t-Bu^{6}-des Gly-NH$_2$^{10}) LHRH ethylamide to human corpus luteum homogenates at different temperatures for periods up to 4h. The patterns of specific binding with time and temperature were similar in 3 separate experiments using different luteal homogenates. Hence the data have been combined (after normalisation to a plateau value of 4000 pg/50µl to correct for differences in homogenate amounts. Points represent mean specific binding (Bo-NSB) ± ranges.

No difference was found in binding characteristics of fresh and frozen tissue (Table 1) and no significant binding could be demonstrated to ovarian tissue from postmenopausal women.

DISCUSSION

These results demonstrate that binding sites for LHRH are present on human luteal tissue and that these sites are specific for LHRH and related peptides. Our studies indicated that for native LHRH, the apparent affinity of binding to the human corpus luteum was similar to value reported for binding to the rat pituitary (11) and gonadal tissue (12). However, these same rat tissues have apparent affinities for the LHRH agonist which are 100- to 1000-fold greater than that of native LHRH (11, 12). In marked contrast, human ovarian binding sites had similar affinities for the two agonists and for native LHRH indicating a clear species difference.

Our findings are in contrast to a recent report in which LHRH binding to human luteal tissue was not detectable (13). The reasons for this discrepancy became

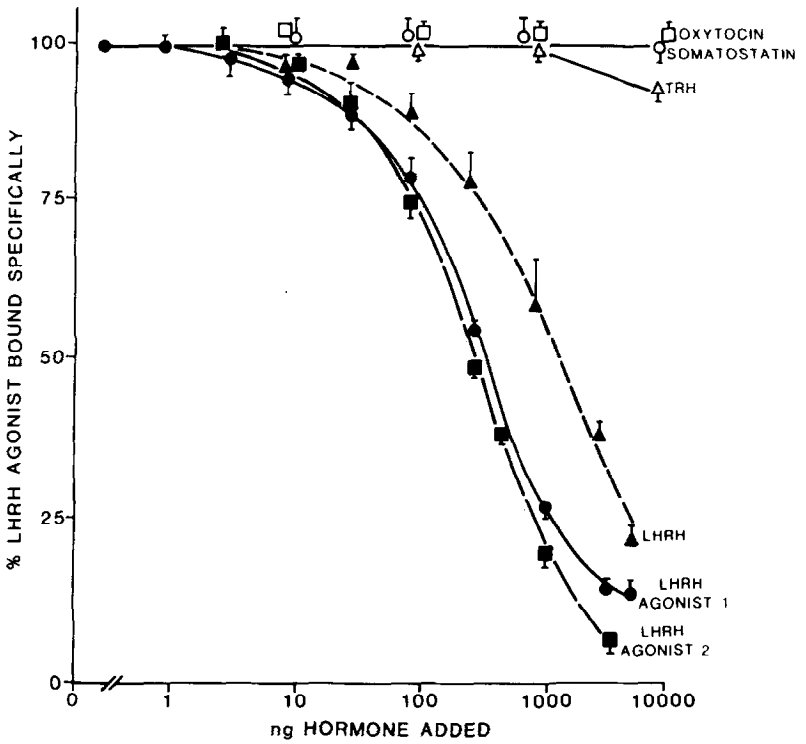

Fig.3 Competition curves for binding of ^{125}I-LHRH agonist in the presence of increasing concentrations of unlabelled LHRH agonist$_1$, (D-ser-t-Bu^6-des-GlyNH_2^{10}) LHRH ethylamide, LHRH agonist$_2$ (D-Nal (2)^6) LHRH, native LHRH, oxytocin, somatostatin and TRH. The figure represents the mean of 3 displacement curves obtained from 3 mid-luteal corpora lutea. The values of Bo for each corpus luteum were 9,500 cpm/75μl, 4,900 cpm/50μl, and 7,100 cpm/40μl.

clear from the data presented here on the nature of the binding site. In order to detect the presence of a relatively low affinity receptor, it is necessary to use both a sufficiently high concentration of radiolabelled ligand and a sufficiently high concentration of unlabelled hormone (10^{-5}-10^{-6}M) to assess non-specific binding. Using the method of Clayton and Huhtaniemi (13) with a lower concentration of both unlabelled (10^{-8}M) and labelled (10-50,000 cpm) ligand we failed to detect binding to a homogenate of human luteal tissue. The same tissue in our own assay specifically bound 37.5pg LHRH agonist/μg DNA.

We suggest several possible explanations for the low apparent affinity of LHRH binding to the human corpus luteum. Firstly, the apparent Ka (10^7M^{-1}) may represent the true affinity of the binding site, in which case its physiological significance remains uncertain due to the relatively high

Table 1

Binding of LHRH agonist to fresh CL, the same CL after freezing and to 2 postmenopausal ovaries.

	Non specific binding	Total binding	Specific binding	Total counts	Percentage binding
	NSB	Bo	Bo-NSB	TC	100 (Bo-NSB)/TC
Fresh CL (41 μg DNA)	8300±200	1500±300	6700	100,000	6.7%
Frozen CL (41 μg DNA)	1700±900	28000±100	11000	160,000	7.0%
Postmenopausal ovary 1(30 μg DNA)	3000±300	2600±200	-	130,000	-
Postmenopausal ovary 2(25μg DNA)	2000±250	2400±300	400	100,000	0.4%

Values represent mean cpm ± ranges of duplicate estimations. The frozen corpus luteum (CL) had been stored at -40°C for 6 months after the assay of the fresh tissue.

concentrations which would be necessary to activate the receptor. Secondly, the true affinity of the receptor may be greater than $10^7 M^{-1}$, but the agonist and native hormone may be degraded to a similar extent (as suggested for human placental tissue (14)) giving rise to apparent low affinity binding. Thirdly, the putative intragonadal factor(s) interacting with such a binding site *in vivo* are unlikely to be identical to hypothalamic LHRH or its agonist. Indeed, LHRH like molecules detected in rat testicular tissue have been shown to be non-identical to LHRH (15,16). Thus, the physiological ligand for such receptors may well have a greater affinity than either native or LHRH agonist.

In addition to our data showing specific binding sites for LHRH in human luteal tissue other groups have reported both extra-pituitary actions of LHRH in the human, eg on human arrhenoblastoma cells (17), granulosa cells (18) and placental cells (19) and the presence of LHRH like activity in extra-hypothalamic sites (19,20). Our findings add to these data and suggest that an intragonadal LHRH molecule may exist which may exert direct gonadal actions in the human.

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