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HUMAN PLACENTAL RECEPTORS FOR LUTEINIZING HORMONE RELEASING HORMONE

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SUMMARY

Plasma membrane preparations of human term placenta bound ^{125}I-labelled luteinizing hormone releasing hormone (LHRH) agonist with high affinity (K_A = $5.5 \times 10^7 M^{-1}$) and specificity. Native LHRH competed for binding with the same affinity (K_A = $6.2 \times 10^7 M^{-1}$) whereas fragments of the LHRH agonist, oxytocin, somatostatin and TRH gave no or minimal displacement. Placenta from 10 week's gestation and membrane preparations from a single hydatidiform mole also contained LHRH receptors with similar characteristics to those found in term placenta. To our knowledge this is the first demonstration of extra-pituitary LHRH receptors in the human.

A luteinizing hormone releasing hormone (LHRH) with apparently similar immunochemical, physiochemical and biological properties to hypothalamic LHRH has been detected in the human placenta (1-4). The placental content of LHRH varies throughout gestation (1) and placental tissue in vitro has the capacity to synthesize the peptide (4,5). It has therefore been suggested that placental LHRH may have a role in the regulation of hCG production and may affect placental steroidogenesis (4).

Immunofluorescence studies have shown placental LHRH to be located in the cytotrophoblast (1,6). On this basis it has been postulated that LHRH is made in the cytotrophoblast and acts on the syncytiotrophoblast to control hormone production. If this is the case, then LHRH receptors must be present on the syncytiotrophoblast membrane to convey the hormonal message. In this paper we demonstrate the presence of LHRH receptors in the human placenta.

Abbreviations: LHRH = luteinizing hormone releasing hormone
TRH=thyrotrophin releasing hormone. hCG = human chorionic gonadotrophin. BSA= bovine serum albumin

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MATERIALS AND METHODS

Collection and Preparation of tissue

Human term placentae were collected from the Simpson Maternity Hospital, Edinburgh, following normal vaginal delivery or Caeserian section and placed on ice. Placentae of 10-12 weeks gestation and a single hydatidiform mole were obtained from suction curettage. Trophoblastic villi were dissected free of the membrane in the case of 10-12 week placentae and vesicles were removed from the hydatidiform mole. In term placentae, cotyledons from different regions were dissected free from the membranes and weighed.

Tissue was homogenized in 25mM Sodium Potassium Phosphate buffer containing 1mM Magnesium Chloride (pH 7.4) using a Polytron Homogeniser (2ml buffer/g tissue). The homogenate was filtered through gauze and the filtrate centrifuged for 20 mins at 1,000g. The pellet was resuspended or frozen until further use. The 1,000g supernatant was further centrifuged at 10,000g for 15 minutes and the resultant pellet resuspended in 5ml buffer. The 10,000g supernatant was then centrifuged at 100,000g for 60 minutes and the pellet resuspended in 2 ml buffer.

Aliquots of the above preparations were assayed for LHRH agonist binding using the methods described below.

Hormones

(D-Ser-t-bu^{6}, des-Gly-NH$_2^{10}$) LHRH ethylamide (LHRH agonist, Hoechst U.K.) and its 4-9 and 6-9 fragments, LHRH, TRH and somatostatin were all provided by courtesy of Dr. J. Sandow (Hoechst, A.G). Oxytocin was a gift from Sandoz Labs.

Preparation of ^{125}I-LHRH agonist

5 µg LHRH agonist was labelled with 2 mCi ^{125}I using lactoperoxidase (7) and purifed on a 40 x 1cm column of Sephadex G25 fine (Pharmacia) as described previously (8). The specific activity of the resulting mono ^{125}I-LHRH agonist varied from 990-1150 mCi/mg as determined by self-displacement of binding to isolated Leydig cells (8).

Measurement of Protein and DNA.

Protein was assayed by the method of Lowry et al.(9) using bovine serum albumin (BSA ; fraction V, Sigma) as standard whilst DNA was measured by the method of Burton (10).

Measurement of the binding of ^{125}I-LHRH agonist.

Unlabelled LHRH agonist or other hormones were dispensed into 63 x 11mm polystyrene tubes followed by ^{125}I-LHRH agonist (100,000cpm) in a volume of 0.05 ml. Aliquots of 0.25ml of placental preparation were then added to the tubes to give a final volume of 0.5 ml. Non-specific binding was determined in the presence of 10^{-5}M unlabelled LHRH agonist. All additions and dilutions were made using 25mM Sodium Phosphate buffer, 1mM Magnesium Chloride (pH 7.4). Hormone solutions also contained 0.5% BSA.

For the 1,000g placental fraction all incubations were performed in triplicate for 90 minutes at 20-22°C and terminated by adding 1ml of ice-cold phosphate buffered saline and centrifuging for 30 minutes at 1,000g. Radioactivity in the pellet was measured in a gamma counter.

The 10,000g and 100,000g membrane preparations were incubated in duplicate for 60 minutes at 20-22°C. 1ml ice-cold phosphate buffered saline was then added to each tube and the contents filtered under vacuum through glass fibre filters (Whatman GF/C) which had been soaked for more than 8h beforehand in 1% BSA. The filters were washed twice with buffered saline and radioactivity measured in a gamma counter.

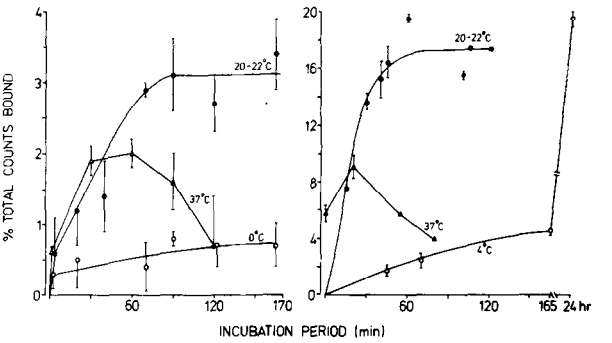

Fig. 1 Binding of ^{125}I-LHRH agonist to the 1,000g fraction (left hand panel) and 10,000g fraction (right hand panel) of term placenta at different temperatures. Each point represents the mean ± range of either duplicate or triplicate incubations and the results are representative of several experiments.

RESULTS

Initially the optimum conditions for assessing the binding of ^{125}I-LHRH agonist to term placentae were determined using tissue which had been stored at -20°C; freezing did not affect the binding activity of the placental tissue. At 20-22°C binding of ^{125}I-LHRH agonist to the 1,000g fraction reached equilibrium after 90 minutes whilst binding to the 10,000g fraction reached equilibrium after 60 minutes (Fig 1). At 4°C maximum binding was not reached until 24 hours of incubation, whilst at 37°C maximum binding was achieved rapidly but soon fell.

For the 1,000g fraction the non specific binding measured in the presence of 10^{-5}M LHRH agonist was 2-7% of the total counts added. The 10,000g and 100,000g placental fractions bound less than 5% of the total counts non specificially. Specific binding in the 1,000g and 100,000g fractions was up to 5% of the total counts whilst the 10,000g bound between 13 and 20% of the total counts added. Under equilibrium conditions the pM of ^{125}I-LHRH agonist bound/mg protein to the 1,000g, 10,000g and 100,000g fractions of placenta was 16.9 ± 5.8 (n=6), 41.2 ± 8.2 (n=3) and 11.3 ± 5.9 (n=2) respectively (mean ± S.D.).

The characteristics of binding of ^{125}I-LHRH agonist under equilibrium conditions were investigated using both the 1,000g and 10,000g preparations.

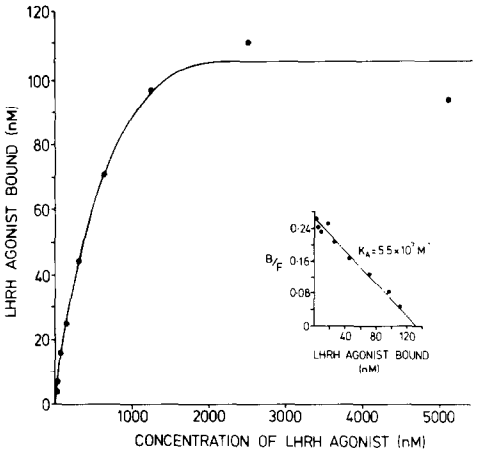

Fig. 2 Binding of ^{125}I-LHRH agonist to 10,000g fraction of term placenta at increasing concentrations of the LHRH agonist. The inset shows the data as a Scatchard plot. Each point is the mean of duplicate incubations. The graph is representative of several experiments.

Binding of LHRH agonist was saturable and to a single class of high affinity binding sites (Fig. 2). For the 1,000g fraction the mean (± S.D.) K_A of binding of the LHRH agonist in three experiments was 4.3 ± 0.7 x $10^7 M^{-1}$. Using the 10,000g fraction the K_A was 5.5 x $10^7 M^{-1}$ (Fig. 2) for LHRH agonist and 6.2 x $10^7 M^{-1}$ for native LHRH (Fig. 3). The binding sites were specific for LHRH agonist and native LHRH, and the 6-9 fragment of LHRH agonist inhibited binding by 20% at 5 x 10^{-5}M (Fig. 3). Oxytocin and somatostatin were largely inactive but gave some displacement of binding at 10^{-4}M. TRH and the 4-9 fragment of LHRH agonist were inactive.

Under similar conditions of incubation ^{125}I-LHRH agonist bound to preparations of 10-12 week placentae. Binding to the 1,000g fraction was compared in term and 10-12 week placentae and when expressed in pM ^{125}I-LHRH bound/mg protein there was little difference between them. However, when binding was expressed relative to the DNA content there was a significant difference ($P < 0.05$). The mean (± S.D.) of 5 observations for term placentae was 355.8 ± 138 pM ^{125}I-LHRH bound/mg DNA and of 4 observations in 10-12 week trophoblast was 163.7 ± 49 pM ^{125}I-LHRH bound/mg DNA.

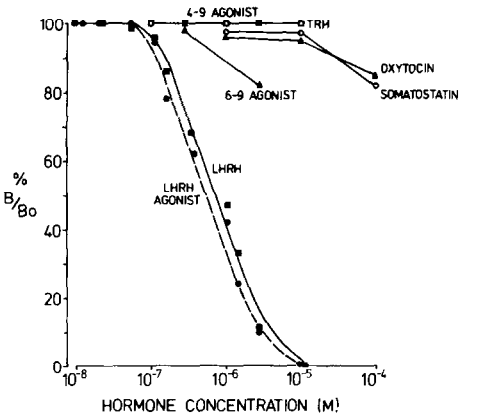

Fig. 3 Specificity of the binding of ^{125}I-LHRH agonist to the 10,000g fraction of term placenta. Each point is the mean of duplicate incubations. The 1,000g fraction gave similar results.

Binding to the 10,000g fraction of 10-12 week placentae with respect to protein was half that found in term placentae. However binding is difficult to quantify due to varying protein concentrations in different placental preparations. Binding was studied at varying protein concentrations (Fig. 4) and did not increase linearly with increase in protein concentration. The plateau observed suggests enzymic degradation of the radio-ligand at high protein concentrations.

Similar binding studies to those detailed above were performed using a 10,000g membrane fraction prepared from an homogenate of isolated vesicles from a single hydatidiform mole. Although the characteristics of binding were similar to that observed for normal term placentae, the mass of ^{125}I-LHRH agonist bound/mg protein was only 16% of binding to term placentae (6.6pM/mg).

DISCUSSION

These results demonstrate that human placentae of 10-12 weeks gestation and at term contain specific, high affinity receptors for LHRH and its agonists. Native LHRH was found to have the same affinity for the placental LHRH receptor as it has for LHRH receptors in pituitary cell membranes (11), rat luteal cells (12) and rat Leydig cells(8). However the LHRH agonist has an affinity 100 times higher in

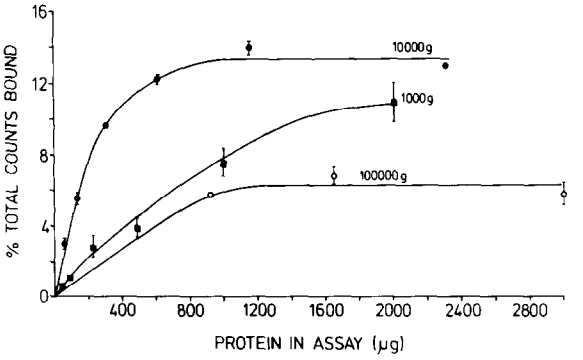

Fig. 4 Binding of ^{125}I-LHRH agonist to 1,000g, 10,000g and 100,000g fractions of term placenta at increasing protein concentration.

these other tissues than in the placenta where its activity is indistinguishable from that of native LHRH.

Native LHRH has five sites for enzymic degradation. By substituting two amino acids at position 6 and 10 the resulting LHRH agonist is protected from degradation at three of these five sites and it is these changes which are believed to increase its affinity for the LHRH receptor (11). LHRH degrading capabilities vary between tissues (13), although the placenta itself has not yet been studied. Possibly the placenta contains large amounts of a protease which attacks LHRH agonist at the sites which are not modified with the result that both the LHRH agonist and native LHRH are degraded similarly. This interpretation is supported by the present observation that the amount of ^{125}I-LHRH agonist bound/mg protein decreases as protein concentration increases making results difficult to quantify. A further possibility is that placental LHRH differs structurally from hypothalamic LHRH, in which case the placental LHRH receptor might have a higher affinity for the correct ligand and a lower affinity for both LHRH and the agonist. However, all of the evidence so far supports the view that placental LHRH is indistinguishable from hypothalamic LHRH (1-4) and further work remains to be done to elucidate the discrepancy between the placenta and other tissues in their differing affinity for the LHRH agonist.

In the testis and ovary, which also have receptors for LHRH and its agonists (8,12), it has been observed that chronic treatment with high doses of LHRH or its

agonists can have a direct inhibitory effect on hormone production (14, 15, 16). The present demonstration of LHRH receptors on the human placenta gives further support to a physiological role of LHRH in placental function. However, it remains to be established whether the placenta could also be a target for the inhibitory effects of chronic LHRH treatment.

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