Properties of the Luteinizing Hormone Receptor of Isolated Bovine Corpus Luteum Plasma Membranes*

(Received for publication, December 11, 1972)

DENIS GOSPODAROWICZ
From The Salk Institute for Biological Studies, P. O. Box 1809, San Diego, California 92112

SUMMARY

Luteinizing hormone binding sites with high affinity and specificity for ovine-luteinizing hormone have been shown to be present in a purified plasma membrane preparation obtained from bovine corpus luteum. The specific binding of $^{125}$I-luteinizing hormone to the membranes is a saturable process with respect to $^{125}$I-luteinizing hormone. Native luteinizing hormone competes for the binding in a way expected from the biological identity of the 2 molecules. Human chorionic gonadotropin and pregnant mare serum gonadotropin, two gonadotropins which have luteinizing hormone activity, compete for the binding site of luteinizing hormone, with an affinity which is less than that of native luteinizing hormone. The same holds for the $\alpha$ and $\beta$ chains of luteinizing hormone which showed, respectively, 100 and 200 times less affinity than luteinizing hormone for its binding site. Follicle-stimulating hormone, which does not have intrinsic luteinizing hormone activity, does not compete for the binding site of luteinizing hormone to an extent greater than its contamination by luteinizing hormone allows.

The binding of $^{125}$I-luteinizing hormone is temperature-dependent and reaches its maximum in 10 min at 37°C. The rate constant of the luteinizing hormone-membrane association ($2.17 \times 10^{6} \text{ M}^{-1} \text{s}^{-1}$) and dissociation ($2.46 \times 10^{-3} \text{ s}^{-1}$) have been measured independently at 23 and 10°C. The dissociation constant ($1.13 \times 10^{-9} \text{ M}$) based on these rate constants is similar to that ($3 \times 10^{-9} \text{ M}$) calculated separately from equilibrium data. Measurement of the rate constants at various temperatures gives similar values for the dissociation constant. This shows that the decrease in dissociation rate is proportionately the same as the decrease in association rate. Binding is maximal at pH 7.6 and is not affected by Ca$^{2+}$ concentration in the range of 0.1 to 20 mm. The effects of different enzymatic preparations on the binding site of luteinizing hormone have been investigated. It is not affected by DNase, trypsin, chymotrypsin, pepsin, and collagenase. Treatment of the membrane preparations by neuraminidase increased the binding capacity for luteinizing hormone by 2-fold. Phospholipase C, as well as phospholipase A, decreases it by half.

Study of the specific binding of $^{125}$I-luteinizing hormone to subcellular fractions of bovine corpus luteum has shown that the specific binding of luteinizing hormone is localized to the plasma membrane fraction (1). Since the disruption of cellular structures leads to the loss of biological activity, the interaction of LH with its receptor site can be best defined by looking at the binding of $^{125}$I-LH to the membranes. One should then make certain that this interaction is specific and that $^{125}$I-LH does not bind to structures other than its receptor site. The present report presents detailed data on the properties of the binding interaction between bovine corpus luteum plasma membrane and $^{125}$I-LH. The kinetic data for binding of $^{125}$I-LH to its receptor site will be further utilized for the isolation of the LH receptor site from plasma membrane of bovine corpus luteum.

EXPERIMENTAL PROCEDURE

Materials—Highly purified ovine LH is prepared by the method of Papkoff et al. (2) and is further purified by chromatography on diethylaminoethyl cellulose to remove contaminating thyrotropin-stimulating hormone as described by Pierce and Carsten (3). The biological activity measured by the ovarian ascorbic acid depletion assay is 275 units per mg (95% confidence limits 2.1 to 3.7) referred to the standard NIH-LH-S17. Thyroid-stimulating hormone activity amounts to 15 to 30 milliunits per mg. Contamination by growth hormone, follicle-stimulating hormone, adrenocorticotropin hormone, or melanocyte-stimulating hormone cannot be detected. Highly purified PMSG (15,000 i.u. per mg) is prepared by the method of Gospodarowicz and Papkoff (4) and Gospodarowicz (5). The isolation of the two subunits of ovine LH is affected in this laboratory by counter current distribution (12 transfers repeated twice) as described by Papkoff and Samy (6) and as modified by Reichert et al. (7).

Amino acid and carbohydrate analyses show the purity of the LH $\alpha$ and LH $\beta$ subunits (6) which is further confirmed by double diffusion in agar, immunoelectrophoresis, quantitative precipitation curves, steroidogenic activity (8), and physicochemical properties (9). Highly purified FSH is prepared by the method of Papkoff et al. (10). Its activity is 30 times the standard NIH-FSH-S1. Residual LH contamination is eliminated by chromatography on an anti-LH IgG Sepharose column, and amounts to 0.005% as determined by radioimmunoassay (11).

* This work was supported by grants from the Population Council (M.72.138C), the National Science Foundation (GB 34115), and the Rockefeller Foundation.

1 The abbreviations used are: LH, luteinizing hormone; PMSG, pregnant mare serum gonadotropin; FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin.
HCG (2500 units per mg) is obtained from Mann Research Laboratories, New York. Bovine serum albumin Fraction V is from Riker's Chemical Co., Chicago, Ill. Lactoperoxidase grade B is from Calbiochem, San Diego, Calif. \(^{125}\)I Na, carrier-free, is obtained from New England Nuclear, Boston, Mass.

Neuraminidase (Clostridium perfringens sp, specific activity 3.6), soybean trypsin inhibitor, trypsin, chymotrypsin, collagenase (1.5 units per mg), pepsin, and DNase are obtained from Worthington Biochemical Co., Freehold, N. J. Phospholipase A (from Vipera russelli and bee venom), hyaluronidase (type II), protease (Streptomyces griseus type VI) are from Sigma Chemical Co., St. Louis, Mo. Phospholipase C (15 units per mg) is from Nutritional Biochemicals Co., Cleveland, Ohio. Cellulose acetate filters EGWP (02500, 0.2 μ) are obtained from Millipore Co., Bedford, Mass.

**Iodination Procedure**—The iodination of LH is performed using lactoperoxidase as the catalytic agent. All reactions are carried out in small glass tubes at room temperature. The reagents are mixed continuously with a small magnetic stirrer. In order to minimize the introduction of more than 1 atom of iodine per LH molecule, iodination is performed with an equimolar ratio of \(^{125}\)I to LH molecule, iodination is performed with an equimolar ratio of \(^{125}\)I to LH, lactoperoxidase, and hydrogen peroxide is added rapidly in the following order and amounts: (a) 250 μCi of \(^{125}\)I Na in 5 μl of 0.1 N NaOH; (b) 588 ng of \(^{125}\)I Na in 100 μl of 0.1 N NaOH; (c) 50 μl of 1 M potassium phosphate buffer, pH 7.3; (d) 120 μg of LH in 50 μl of 0.25 M potassium phosphate buffer, pH 7.3; (e) 5 μg of lactoperoxidase in 5 μl of the same buffer. The reaction is initiated by adding 70 μg of hydrogen peroxide in 10 μl of water. To sustain the reaction, 70 μg of hydrogen peroxide are added four times at intervals of 2 min. At 1-min intervals starting at zero time, 5 μl of the iodinated solution are withdrawn, mixed with 0.1 ml of 0.1% bovine serum albumin (w/v), and precipitated with 10% trichloroacetic acid. Precipitates are washed twice with cold 10% trichloroacetic acid, dissolved in 0.2 ml of 0.2 N KOH, and their radioactivity determined in a liquid scintillation counter (Nuclear Chicago model Unilux II). Usually in 10 min, 90 to 95% of the \(^{125}\)I trichloroacetic acid-precipitable. When iodinated LH of high specific activity is applied on an anti-LH IgG-Sepharose column (11), 93% is retained on the column, thus proving that the antigenic sites of LH are intact (Fig. 1C). Ninety per cent of the adsorbed \(^{125}\)I-LH is eluted with 6 M guanidine HCl, pH 1.5. The unadsorbed fraction may represent either iodinated peroxidase or denatured LH. When the biological activities of LH and \(^{125}\)I-LH are compared by the ovarian ascorbic acid depletion assay, their activities are indistinguishable (Table I). Their ability to stimulate progesterone biosynthesis (Fig. 2A), as well as release of progestins in vitro from bovine luteal cell suspension (Fig. 2B), is also identical over a 1000-fold range of concentration.

These different criteria indicate that iodination of bovine LH using the lactoperoxidase as a catalytic agent produces no noticeable denaturation of the hormone.

The final activity of ovine LH is 1800 Ci per mm, corresponding to an average incorporation of 1 atom of \(^{125}\)I per molecule of LH. With 90% incorporation of \(^{125}\)I into LH, no further purification of LH by ion exchange chromatography is required to separate LH from \(^{125}\)I-LH.

By increasing the concentration of \(^{125}\)I, an average of more than 1 atom of \(^{125}\)I can be incorporated by a molecule of LH. Since a decrease in biological activity of ovine LH is observed when more than 2 atoms of iodine are incorporated into each molecule.
of LH, the iodination procedure has been designed to limit the incorporation of iodine to 1 atom per molecule.

The conditions of iodination described by Miyachi et al. (18) for the iodination of human LH or HCG using lactoperoxidase as a catalytic agent are not satisfactory for ovine LH. With their conditions, 90% of the ovine LH is denatured and aggregated. This is mostly secondary to the pH of 5.0 at which the iodination is performed. In contrast to human LH or HCG, ovine LH dissociates easily at acidic pH (17) and iodination of tyrosine in the α chain, as well as in the β chain, occurs resulting in denaturation of ovine LH. Furthermore, high concentration of hydrogen peroxide has a deleterious effect on the biological activity of ovine LH (19) even though it may not have any effect on HCG or human LH.

Preparation of Plasma Membrane Fraction from Bovine Corpus Luteum—Plasma membrane fraction is prepared as described by Gospodarowicz (11). Fractions F1 and FI1 are used as a source of plasma membrane. Proteins are determined according to the method of Lowry et al. (14) using bovine serum albumin as a standard.

Binding Assay—The assay for specific binding of \(^{125}\text{I}-\text{LH}\) to membranes is a slight modification of that used to measure specific binding of insulin to intact fat cells (20, 21) and fat cell membrane (22).

Briefly, membranes are incubated at 23° to equilibrium in 0.2 ml of Krebs-Ringer-bicarbonate buffer containing 1% albumin (w/v) and \(^{125}\text{I}-\text{LH}\) (10–11 to 10–7 M). Three milliliters of ice-cold Krebs-Ringer bicarbonate buffer containing 1% albumin (w/v) are added, and the contents are passed through cellulose acetate EGWP filters positioned with vacuum. The filters are washed under vacuum with 3 times 3 ml of ice-cold Krebs-Ringer bicarbonate buffer containing 1% albumin (w/v). Every determination of binding is performed in triplicate, and for every such determination parallel, triplicate samples are performed in the presence of native LH (100 μg per ml) to determine the correction for nonspecific binding of LH. As it has been stressed by others (22), it is imperative that such corrections be performed in order to determine "specific" LH binding accurately. Nonspecific binding to EGWP filters in the absence of membranes is 0.3% of the input. Plasma membranes which have been heated to 90° for 5 min do not bind more than 0.5% of the input. The filters are dissolved in 15 ml of Bray’s scintillation fluid containing 4% Cab-O-Sil and counted in a liquid scintillation counter as described. Counting efficiency is 60%.

Enzyme Effect—Specific procedures used for digestion of plasma membranes with enzymes are described in the appropriate tables. Generally, the membranes are incubated with the enzyme in Krebs-Ringer bicarbonate buffer containing 1 to 0.1% albumin (w/v) for 10 to 20 min at 37°, then washed twice by centrifugation using the same buffer (18,000 x g for 10 min). The pellet is resuspended in Krebs-Ringer bicarbonate buffer containing 1% albumin (w/v), and binding of \(^{125}\text{I}-\text{LH}\) is then performed.

RESULTS

Binding as Function of \(^{125}\text{I}-\text{LH}\) Concentration—The specific binding of \(^{125}\text{I}-\text{LH}\) to purified plasma membranes obtained from bovine corpus luteum is a saturable process with respect to \(^{125}\text{I}-\text{LH}\) concentration (Fig. 3). Specific binding of \(^{125}\text{I}-\text{LH}\) can be detected at a concentration as low as 3 μg per ml (1 x 10–10 M), and saturation is obtained at 300 μg per ml (1 x 10–8 M).

Displacement of Bound \(^{125}\text{I}-\text{LH}\) by Native LH and Other Gonadotropins—\(^{125}\text{I}-\text{LH}\) specifically bound to plasma membrane is displaced by increasing concentrations of native LH in a fashion predicted by the near-identity of these 2 molecules (Fig. 4). Other polypeptide hormones which possess LH activity such as HCG and PMSG are able to compete for the receptor site of LH. However, their affinity is about 10 times less than that of LH. Of the two gonadotropins, HCG is the most potent (Fig. 5). Hormones which are free of LH activity such as FSH (Fig. 6) compete very poorly for the LH binding site. Its affinity appears to be 10,000-fold less than that of LH and can be explained by LH contamination, which is 0.005%; on the basis of radioimmunoassay, LH α and LH β, the two subunits of LH, have, respectively, 50- and 100-fold less activity on a weight basis than LH, thus confirming the fact that to get full steroidogenic effect the two subunits of LH must be combined (8).

Binding as Function of Plasma Membrane Concentration—The binding of \(^{125}\text{I}-\text{LH}\) to plasma membrane is directly proportional to the plasma membrane concentration over the range of concentrations that can be used in these procedures (Fig. 7A). This linear relationship in the absence of saturating concentration, reflects the high affinity of the interacting species and can be
FIG. 3. Specific binding of \(^{125}\)-LH to bovine corpus luteum plasma membranes as a function of the concentration of LH. Plasma membranes (100 \(\mu\)g of protein) are incubated at 23\(^\circ\) for 20 min, in 0.2 ml of Krebs-Ringer bicarbonate buffer, 1% albumin (w/v), and various concentrations of \(^{125}\)-LH. Specific binding and correction for nonspecific adsorption of LH is determined as described in the text.

compared to the early portion of the curve of Fig. 3 (10\(^{-10}\) to 10\(^{-9}\) M). It is impossible to achieve high enough concentrations of plasma membrane to see a plateau of binding since at high membrane concentration the filter clogs.

**Binding as Function of Time**—The specific binding of \(^{125}\)-LH to plasma membrane of corpus luteum at 23\(^\circ\) reaches a maximum at 12 min and then stays constant. In contrast, nonspecific binding reaches a maximum at 4 min (Fig. 7B).

**Effect of Temperature on Rate Constant of \(^{125}\)-LH Receptor Interaction**—The rate of binding of \(^{125}\)-LH to its receptor increases with increasing temperature. It reaches a maximum at 37\(^\circ\) (Fig. 8). The rate constants of association can be calculated from these data since they obey second order kinetics. At 23\(^\circ\) the rate of association \(k_1\) is 2.17 \(\times\) 10\(^{9}\) M\(^{-1}\) s\(^{-1}\) while at 10\(^\circ\) it is 4.2 \(\times\) 10\(^{8}\) M\(^{-1}\) s\(^{-1}\). The rate of dissociation decreases with decreasing temperature (Fig. 8). The dissociation data obey first order kinetics, and the half-life of the complex is 5 min at 23\(^\circ\) and 16 min at 10\(^\circ\). At 23\(^\circ\) the dissociation constant is 2.4 \(\times\) 10\(^{-3}\) s\(^{-1}\), while at 10\(^\circ\) it is 7.18 \(\times\) 10\(^{-4}\) s\(^{-1}\). Values of the equilibrium constant indicate similar binding of LH to its receptor at low temperatures as compared to high temperatures (Table II).

The dissociation constant has also been computed from equilibrium data. With increasing concentration of \(^{125}\)-LH the hormone binding to plasma membrane follows the equation \(Y = \frac{S_{LM} U}{U + 1/K_{LM}}\) where \(S_{LM}\) is the number of LH binding sites, \(U\) is the unbound LH, and \(K_{LM}\) is the equilibrium constant of the LH-receptor interaction. \(K_{LM}\) is measured as 3.4 \(\times\) 10\(^{9}\) M\(^{-1}\) and \(S_{LM}\) 474 fmoles per mg of membrane protein (Fig. 9A). Binding data can also be plotted in the form of a Scatchard
FIG. 7. A, specific binding of 125I-LH to plasma membranes of bovine corpus luteum as a function of the concentration of plasma membranes in the medium. The incubation medium contains the various amounts of plasma membranes in 0.2 ml of Krebs-Ringer bicarbonate buffer, 1% albumin (w/v), and 1 x 10^{-9} M 125I-LH. After incubation for 20 min at 23°C, the specific binding (O-O) and nonspecific binding (O-O) of LH is determined as described in the text. B, time dependence of specific (O-O) and nonspecific (O-O) LH binding to plasma membranes of bovine corpus luteum. The incubation medium containing 150 pg of plasma membrane in 0.2 ml of Krebs-Ringer bicarbonate buffer, 1% albumin (w/v), and 1 x 10^{-9} M 125I-LH. Specific and nonspecific binding is determined at various times as described in the text.

plot (Fig. 9B). This requires the assumption that the steady-state in binding is achieved after 15 min at 23°C. From the slope of such a plot an apparent dissociation constant of 3 x 10^{-9} M is obtained which agrees well with the dissociation constants computed by double inverse plot or computed directly from the rate of association and dissociation of the 125I-LH-receptor complex.

Effect of Salts and pH on LH Binding—The effect of different ionic constituents of the Krebs-Ringer bicarbonate buffer on the binding of 125I-LH to plasma membrane of corpus luteum is shown in Table III. Ca^{2+} has no effect on the binding of 125I-LH to its receptor between 1 and 10 mM, but concentrations higher than 20 mM reduce the binding by 20%. When NaCl is present in a concentration higher than 0.5 M, the binding capacity is considerably reduced, and at 2 M no binding of 125I-LH is observed. KCl has the same effect. The specific binding of 125I-LH to plasma membrane occurs over a relatively narrow range of pH (Fig. 10). Maximum binding is observed at pH 7.4 and 7.6. At pH 6 or 8 the binding capacity is only 30% of the binding capacity observed at pH 7.6. However, the low binding capacity of plasma membrane at low pH values such as pH 5 or high pH values such as pH 9 is fully reversible since incubation of plasma membrane at those pH values followed by washing and resuspension in pH 7.6 fully restores their capacity to bind 125I-LH.

Effect of Enzymes on LH Binding—The effects of several enzyme preparations on the binding of 125I-LH to plasma membranes of corpus luteum are shown in Table IV. Purified preparations of trypsin, chymotrypsin, and pepsin do not affect it. Digestion of plasma membrane preparations with neuraminidase increases the binding capacity of the plasma membrane, while digestion with phospholipase lowers it to half the control value. Collagenase, one of the enzymes we used to dissociate luteal cells
The pH of all buffers is adjusted at 7.4. Results are expressed in femtomoles per mg of protein.

**Table III**

Effect of various salts on specific binding of $^{125}$I-LH (m) to plasma membranes of bovine corpus luteum

| Salt conditions | Specific binding $^{125}$I-LH |
|-----------------|------------------------------|
| 0.1 M Tris-HCl  | 100 ± 1.3                    |
| + 1 mM CaCl$_2$ | 96 ± 1.4                     |
| + 10 mM CaCl$_2$| 100 ± 1.5                    |
| + 20 mM CaCl$_2$| 88 ± 1.8                     |
| 0.1 M Sodium phosphate | 100 ± 1.2 |
| + NaCl 0.15 M  | 104 ± 1.2                     |
| + NaCl 1 M     | 50 ± 1.2                     |
| + NaCl 2 M     | 24 ± 1.2                     |
| + KCl 0.15 M   | 17 ± 1.2                     |
| + KCl 2 M      | 20 ± 1.8                     |

**Table V**

Effect of proteolytic enzymes, hyaluronidase, collagenase, and neuraminidase on binding of $^{125}$I-LH (m) to plasma membranes of bovine corpus luteum

| Enzyme             | Specific $^{125}$I-LH binding |
|--------------------|-------------------------------|
| Control            | 102 ± 9                       |
| Trypsin (100 µg per ml) | 107 ± 8                      |
| Chymotrypsin (100 µg per ml) | 98 ± 7                       |
| Pepsin (100 µg per ml)          | 102 ± 8                      |
| Protease (100 µg per ml)        | 64 ± 4                       |
| Collagenase (250 µg per ml)     | 98 ± 8                       |
| Hyaluronidase (100 µg per ml)   | 74 ± 6                       |
| DNase (100 µg per ml)           | 112 ± 8                      |
| Neuraminidase (100 µg per ml)   | 224 ± 3                      |

**DISCUSSION**

Study of the interaction of LH with its receptor site has been made possible by obtaining preparations of $^{125}$I-LH with high specific activity (1800 Ci per m& of) which exhibits normal immunological and biological activity. Our earlier attempts to study the interaction of $^{125}$I-LH with its receptor site failed because we had labeled the hormone using the chloramine T method. Even though we have attempted to minimize the damage to the proteins caused by chloramine T by working with an equimolar ratio of chloramine-T in L-H, the specific activity of the hormone (300 Ci per m& of) is low, as is the incorporation of $^{125}$I (11). To raise the specific activity of $^{125}$I-LH, chromatography on a diethylaminoethylcellulose is required to separate noniodinated from iodinated molecules. This purification step is time-consuming and difficult to do in view of the quantity of radioactivity manipulated. Also, even though the immunological activity of the final product is not greatly altered (except that the precipitin curve of the iodinated LH does not coincide with that of LH), its biological activity is on an average 20% lower than that of LH, and when binding studies are conducted, saturation of sites even at high concentrations of LH (10$^{-7}$ to 10$^{-6}$ M) is not observed. So denaturation, which is manifested in only small changes in immunological or biological activity, results in large amounts of spurious binding. Also, in the case of human LH or HCG, when chloramine T is used as an iodinating agent, the ratio of chloramine T to hormone, the length of exposure, and the temperature are critical factors for the iodination of human LH or HCG if binding studies are to be conducted with it after-
ward (23). Since ovine LH is a much more fragile molecule than human LH or HCG in that its two subunits are easily dissociated by mildly acidic conditions, it is not astonishing that the damage done by exposing ovine LH to chloramine T is more extensive than for HCG or human LH. Using lactoperoxidase as a catalytic agent we have, in contrast to the chloramine T method, been able to incorporate 90 to 95% of the 125I into LH. Since we are working in an equimolar ratio of iodine to LH, this means that on the average 1 molecule of LH contains 1 molecule of 125I. The immunological and biological properties of 125I-LH are similar to those of the native hormone and when binding studies of 125I-LH with its receptor, present in plasma membrane of corpus luteum, are investigated, saturability of the receptor site is obtained.

Evidence for the specificity of the LH-cell interaction is further strengthened by the failure of several polypeptide hormones to compete with LH for binding to cells. We observe no displacement of 125I-LH (2 × 10^{-10} M) by growth hormone, prolactin, adrenocorticotropin, or glucagon at concentrations as high as 3 × 10^{-8} M. However, native LH is able to compete with 125I-LH for binding. Two other gonadotropins which possess LH activity, HCG and PMSG, are able to compete for the receptor site with an activity 10 times less than that of ovine LH, hardly an astonishing fact since we are working with a homologous system. Ovine LH and bovine LH have been shown to be similar, and the receptor site of plasma membrane of bovine corpus luteum should then have a higher affinity for ovine LH than for gonadotropin of unrelated species such as human or horse. The binding competition by LH α and LH β has also been analyzed. It is known that LH α and LH β have a low stereogenic activity (9) and ascorbic acid depletion activity (6, 8, 24, 25) when compared to LH. This is reflected in their low binding affinity for the LH receptor site present in plasma membrane fraction of bovine corpus luteum. Their ability to compete with LH is 2% for LH α and 1% for LH β. These values represent, respectively, a 100- and 200-fold molar excess over LH and suggest that neither subunit alone is sufficient to convey significant receptor activity. The binding activity of LH α and LH β is comparable to the values reported by us for their stereogenic activity in bovine corpus luteum (8) and similar to their activity in the radioligand receptor assay2 (23). The competition of ovine FSH is negligible. Displacement is observed only at a 10,000-fold excess of FSH over LH and most probably reflects the residual contamination of this hormone by LH. Kammerman et al. (26), looking at the competitive binding of HCG and FSH in porcine granulosa cells, have reported similar data.

Of special interest to us is the effect of different enzymes on the binding of 125I-LH to its receptor in plasma membrane fraction of bovine corpus luteum. We have recently reported the isolation and the maintenance in tissue culture (26) of metabolically active bovine luteal cells, obtained from corpus luteum by an enzymatic treatment (16). Of the three enzymes that we are using for the cellular dissociation, two, collagenase, and trypsin, do not affect the binding site of LH. The third, hyaluronidase, reduces the binding affinity for LH by a third. While it is not yet clear whether this effect is due to proteolytic activity present in this crude enzyme preparation or to hyaluronidase itself, it is evident that its use for cell dissociation presents some dangers if one wants to study the binding of LH to luteal cells. However, the kinetics of binding 125I-LH to cell suspensions obtained from corpus luteum shows the same characteristics3 as those described for the binding of 125I-LH to plasma membrane. It is possible that the hyaluronidase can affect many more receptors in isolated plasma membranes than it can in intact cells when they are associated together or in suspension. Among the other enzymes which reduce the binding capacity of plasma membrane is lecithinase, which reduces it by half. A similar effect of lecithinase is observed on the binding of glucagon to plasma membranes of rat liver (28). In contrast, it has been reported that the binding of insulin to fat cell membrane is increased 3-fold after treatment by lecithinase (29). Among the enzymes which increase the binding capacity of bovine plasma membrane fraction is neuraminidase, which gives a 2-fold increase.

The dissociation constant for LH in bovine plasma membrane calculated from equilibrium kinetics (3 × 10^{-9} M) is comparable to that (1 × 10^{-9} M) calculated from the ratio of the rate constants. Both of these are similar to the values obtained for the stimulation of progesterone release in bovine luteal cells (9). The association of 125I-LH to membrane is a rapid phenomenon which takes no more than 12 min to be completed at 23°. The rate of dissociation at the same temperature is also rapid, and the half-life of the complex is 5 min at 23°. These results are in contrast with those of Catt et al. (30) who have studied the binding of another gonadotropin (HCG) to a testis homogenate. They find that the uptake of 125I-HCG at 24° continued to increase for 24 hours. The half-life of the complexes at 20° is also of the same order of time. The association rate constant calculated from his data for the initial reaction velocity is 10 times lower than the association rate constant for the binding of 125I-LH to receptor site in plasma membrane of corpus luteum (12.7 × 10^{6} M^{-1} s^{-1} versus 2.17 × 10^{5} M^{-1} s^{-1}). However, due to the slow rate of dissociation of the complexes the equilibrium constant (6 × 10^{-9} M) determined from the association and dissociation rate constants is similar to the one we find (1 × 10^{-9} M). The dissociation constant of the LH-receptor complex can also be compared to that found by Leidenberger and Reichert for the binding of HCG to rat testicular homogenate (3 × 10^{-10} M) (10). In the case of lutinizing hormone, the specific binding has been studied by others using Leydig tumor cells with radioimmunooassay as a means to detect the specific binding of LH (31) or ovarian slices (32). In the case of the Leydig tumor cells a binding constant of 1.5 × 10^{-9} M was obtained, and for the ovarian slices a value of 3.6 × 10^{-8} M was given. These results obtained by different laboratories can be greatly affected by the systems used, the method to determine the binding of the hormone to its receptor, and the way the hormone is labeled. In this context, kinetic studies done with plasma membrane fractions, where proteolytic activity ordinarily present in crude extracts is minimized and where we can eliminate the possibility of nonspecific binding to intracellular structure membrane and to connective tissue structures, have a definite advantage over those done with crude homogenate and slices of organs. Also, the method we use permits direct and quantitative observations of complex formation between LH and its receptors at concentrations of hormone normally present in biological fluid. Direct measurement of the dissociation equilibrium constant is possible, based on the rate constant for association and dissociation, rather than indirect measurement based on equilibrium conditions which give apparent dissociation constants.

The interaction of LH with its receptor sites present in plasma membrane is identical with that found with isolated bovine luteal cells and gives us reasonable assurance that this interaction is biologically significant and represents the initial interaction that leads to the activation of the adenylate cyclase present in
plasma membrane. Despite the loss of biological activity when the cell is disrupted, the specific binding of LH to its receptor site should permit its identification during the purification of the receptor sites which will eventually lead to its isolation.

Note Added in Proof—Since this manuscript was submitted for publication Channing and Kammerman (33) have described the characteristics of gonadotropin receptors of porcine granulosa cells and Lee and Ryan (34) have reported the binding of human LH to homogenates of luteinized rat ovaries. \(K_D = 7.9 \times 10^{-10} \text{ M}\) was found.

Acknowledgments—I express appreciation to Mrs. F. Gosподірович and to Mr. P. Curtiss for excellent technical assistance. I thank the Talone Meat Co. for providing the bovine corpora lutea. Without their cooperation this work would not have been possible. I also thank Dr. K. Jones for his invaluable assistance and constructive criticism of the manuscript drafts.

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Denis Gospodarowicz

J. Biol. Chem. 1973, 248:5042-5049.

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