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# Preferential Establishment of a Slowly Dissociable Component in Plasma Membrane Compared to Intracellular Prolactin Receptors (41549)

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*Abstract.* Prolactin dissociates more readily from rat liver than from rabbit mammary prolactin receptors. The rate of dissociation is dependent in the time of association. In rat liver, prolactin dissociates from receptors at the cell periphery (plasma membrane, PM) more rapidly than those of microsomes, a major component of which is the intracellular Golgi membranes. The dissociation curves following 1 hr of association can be resolved into a fast and slow component by logarithmic transformation, with a greater than twofold increase in the fast componment of the dissociation rate constant ($k_2$) in PM compared to microsomal membranes. With longer association times (10 hr), plasma membranes develop a more slowly dissociable component, with dissociation characteristics (rate constants) similar to those observed in microsomes following 1 hr of association. Another means of visualizing this difference is by a Scatchard plot of prolactin binding to PM, microsomal, and Golgi membranes. The affinity constants in the microsomal and Golgi fractions are identical whereas that for the PM fractions more than twofold lower. The decreased affinity in the PM would allow prolactin to more readily dissociate from its receptor than from receptors with higher affinity. Although the differences between PM and Golgi receptors observed are small, they are confirmed by direct measurements of affinity constants and dissociation rate constants. Therefore, it appears that receptors with lower affinity at the cell periphery are those involved in the initial mechanism of action of prolactin.

The interaction of a hormone with its receptors is the initial event leading to the formation of a hormone receptor complex. Under equilibrium conditions, this reaction has been considered to be freely reversible (1, 2). However, for an increasing list of hormones, including PRL, hGH, TSH, insulin, LH, and $\beta$-adrenergic (3–10), it appears that dissociation of the hormone receptor complex is difficult and that a slowly reversible complex is established with an increase in the time of association of the hormone with its receptor.

The present studies were undertaken to examine differences in prolactin receptor dissociation between two target tissues, rat liver and rabbit mammary gland, and to compare the rate of dissociation between various subcellular binding components.

**Materials and Methods.** *Animals.* Mammary glands of 6-day lactating rabbits and livers from ovariectomized female Sprague-Dawley rats weighing 200 g, treated for 7 days with 5 $\mu$g estradiol-17$\beta$, were removed. CB-154 (Sandoz, Basel) was injected just prior to sacrifice in both rabbits and rats to increase receptor content (11).

*Membrane preparation.* Crude microsomes were prepared from rabbit mammary glands and rat liver by differential centrifugation (10, 12). Purified plasma membranes (PM) were prepared by the method of Ray (13) and Golgi membranes by the method of Bergeron *et al.* (14). The purity of the subcellular fractions was assessed by determining 5′-nucleotidase (15) and galactosyl transferase (16) activities, marker enzymes for plasmalemna and Golgi membranes, respectively. Protein was measured by the Lowry method (17) using bovine serum albumin (BSA) as a standard.

*Hormones.* Ovine prolactin (oPRL, NIH-P-S13, 35 U/mg) was kindly provided by the Hormone Distribution Program of the NIAMDD, NIH.

*Iodination of oPRL.* $^{125}$I-oPRL was prepared utilizing low concentrations of chloramine-T (500 ng) and 5 $\mu$g of oPRL and purified on a column of Sephadex G-75 (18). The specific activity ranged from 40 to 60 $\mu$Ci/$\mu$g. The integrity of the labeled hormone

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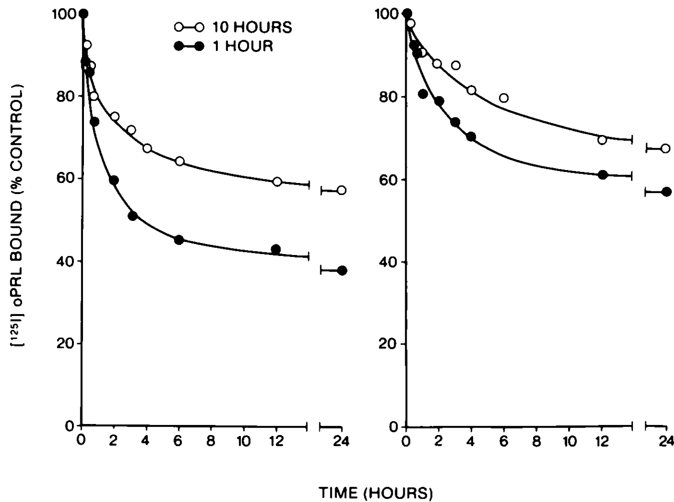


FIG. 1. Dissociation of $^{125}$I-oPRL from microsomal membranes of rat liver (left panel) and rabbit mammary gland (right panel) at 23° following either 1 or 10 hr of association of the labeled hormone with the receptor. Specific binding at time 0 for rat liver is 7520, and 18,970 cpm for 1 and 10 hr association, respectively; and for rabbit mammary microsomes, specific binding is, respectively, 17,300 and 38,500 cpm for 1 and 10 hr of association.

at the outset of the studies was verified with a laboratory control receptor preparation of rabbit mammary gland. Labeled prolactin bound >25% of the added hormone with 200 μg of membrane protein and >75% in the presence of excess membranes. Iodinated hormone dissociated from membrane fractions retained its capacity to bind to control receptor preparations.

*Assay and dissociation procedures.* $^{125}$I-oPRL (approximately 100,000 cpm) was incubated with rabbit mammary gland or rat liver membranes (200 μg) for either 14 or 16 hr at 23°, which is the time required for maximal binding to be obtained or for the times indicated in the figures and tables. The final volume was 0.5 ml in an assay buffer of 25 m*M* Tris, containing 10 m*M* $MgCl_2$, and 0.1% BSA. Duplicate tubes, two in the absence and two in the presence of excess (1 μg) oPRL, were used. Bound and free hormones were separated by centrifugation at 2300*g* for 15 min following the addition of 3 ml assay buffer to each tube. Specific binding was the difference between the total counts per minute (cpm) and nonspecific cpm and expressed as cpm or a percentage of the total radioactivity added.

Dissociation (at 23°) of prolactin from its receptor was initiated in tubes in which the labeled prolactin had been allowed to associate with the receptor for 1 or 10 hr by the addition of 3 ml assay buffer containing 10 μg/ml oPRL. The dissociation reaction was stopped by placing the tubes in ice water and immediately centrifuging them at 2300*g* for 15 min at 4°.

Tubes were counted in a LKB gamma spectrometer with a counting efficiency of 65%. For dissociation experiments, results are expressed as a percentage of the radioactivity bound at time 0 (% of control). Affinity constants and binding capacities (N) were calculated by Scatchard analysis (19) under equilibrium conditions.

**Results.** The difference in the dissociability of prolactin from rat liver and rabbit mammary gland prolactin receptors is shown in Fig. 1. It can be seen that $^{125}$I-oPRL dissociates more readily from rat liver (left panel) than from rabbit mammary prolactin receptors as has been previously reported (3, 4, 20). In addition, the longer that a labeled hormone is allowed to associate with its receptor, the more tightly it appears to be bound resulting in the decreased ability to dissociate. This relationship has been established for a number of hormone–receptor systems (3–10).

In rat liver, where dissociability can be more readily measured, there is a marked difference

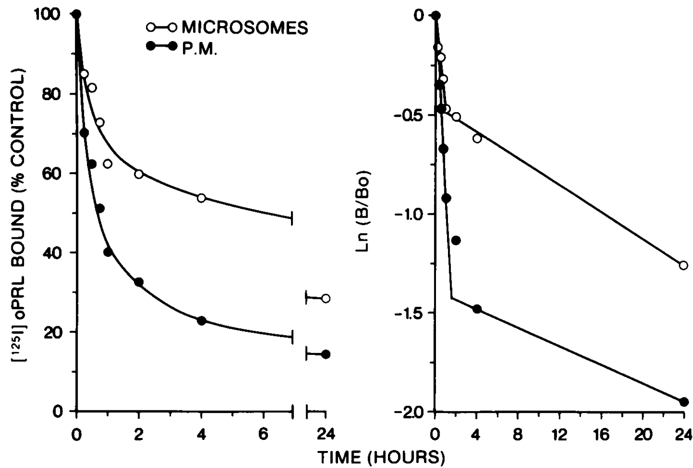


FIG. 2. Dissociation of $^{125}$I-oPRL from rat liver microsomes and purified plasma membranes (PM) at 23° following 1 hr of association. The left panel shows the dissociation as a percentage of specific binding at time 0 (after 1 hr of association) which is 11,180 and 8980 cpm for microsomes and PM, respectively. The right panel shows the data transformed to linearize the curve. The slope of these curves is used to calculate the dissociation rate constant ($k_2$).

in the ability of prolactin to dissociate from receptors normally found at the cell periphery (plasma membrane) than from microsomes, of which a major component in rat liver preparations is the intracellular Golgi membranes (14, 16). Figure 2 shows that $^{125}$I-oPRL dissociates from plasma membrane receptors much more readily than from microsomes following 1 hr of association.

The dissociation curves, which can be resolved into fast and slow components based on logarithmic transformation (21), reveal a greater than twofold increase in the faster component of the dissociation rate constant ($k_2$) in plasma membranes compared to microsomes with no change in the slow component (Table I).

However, with increased association times, plasma membranes preferentially develop a slowly dissociating component. Figure 3 shows a similar dissociation study carried out with membranes which have been allowed to associate with labeled prolactin for 10 hr. The difference between plasma membranes and microsome component is less apparent. It appears that with longer periods of association (10 hr), prolactin receptors in plasma membranes develop dissociation characteristics similar to those observed in microsomes following just 1 hr of association. This shift is confirmed in Table I.

The differerences in dissociation rate constants can be seen by other means. Figure 4 shows a Scatchard plot of PRL binding in

TABLE I. DISSOCIATION RATE CONSTANTS ($k_2$) OF $^{125}$I-oPRL FROM RAT LIVER MICROSOMES AND PURIFIED PLASMA MEMBRANES AS A FUNCTION OF TIME THE LABELED LIGAND WAS ALLOWED TO ASSOCIATE WITH THE BINDING SITES

| | Rate constant, $k_2$ (hr$^{-1}$) | | | |
|---|---|---|---|---|
| | 1-hr association | | 10-hr association | |
| Cell fraction | Fast component | Slow component | Fast component | Slow component |
| Microsomes | 0.44 | 0.03 | 0.34 | 0.02 |
| Plasma membranes | 0.91 | 0.02 | 0.38 | 0.03 |

*Note.* Rate constants were calculated from the dissociation data shown in Figs. 2 and 3 (21). These data are the mean of two separate dissociation experiments.

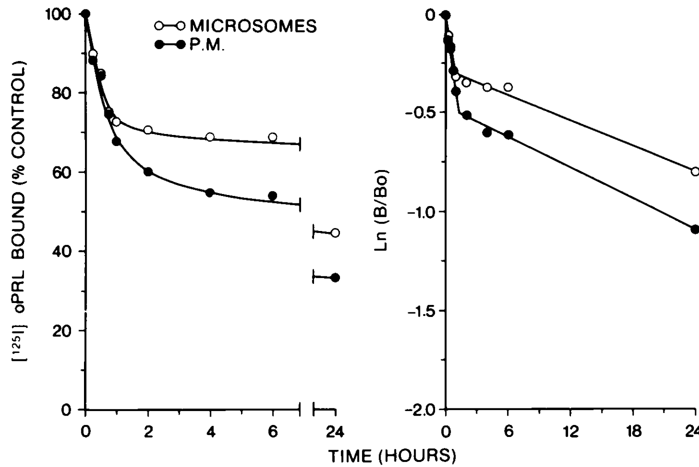


FIG. 3. Dissociation of $^{125}$I-oPRL from rat liver microsomes and purified plasma membranes (PM) at 23° following 10 hr of association. Conditions are as described in legend to Fig. 2. Specific binding at time 0 (after 10 hr of association) is 25,800 and 18,380 cpm for microsomes and PM, respectively.

microsome, plasma, and Golgi membranes. As can be seen in Table II, the plasma membrane fraction contains the greatest concentration of 5′-nucleotidase activity and the Golgi intermediate is by far the richest in galactosyl transferase, the affinity constants in microsomes and Golgi fractions are identical, whereas the affinity constant for plasma membranes is slightly more than twofold lower. The decreased affinity in PM would allow prolactin to more readily dissociate from its receptor than from receptors with higher affinities.

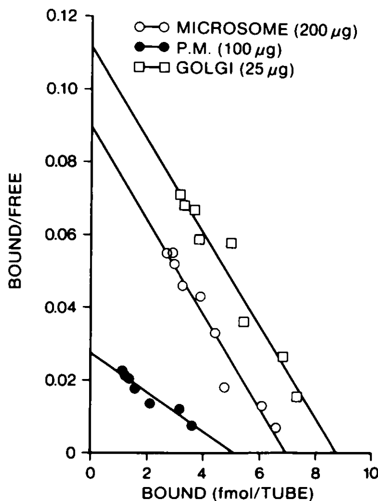


FIG. 4. Scatchard plots of prolactin binding to microsome, plasma membrane (PM), and Golgi membrane fractions.

**Discussion.** Prolactin more readily dissociates from receptors in rat liver than from receptors in rabbit mammary gland (Fig. 1) as we and others have previously observed (3, 4, 11, 12, 20). As well, there appears to be an increase in affinity with longer association times for mammary receptors as occurs for rat liver receptors. However, since lactating mammary gland preparations are greatly enriched in Golgi membranes (22) and it is difficult to obtain purified plasma membrane fractions from the mammary gland, further comparisons regarding differences between rat liver and rabbit mammary gland have not been made.

These studies could have important implications in terms of cellular function. A very large percentage of prolactin receptors in rat liver is located within the cell (23). The present results confirm the differential subcellular localization of PRL receptors and suggest that receptors in the PM and Golgi are in different forms, with the affinity of the receptor being dependent on the subcellular localization.

Prolactin receptors are down-regulated both *in vivo* and *in vitro* (24, 25) with a passage of the hormone-receptor complex inside the cell (23). The present data suggest that internalization of the hormone-receptor complex is preceded by a tightening of the linkage between the hormone and its receptor, as has

TABLE II. BINDING CONSTANTS OF PROLACTIN RECEPTORS FROM DIFFERENT CELLULAR FRACTIONS OF RAT LIVER CALCULATED BY SCATCHARD ANALYSIS

| Cell fraction | 5′-Nucleotidase (μmole/hr/mg protein) | Galactosyltransferase (nmole/hr/mg protein) | $K_a$ ($nM^{-1}$) | $K_d$ ($nM$) | $N$ (fmole/mg protein) |
|---|---|---|---|---|---|
| Microsomes | 2.6 | 20.0 | 6.4 | 0.16 | 34.7 |
| Golgi intermediate | 14.1 | 178.0 | 6.4 | 0.16 | 348.4 |
| Plasma membrane | 34.1 | 6.3 | 2.6 | 0.38 | 50.8 |

*Note.* 5′-Nucleotidase activity is in units of $P_i$ and galactosyltransferase activity is in units of galactose transferred. These data are the means of two separate experiments.

been suggested by Catt (26). The role of internalization of the prolactin–receptor complex at the present time remains unclear. It is possible it represents simply a scavenger process for the cell.

Following the binding of insulin to cell surface receptors, a ligand-specific internalization of the hormone–receptor complex occurs. Pastan and his co-workers have characterized these events in fibroblasts (27) and have demonstrated that following internalization, hormone–receptor complexes form vesicles in the cytoplasm which have been identified as "receptosomes." These structures appear to accumulate in the Golgi region, and finally the hormone–receptor complexes are degraded in the lyosomes. Recently, Marshall *et al.* (28) have reported evidence suggesting that following binding of insulin to adipocytes, there is a degradation in lysosomes, primarily of the ligand, whereas receptors are rapidly recycled back to the cell surface. It is interesting to speculate that for prolactin receptors, differences in affinity between receptors at the cell periphery and within the cell might be a reflection of the cellular ability to regulate either or both the number of receptors or the degradation of prolactin.

Although the difference in affinity between PM and Golgi receptors is small, it is confirmed by differences in the rate of dissociation of labeled hormone from the receptor. It is possible that if a change of affinity occurs following the binding of prolactin to its PM receptors, that it could be related to the production of an intracellular message reponsible for the action of prolactin. (29, 30). It appears that it is the receptors with the lower affinity found at the periphery of the cell that are most important in the initial mechanism of action of prolactin.

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