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KINETIC PROPERTIES OF RAT HEPATIC PROLACTIN RECEPTORS

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Binding of ^{125}I-labelled ovine prolactin to female rat liver membranes under-equilibrium conditions showed an apparent K_d of 200 pM, and a Hill coefficient of 1.0. The association rate was second order, with a rate constant K_1, of 2.1×10^7, 1.4×10^7, 1.2×10^7 and 4×10^6 $M^{-1} \cdot min^{-1}$ at 37, 30, 24 and 4° respectively. At 24° there were two components to the dissociation; a faster phase with $K_{-1}=1.26 \times 10^{-2} \cdot min^{-1}$ ($T_{\frac{1}{2}}=55$ minutes) and a slower phase with $K_{-1}=1.103 \times 10^{-3} \cdot min^{-1}$. The apparent K_d (from K_{-1}/K_1) was 1.05 nM for the faster phase and 87.5 pM for the slower phase. These data suggest that there is a conformational change following hormone binding which results in an increased receptor affinity, which effectively prevents release of bound hormone.

INTRODUCTION

Although the liver is not traditionally considered to be a primary prolactin (Prl[1]) target organ, a substantial number of receptor sites with high affinity for lactogenic hormones (Prl, GH, PL) are in fact present. The rat liver PrlR has been studied in terms of the number of binding sites (1,2) and affinity for radiolabelled lactogenic hormones, but the kinetics of the receptor-hormone interaction have not been fully characterized previously. In this report the binding kinetics of the membrane-bound rat hepatic PrlR are described.

MATERIALS AND METHODS

Purified hGH (1.7 IU/mg, HS 216 OE) and oPrl (35 IU/mg, S12) were obtained through the NIAMDD Hormone Distribution Program. The source of other materials: Lactoperoxidase, Sigma, St. Louis, Missouri; Sephadex G-100, Pharmacia, Piscataway, New Jersey; {^{3}H}5'AMP, New England Nuclear, Boston, Massachusetts; {^{125}I}Na (carrier free) and Aqueous Counting Scintillant, Amersham-Searle, Arlington Heights, Illinois; other chemicals, Fisher Scientific Company, Fair Lawn, New Jersey; rats, A.R. Schmidt, Madison, Wisconsin.

[1] Prl, prolactin; oPrl, ovine prolactin; PrlR, prolactin receptors; GH, growth hormone; hGH, human growth hormone; PL, placental lactogen; hCG, human chorionic gonadotropin; LH, lutenizing hormone.

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Preparation of Iodinated hGH. hGH was iodinated to a specific activity of 150-185 μCi/μg using equimolar concentrations of hGH and lactoperoxidase (5). The iodinated hGH was purified by passage over a Sephadex G-100 column (0.7 x 20 cm) (4).

Membrane Preparation and Binding Assay. Partially purified membranes were prepared from the livers of 50-60 day old female rats using discontinuous sucrose density gradients as described (6). The membranes were frozen with liquid nitrogen and stored at -70°C. Protein was assayed according to the method of Lowry (7) using BSA as a standard. 5'-nucleotidase was assayed using {^{3}H}5'-AMP (8). PrlR were assayed as we have previously described (6) using radiolabelled hGH. Nonspecific binding was determined using unlabelled oPrl (2000 fold labelled hGH concentration).

RESULTS.

Membrane-bound PrlR Preparation. A plasma membrane fraction which was enriched in PrlR was prepared by passing the pellet from the high-speed centrifugation (80 min at 105,000 g) over discontinuous sucrose gradients. The particulate material that collected at the sample and 20% sucrose interface (Band I), the 20% and 30% sucrose interface (Band II) and the 30% and 40% sucrose interface (Band III) was analyzed for 5'-nucleotidase activity and PrlR. The majority of the 5'-nucleotidase activity and the PrlR was associated with Bands II and III. The concentration of PrlR and 5'-nucleotidase in Band I was negligible. The fold purification of the 5'-nucleotidase activity was 4.9 and 8.5, and of the PrlR 5.1 and 4.4 for Bands II and III, respectively.

Kinetics of Association of Membrane-bound PrlR. The effect of temperature on the association rate of Prl with its receptor(s) was assessed at 37°C, 30°C, 24°C, and 4°C. The time required to reach equilibrium reflects the temperture dependence of the association rate. At 37°C, equilibrium was reached at approximately 2 hours, while at 30°C, equilibrium was reached only after 4 hours of incubation. The $t_{1/2}$ of association, calculated from a semilogarithmic plot of fmoles bound *vs* time, was 27 minutes at 37°C and 63 minutes at 30°C. The rate of association at 24°C was considerably slower than at the higher temperatures; the time to reach equilibrium being on the order of 22 hours (Fig. 1A). From the semilogarithmic plot (Figure 1B), the $t_{1/2}$ of association at this temperature was 180 minutes. The initial rate of hormone binding was also profoundly affected by temperature. The calculated rates of association (K_1) in $M^{-1}.min^{-1}$ are 2.07×10^7 at 37°C; 1.42×10^7 at 30°C; 1.16×10^7 at

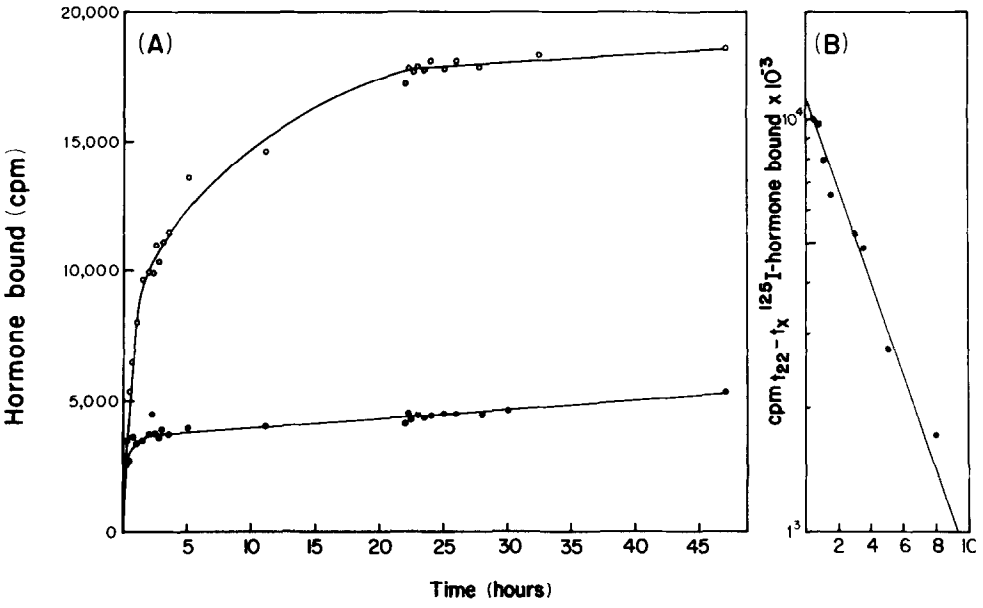

Figure 1. The rate of association between rat liver membranes and human growth hormone.

Membranes were incubated at 24°C with 88 pM {^{125}I}hGH in the presence (open circles) and absence (closed circles) of 2·5 μM unlabelled prolactin. One hundred and fifty microliters of the reaction mix containing 100 μg of protein were withdrawn at the times indicated and transferred to tubes containing one volume of cold (4°C) incubation buffer. In the left panel is shown the raw binding data. Each point represents the mean of triplicate determinations. The half-time of association was calculated from the semi-logarithmic plot (right panel) $K_1 = 1.6 \times 10^7\ M^{-1}.min^{-1}$.

24°C and 4.0×10^6 at 4°C. The strong temperature dependence of the initial association rates is illustrated in the Arrenhius plot (Figure 2).

Kinetics of Dissociation of Membrane-bound PrlR. The dissociation of labelled hGH from the PrlR at 24°C was assessed by the addition of a 5,000-fold excess of unlabelled Prl to the reaction mixture at equilibrium. Dissociation of the complex occurred extremely slowly and two phases for dissociation were discovered (Figure 3A). Graphical resolution of the two components revealed a first order dissociation rate constant (K_{-1}) of $1.26 \times 10^{-2} min^{-1}$ and a $t_{1/2}$ of dissociation equal to 55 minutes (Figure 3B). The second more slowly dissociating component had a $K_{-1} = 1.03 \times 10^{-3}\ min^{-1}$.

Once the association and dissociation rates are known, the K_d for the hormone-receptor complex can be calculated ($K_{-1} = \frac{0.699}{T_{1/2}^{diss}}$ and $K_d = K_{-1}/K_1$). From this equation the K_d is found to be 1.05 nM when the K_{-1} for the more

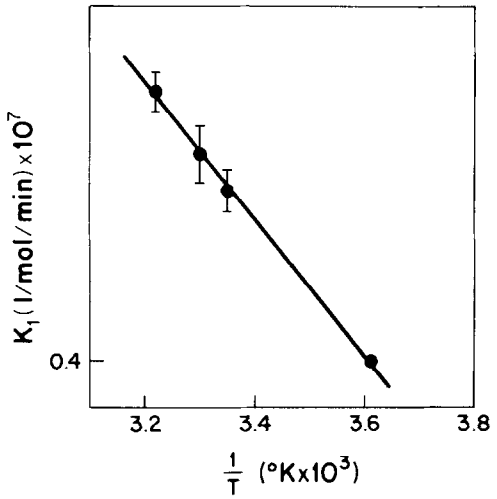

Figure 2. Dependence of the association rate constants on temperature in the range 4°C-37°C.

The association rates calculated from several experiments have been combined. Each point (except 4°) represents the average rate from 3 separate experiments performed in triplicate.

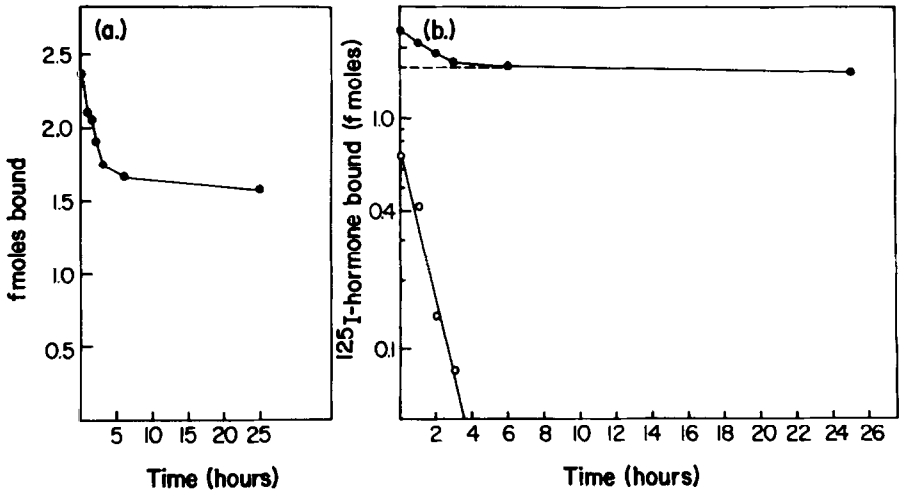

Figure 3. The rate of dissociation of human growth hormone from rat liver membranes.

Membranes were incubated for 22 hours at 24°C with 84 pM {^{125}I}hGH in the presence or absence of unlabelled prolactin. Unlabelled prolactin 2.5 µM was then added. One hundred and fifty microliter aliquots (200 µg protein) were withdrawn at the times indicated and transferred to tubes containing one volume of cold (4°C) incubation buffer. The amount of {^{125}I}hGH bound was determined as described in Methods. In the small frame (a) is shown the complete dissociation curve and in (b) the analysis of the faster dissociating component. Each point represents the average specific binding from triplicate determinations. The half-time of dissociation was calculated from the semilogarithmic plot.

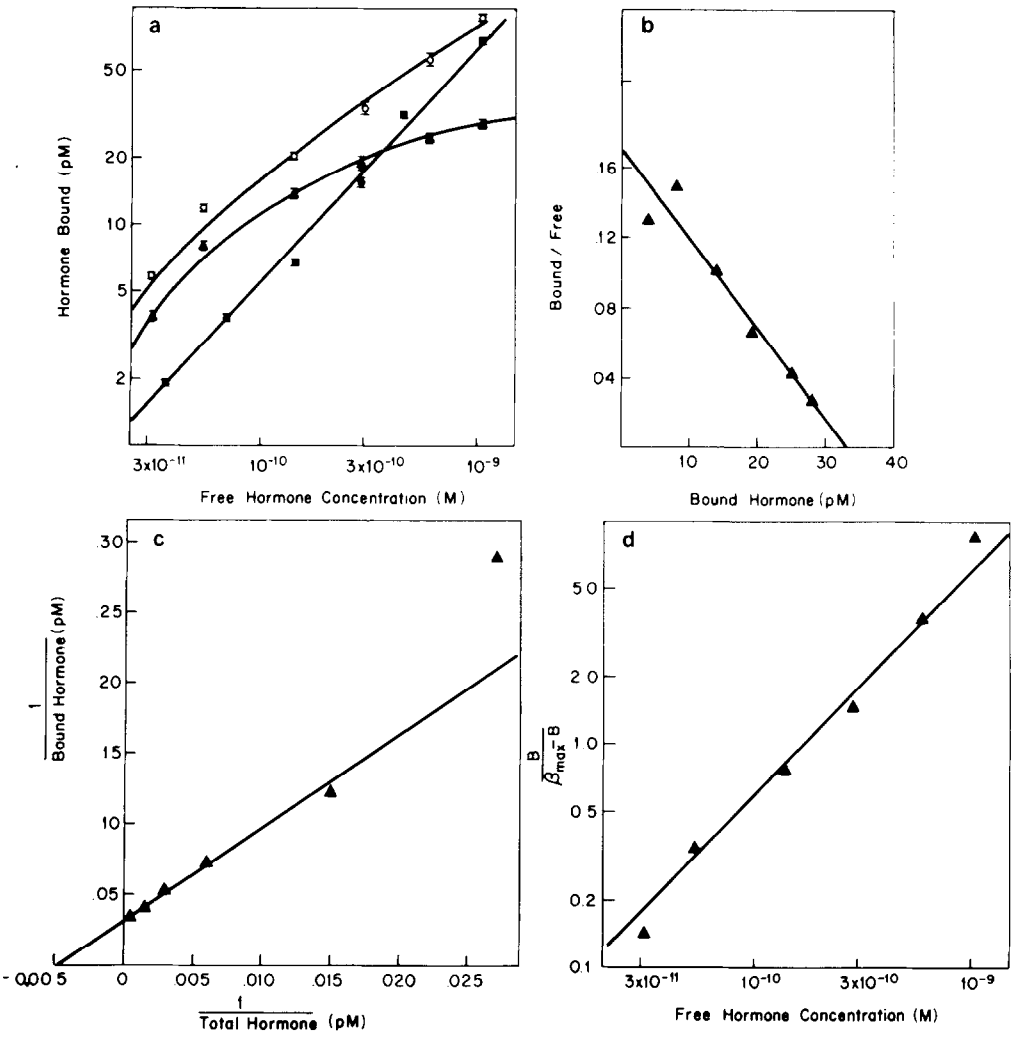

Figure 4. Binding of {^{125}I}hGH to partially purified liver membranes as a function of hormone concentration.

Fifty micrograms of membrane protein were incubated with {^{125}I}hGH between (84 pM to 5.3 nM) in the presence or absence of 450 nM unlabelled ovine Prl for 22 hours at 24°C. Total assay volume was 150 microliters. In (a) is shown the raw data: total counts bound (open circles); nonspecific binding (closed circles); and specific binding (closed triangles). Each point represents the mean (±SEM) of triplicate determinations; (b) the specific binding is analysed according to the method of Scatchard (21); (c) the specific binding is analysed by double reciprocal plot; (d) the specific binding is analysed according to the method of Hill (10).

rapidly dissociating component is used, and 90 pM when the more slowly dissociating component is used.

Equilibrium Studies. The relationship of hormone concentration to the formation of PrlR was investigated by the method of Scatchard (9) and by double-reciprocal plots. The rat liver receptor becomes saturated at a labelled hormone

TABLE 1

VALUES FOR DIFFERENT KINETIC CONSTANTS DETERMINED WITH MEMBRANE-BOUND PrlR

	Unit	24°C
Association rate constant (K_1)	$M^{-1}min^{-1}$	1.2×10^{7}
Dissociation rate constant (K_{-1})	min^{-1}	
Fast component		1.26×10^{-2}
Slow component		1.03×10^{-3}
Dissociation constant (K_d)	M	
From double reciprocal plot		210 pM
From Scatchard plot		190 pM
From Hill plot		170 pM
From K_{-1}/K_1		
using K_{-1} fast component		1.05 nM
using K_{-1} slow component		87.5 pM
Association constant (K_a)	M^{-1}	
From 1/K		5×10^{9}
From K_1/K_{-1}		
using K_{-1} fast component		$.95 \times 10^{9}$
using K_{-1} slow component		11.4×10^{9}

concentration of approximately 1.2 nM (Fig. 4A). When the concentration of labelled hormone is increased, a parallel increase in both total and nonspecific binding is seen. The nonspecific sites are apparently unsaturable. Scatchard (9) analysis (Fig. 4B) indicates an apparent K_d of 190 pM and 99 fmol of binding sites/mg of membrane protein. These results are comparable to those obtained from the double reciprocal plot (Fig.4C)(K_d=210 pM and β_{max}=92 fmol/mg of membrane protein). A Hill plot (10) of these data yields a slope of 1.0 and K_d=170 pM (Fig.4D). Table 1 is a summary of the kinetic constants of the PrlR.

DISCUSSION

The rates of association of PrlR in rat liver are slow and exhibit a strong temperature dependence. These slow rates of association are similar to those reported for PrlR in rabbit mammary glands (11) and to those for

some other peptide hormone-receptor interactions: Dufau, et al. (12) in an extensive study of the gonadotropin receptors for hCG and LH in the rat ovary and testis also found slow rates of association of hormone and receptor.

The interaction between the liver PrlR and labelled hGH at the concentrations tested follows second order kinetics. However, the dissociation of the ligand does not follow strict first order kinetics: 35% of the bound ligand dissociates in a 24-hour period and clearly shows two phases of dissociation. This resistance to dissociation has also been observed with PrlR from rabbit mammary gland (14,15), rat mammary tumors (16), as well as with gonadotropin receptors in rat ovary and testis (12).

The long association time for the receptor-hormone complex is in marked contrast to the association times reported for insulin-receptor complexes and β-adrenergic receptor complexes. The β-adrenergic receptor in canine heart and liver and the insulin receptor have comparable equilibrium constants (K_d=0.1 nM) to that of the PrlR but the $t_{1/2}$ of dissociation of the β-receptor-ligand complex is approximately 18 minutes (17) and the half-life of the insulin-receptor complex is about 16 minutes (18).

The lack of reversible binding suggests that either the ligand is covalently bound to the receptor or, perhaps, the hormone-receptor binding produces a change in the conformation of the receptor, resulting in a higher receptor affinity. The slow rate of association would be consistent with the hypothesis that a conformational change takes place upon ligand binding. Calculation of the K_d from the values for K_1 and K_{-1} suggests two classes of sites; however, analysis of the saturation isotherm by the method of Scatchard (9) yields a straight line. These data are consistent with only one class of binding sites over the range of ligand concentrations used.

Analysis of the saturation isotherm data by the method of Hill (10) revealed a Hill coefficient of 1.0 which implies that there is no cooperativity between sites which could explain the dissociation data. Further experiments examining the dissociation following longer periods of incubation before interruption of the equilibrium should reveal whether a second class of recep-

tors is formed upon prolonged contact with the hormone. Studies on the effects of endogenous Prl on subsequent assay of PrlR would support the hypothesis of a conformational change taking place (16).

In the intact cell, it has been reported that Prl is internalized (19). If these membranes form vesicles, then it is possible that the majority of the hormone has been transported to the inner membrane surface and is no longer available for exchange. Studies on the dissociation kinetics of soluble receptors may aid in deciphering this question: the same two-phase dissociation would be expected if there is a conformational change associated with ligand binding, but if "transport" alone is involved, then first order kinetics should be observed with the soluble receptor.

The present studies characterize PrlR in partially purified rat liver membranes. The binding sites have properties consistent with biological significance. These data provide a basis for future studies on the role of these receptors in the mammalian liver.

ACKNOWLEDGEMENT

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