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PROLACTIN MODIFIES THE PROSTAGLANDIN SYNTHESIS, PROLACTIN BINDING AND FLUIDITY OF MOUSE LIVER MEMBRANES

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SUMMARY: The objective of these studies was to determine if prolactin, known to induce its own receptors, alters the prostaglandin (PG) synthesis which could, in turn, modify the fluidity of the membrane and thus alter the functionality of prolactin receptors. Adult male C_3H mice were injected subcutaneously with 100 µg of oPRL every 4 h for 0, 24 or 48 h and sacrificed 8 h after receiving the last injection. Liver 100,000 x g membrane pellets were used in the measurement of these parameters. The amount of binding of prolactin to these membranes increased with the duration of injections, the values being 179 and 244% of control values after 24 and 48 h of injections, respectively. The amounts of $PGF_{2\alpha}$ and PGE synthesized also increased after these injections, the values being 127 and 270% of control for $PGF_{2\alpha}$ and 634 and 695% of control values for PGE after 24 and 48 h of injections, respectively. Fluorescence polarization, an index of microviscosity, was decreased by 14 and 20% after 24 and 48 h of PRL administration, respectively. Previous studies have demonstrated simultaneous in vitro effects of prostaglandin on both prolactin receptors and membrane fluidity. The current data are in agreement with those observations and suggest that prolactin may modulate its own receptor by increasing the fluidity of the membrane in which it exists by alterations within the PG cascade. Such biochemical changes may then modify existing restraints and allow the hormone receptor to assume a more functional configuration.

The functionality of membrane-associated polypeptide hormone receptors is modulated by many factors (1-6). Prolactin is one such factor known to modify its own receptors under a variety of physiological and pathological conditions. Recently we have reported that prostacyclin and an inhibitor of prostaglandin (PG) synthesis concomitantly alter both the membrane microviscosity and the number of prolactin receptors (6,7). This raises the possibility that the physical state of the membrane may regulate the detectability and consequent functionality of prolactin receptors by modifying the ease with which these receptor proteins move within the lipid bilayer. Mouse microsomal membranes with low microviscosity, determined by measuring the

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mobility of a fluorescent probe inserted into the lipid domain of the membrane, were found to have more prolactin binding capacity whereas less fluid membranes had smaller numbers of prolactin receptors (5-8).

These studies suggested that the prolactin receptors may be controlled directly or indirectly via changes in the membrane fluidity and further suggest that changes in local prostaglandin level and synthesis activity may influence the fluidity of these membranes. These observations prompted the studies reported herein to determine if prolactin modulates its own receptor by changing the fluidity of the target cell membrane and if such changes correlated with the ability of the membrane preparations to synthesize prostaglandins.

MATERIALS AND METHODS

Animals: Male C_3H mice, used at 14-16 weeks of age, were maintained in a 12-h light-dark cycle with water and Purina rat chow available ad lib.

Prolactin injections: Ovine prolactin (oPRL, NIH-S-13), obtained from the National Pituitary Agency, was dissolved in 0.9% NaCl-25 mM Tris buffered to pH 7.6. Mice were injected subcutaneously with 100 µg of oPRL every 4 h for 0, 24 or 48 h and were sacrificed 8 h after receiving the last injection.

Iodination procedure: oPRL was iodinated by a lactoperoxidase method as described elsewhere (9).

Tissue preparation: After decapitation, the livers were excised, frozen in liquid nitrogen, pulverized, and then homogenized (Polytron, Brinkmann) individually at a setting of 6 for 1 min at 4° in approximately 10 vol of 0.3 M sucrose buffered to pH 7.6 with 25 mM Tris-HCl. The supernatant from a preliminary 20 min, 15,000 x g centrifugation was recentrifuged at 100,000 x g for 1 h. The resultant microsomal/plasma membrane pellets were then resuspended in buffer and used for PRL binding assay, PG synthesis assay and microviscosity measurement.

Prolactin binding assay: The PRL binding activity was determined under the following assay conditions: 70,000-75,000 cpm of ^{125}I-oPRL with or without unlabeled PRL (1 µg) in a final volume of 0.5 ml of buffer (10 mM $MgCl_2$/0.1% bovine serum albumin/25 mM Tris-HCl, pH 7.6) were incubated with the liver membrane preparations (200-300 µg protein/tube) (10). After overnight incubation at room temperature, the tubes were plunged into an ice-water bath, diluted with 1 ml aliquots of chilled buffer, and centrifuged at 2,500 x g for 20 min at 4°. The resultant pellets were rinsed with an additional 1 ml of cold buffer and then recentrifuged. The supernatant was decanted and the radioactivity of the pellet was measured in a Packard Gamma Counter. The cpm bound in the presence of 1 µg of unlabeled hormone represented nonspecific binding and was subtracted from the total counts in the remaining tubes to yield the specific binding.

Microviscosity measurement: Membrane microviscosity was studied by fluorescence polarization at 24° with the lipid probe 1,6-diphenylhexatriene

(DPH). Membrane preparations containing 50 µg of protein per 1.5 ml were incubated for 1 h at 25° with 1.5 ml of 2 µM DPH dispersed in phosphate-buffered saline (PBS), pH 7.1. These samples were then subjected to polarization analysis (Aminco) by measuring the peak of emission fluorescence between 380-460 nm while holding the excitation wavelength at 366 nm. The polarization constant, an index proportional to the membrane microviscosity, was calculated according to the equation $P = (I_V - T \cdot I_H)(I_V + T \cdot I_H)$, in which I_V and I_H are the relative fluorescence intensities measured at an angle of 90° to the incident beam with the emission polarization in the vertical and horizontal positions, respectively, and $T = I_V/I_H$ measured with the excitation polarization in the horizontal position (11).

PG synthesizing activity: Membrane pellets were resuspended in ice-cold buffer (mM Tris-HCl, 10 mM glucose, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, and 115 mM NaCl, pH 8.1). One-ml aliquots were placed in 6 conical, ground glass-stoppered centrifuge tubes containing 1 ml buffer, which, in addition to the above components, also contained 2 mM reduced glutathione (Sigma Chemical Co., St. Louis, MO) and 0.17 mM hydroquinone (Fisher Scientific, Waltham, MA), 3 with and 3 without 0.5 mM indomethacin (Sigma). After incubating the membranes at 37° for 2 h, the reaction was terminated by plunging the tubes into an ice-water bath and adding 10 µl concentrated formic acid. Approximately 1,000 cpm [^{3}H] $PGF_{2\alpha}$ (New England Nuclear Corp., Boston, MA; NET433, [5,6,8,9,11,12,14,15(N)-^{3}H] $PGF_{2\alpha}$; 100-150 Ci/mmol) and [^{3}H] PGE_2 (New England Nuclear, NET-428, [5,6,8,11,12,14,15-(N)-^{3}H] PGE_2; 100-200 Ci/mmol) were then added to each tube to permit individual estimates of recovery. Prostaglandins were then extracted from the aqueous suspension into chloroform, dried under nitrogen, and then dissolved in 0.2 ml benzene-ethyl acetate-methanol (B-EA-M) solution mixed in a ratio of 60:40:20 by volume. A 1-g silicic acid (Sigma, SIL-A-200) column was pre-washed with 60:40:20 B-EA-M and then benzene before application of the sample. An initial flush with 1 ml benzene and then 18 ml 60:40:0 B-EA-M eluted the less polar compounds, which included PGA and PGB, and was discarded. Subsequent elution with 20 ml 60:40:20 B-EA-M yielded the PGE + $PGF_{2\alpha}$ fraction, which, after drying, was incubated with 1 ml 0.1 M KOH in absolute methanol for 45 min to convert PGE to PGB. This permitted the separation of PGE (as PGB) from $PGF_{2\alpha}$ by repeating the above column chromatography with fresh columns. This procedure essentially duplicates that of Alexander et al. (12).

The partially purified PGE (as PGB) and $PBF_{2\alpha}$ were thus separated before quantitation by dextran-charcoal RIA (20) using anti-PGB antibody (provided by Drs. John Pisano and Perry Halushka) and anti-$PGF_{2\alpha}$ antibody (New England Nuclear, NEA-057B). The cross-reactivities of the various PG's with these antibodies have been shown previously (13). The amounts of PG synthesized during the 2-h incubation were determined by first correcting for recovery and then subtracting the corrected quantities present in the indomethacin-containing tubes from the corrected quantities present in the tubes containing no indomethacin. These values were normalized for the amount of membrane protein present in each incubation tube.

RESULTS

The values for prolactin binding and fluorescence polarization constant, p, of hepatic microsomal membranes from C_3H male mice injected with 100 µg oPRL every 4 h for 0, 24 or 48 h are shown in Figure 1. Prolactin-binding was increased significantly after prolactin administration, the values of specific binding being 20984 ± 2383, 37508 ± 2592 and 51246 ± 3417 cpm/mg

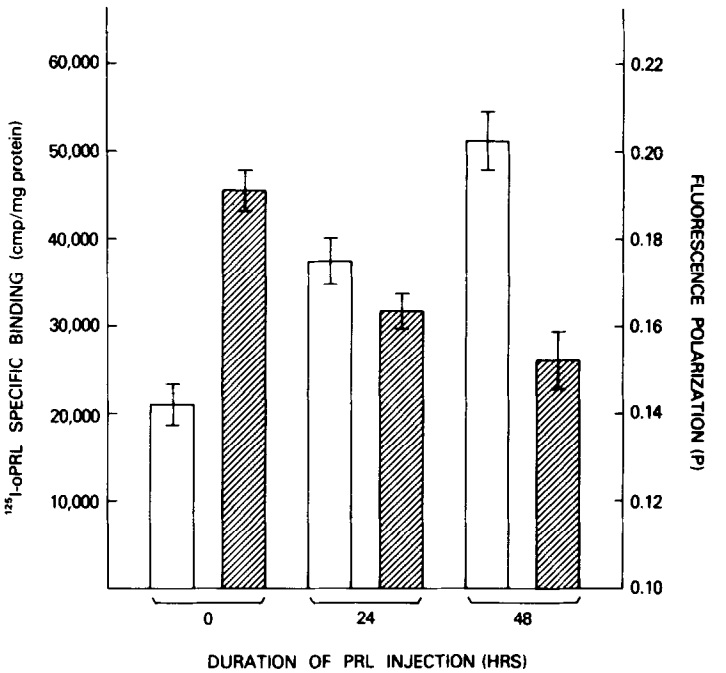

Figure 1

Specific binding of prolactin (open bars) to hepatic membranes increased with duration of in vivo prolactin treatment. Fluorescence polarization measurements (cross-hatched bars), an index of membrane microviscosity, decreased concomitantly with duration of prolactin treatment. Four-five animals were in each group and each membrane sample was assayed in triplicate. Vertical bars = 1 SEM.

protein at 0, 24 or 48 h of 4-hourly prolactin administration, respectively. As also shown in Figure 1, membrane microviscosity, as determined by fluorescence polarization, decreased significantly after prolactin treatment, the values of fluorescence polarization being 0.191 $\pm$ 0.0046, 0.165 $\pm$ 0.0038 and 0.152 $\pm$ 0.0066 at 0, 24, or 48 h after prolactin injections, respectively.

The amount of prostaglandin synthesized in vitro by the membranes obtained from these treatment groups is shown in Figure 2. The amounts of PGE and $PGF_{2\alpha}$ synthesized increased significantly after prolactin administration. The values for PGE were 0.200 $\pm$ 0.027, 1.269 $\pm$ 0.056, and 1.391 $\pm$ 0.023 ng/mg protein-2 h, respectively, for 0, 24 or 48 h of prolactin treatment while $PGF_{2\alpha}$ was synthesized in amounts of 14.07 $\pm$ 0.77, 17.84 $\pm$ 3.48, and 37.99 $\pm$ 3.96 ng/mg protein-2 h for the 0, 24, or 48 h groups, respectively.

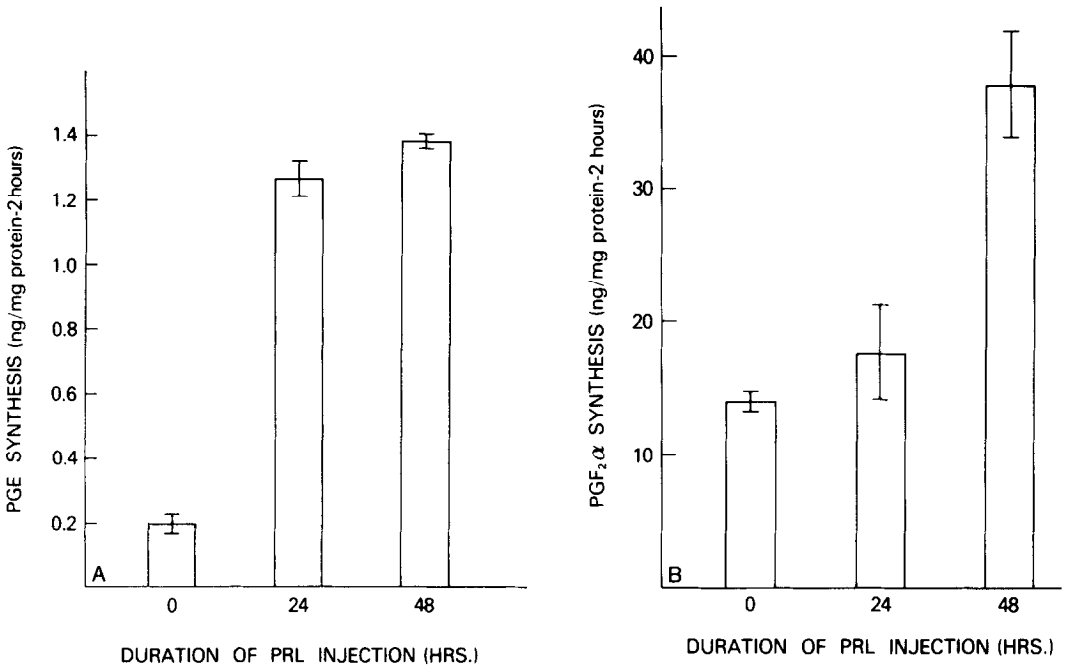

Figure 2A
Synthesis of PGE by hepatic membranes in vitro increased within 24 h after the start of in vivo prolactin injections.

Figure 2B
Synthesis of PGF_2 by hepatic membranes in vitro was seen to increase after 48 h of in vivo prolactin injections. The membrane preparations from 3-4 animals for each group were pooled. Each point represents 3 incubations with or without indomethacin, assayed in duplicate. Vertical bars = 1 SEM.

DISCUSSION

The studies reported herein demonstrate that prolactin induces significant changes in the prolactin binding capacity, PG synthesis and fluidity of mouse liver membranes in vivo.

Cell membranes exist as a dynamic matrix which responds to various physiological or pathological stimuli by modifications of its physical state. This allows conformational changes or movement of membrane-associated proteins within the membrane, which can then be expected to exhibit functional changes in their ability to bind and/or respond to various agents. One such alteration is the ability of the membrane to bind a variety of antigens or hormones as exemplified by present data demonstrating an increase in prolactin binding capacity after prolactin administration. This is consistent with previous studies reported by this laboratory and by other investigators (8, 9,14).

Earlier studies carried out in this laboratory have shown that prolactin receptors are influenced by changes in membrane fluidity: mouse liver membranes with high microviscosity were found to have lower prolactin binding capacity and vice versa (5-8). Injections of graded doses of exogenous prolactin into hypophysectomized, PMSG/hCG-treated rats induced changes in both the viscosity and prolactin binding of hepatic membranes (8) with decreased membrane microviscosity correlating with the increased numbers of detectable prolactin receptors. These changes were found to be bi-phasic with both relatively lower and pharmacologic amounts of administered prolactin decreasing the receptor levels. Such manipulations have also resulted in changes in the rate of prostaglandin synthesis by granulosa cells (13), supporting the suggestion that the mechanism by which effective doses of prolactin exert their physiological effects is through modulation of PG cascade (15-17).

In further support of this thesis, recent studies from this laboratory have shown that prolactin binding capacity, fluidity and PG synthesizing ability of hepatic membranes obtained from female mice were significantly increased during mid-late gestation period (18,19).

In the studies described herein, we have again observed a very close correlation between these parameters. Male C_3H mice injected with 100 µg oPRL every 4 h for 24 or 48 h have higher values of prolactin binding, an elevation that may be due to increased fluidity of the membranes. The changes in the membrane fluidity may also be the result of a change in the ratio of phospholipids to cholesterol, since alterations of this ratio were observed in earlier studies (8) after injections of graded doses of prolactin into hypophysectomized rats. This effect may play a role in maximal exposure of receptors already present. These changes in microviscosity may be brought about by increases in the ability of the membranes to synthesize prostaglandins. This observation is consistent with the earlier studies carried out in this laboratory in which prostacyclin and PG synthesis inhibitor, indomethacin, were found to modify both the prolactin binding capacity and fluidity of mouse liver membranes (6,7).

The results of the present study are thus compatible with the hypothesis that prostaglandin synthesis is involved, either directly or indirectly, in the induction and maintenance of the prolactin receptor and that modification of the physical characteristics of the surrounding lipid matrix modulates the functionality of this receptor.

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