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PROLACTIN MODIFIES THE FLUIDITY OF RAT LIVER MEMBRANES

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SUMMARY: The objective of this study was to determine the effect of prolactin upon the fluidity of hepatic membranes and subsequent modification of the prolactin receptors. Hypophysectomized, immature, female rats (HIFR) having subcutaneous diethyl stillbesterol implants were treated with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). In addition, graded doses of 0, 0.25, 0.5, 1.0, 2.5 or 50 µg oPRL were injected hourly for the 48 hours prior to sacrifice at 28 days of age. Both the lipid fluidity as determined by 1,6-diphenylhexatriene (DPH) fluorescence polarization and phospholipid/cholesterol (P/C) ratio were measured in hepatic microsomal membranes. A biphasic response was observed in both of these parameters as the dose of oPRL was increased. P/C values were 91,113, 117, 88, and 93% of control values for the 0.25, 0.5, 1.0, 2.5 and 50 µg oPRL groups, respectively, the maximal value being significantly different from the saline injected controls. These ratios were inversely proportional to the corresponding values of fluidity which also varied in a biphasic manner, with fluorescence polarization equal to 97, 90,82, 100 and 101% of control values. The value for the 1.0 µg group was, again, significantly different from control. No significant specific ^{125}I-oPRL binding was detectable in any of the groups after treating the membranes with distilled water, however, pretreatment of membrane preparations with 4 M $MgCl_2$ for 5 min resulted in a significant increase in the oPRL-binding in the 0.5 and 1.0 µg oPRL groups. The values being 273 and 521% of control, respectively in these two groups. These data demonstrate that prolactin modifies the viscosity of hepatic membranes and suggests that this phenomenon may be responsible for the autoregulation of detectable prolactin receptors.

INTRODUCTION

Cell membranes exist in a state of continual change, influenced by dietary, pharmacologic and hormonal factors. Various hormones control the number or affinity of their own receptors or those of other hormones. Prolactin is one such hormone, inducing or increasing the number of its own receptors in animals (1). However, the mechanisms by which this regulation occurs is unknown.

The proteins or glycoproteins embedded within the phospholipid bilayer of such membranes comprise the various enzymatic, receptor, and antigenic determinants of the particular cell type. While direct modifications of these constituents will alter their functionality and consequent cellular behavior, modifica-

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tion of their phospholipid matrix will also alter their functionality, perhaps, by inducing positional or conformational changes. The ease with which such components move within the lipid bilayer can, in theory, therefore, modify their functionality and detectability. Such a characteristic can be inferred from measurements of the fluidity of the membranes, accomplished by determining the mobility of a fluorescent probe inserted into the lipid domain of the membrane.

Such studies have demonstrated that prostaglandin I_2 modifies the fluidity of hepatic membranes while simultaneously increasing the number of prolactin receptors (2). This implies a causal relationship, that the receptors may be controlled directly or indirectly via the products of the prostaglandin cascade. This hypothesis is strengthened by a recent report of an in vivo effect of exogenous prolactin on prostaglandin synthesis by rat granulosa cells (3). These observations prompted the experiments reported herein: to determine if prolactin modulates its own receptor by changing the fluidity of the target cell membranes.

MATERIALS AND METHODS

Reagents: Ovine prolactin (oPRL, NIH-S-13) was obtained from the National Pituitary Agency, diethyl stillbesterol (DES) from Sigma, pregnant mare serum gonadotropin (PMSG) from Organon and hCG from Ayerst. 1,6-diphenylhexatriene (DPH) was purchased from Aldrich Chemical Company, Milwaukee, Wis. Other chemicals were of reagent grade.

Iodination: oPRL was iodinated by a lactoperoxidase method as described elsewhere (4).

Animals: Twenty-one day old female, Sprague-Dawley rats were hypophysectomized and implanted subcutaneously with a 1 cm length of silastic tubing filled with diethyl stillbesterol (5). They were sacrificed 7 days later after having received 3 subcutaneous injections of 50 I.U. PMSG at 12, 24 and 36 hours before sacrifice, and hourly doses of 50, 2.5, 1.0, 0.5, 0.25 or 0 µg oPRL in saline buffer for the 48 hours before sacrifice at 28 days of age.

Tissue preparation: Animals were decapitated, their livers immediately excised, frozen in liquid nitrogen, and then pulverized. After a 1 minute homogenization (Polytron, Brinkmann) at setting = 6 in approximately 10 volumes of cold 0.3 M sucrose buffered to pH 7.6 by 25 mM Tris-HCl. The homogenates were then centrifuged at 4°C at 15,000 x g for 20 min. The supernatants from this preliminary spin were then centrifuged at 100,000 x g for 1 hr. The membrane pellets, resuspended in buffer, were used for oPRL-binding, fluidity studies, and measurement of cholesterol and phospholipid. The oPRL-binding activity was determined as described in earlier studies (4). Membrane microviscosity

was studied by fluorescence polarization at 24°C using the lipid probe 1,6-diphenylhexatriene (DPH). This was accomplished by incubating membrane preparations containing 50 µg protein (6) per 1.5 ml with 1.5 ml 2 µM DPH dispersed in phosphate-buffered saline, pH 7.1 for 1 hr at 25° C. These samples were then subjected to polarization analysis by measuring the emission fluorescence between 380-460 nM while keeping the excitation wavelength constant at 366 nM (Aminco). The fluorescence polarization was calculated according to the equation: $P = (I_V - T\ I_H)/(I_V + T\ I_H)$, where I_V and I_H are the relative fluorescence intensities measured at an angle 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 (7). For the determinations of phospholipids and cholesterol, lipids were extracted from the microsomal membrane preparations into a mixture of chloroform and methanol (2:1, v/v) as described by Folch et al (8). Total phospholipids were determined by phosphorus analysis (9) and this value multiplied by 25 to give the value for total phospholipids. Cholesterol was estimated by the method of Bowman and Wolf (10).

RESULTS

Since its half-life in the circulation is short, prolactin levels were sustained within the hypophysectomized, DES-implanted rats by repeated injections of 0, 0.25, 0.5, 1.0, 2.5 or 50 µg oPRL every hour for the 48 hours prior to sacrifice. The animals were also supplemented with 3 subcutaneous injections of PMSG at 12, 24 and 36 hours before sacrifice and hCG at 12 hours before sacrifice.

The phospholipids/cholesterol (P/C) ratios in the liver microsomal membrane preparations, presented in Fig. 1, show a dose-response pattern with the peak ratio observed in the group injected with 1.0 µg oPRL every hour. The suggestion that this treatment induced the most fluid state was confirmed by measurement of DPH fluoresccence polarization which showed an 18% decrease in the same membrane preparation. The phospholipid/cholesterol ratios of 4.48 ± 0.52, 4.09 ± 0.55, 5.08 ± 0.37, 5.26 ± 0.13, 3.95 ± 0.19 and 4.18 ± 0.33 were obtained for 0, 0.25, 0.5, 1.0, 2.5 and 50 µg oPRL groups, respectively. The values for 0.5 and 1.0 µg oPRL groups were significantly different from saline-control group at $p < 0.01$ and $p < 0.001$. As expected, the corresponding values of fluidity also varied in a similar manner, the values of fluorescence polarization being inversely proportional at 0.235 ± 0.004, 0.227 ± 0.008, 0.210 ± 0.009, 0.193 ± 0.007, 0.235 ± 0.007 and 0.238 ± 0.004. The values for the 0.5 and 1.0 µg oPRL groups were significantly different from the control at $p < 0.001$, indicating

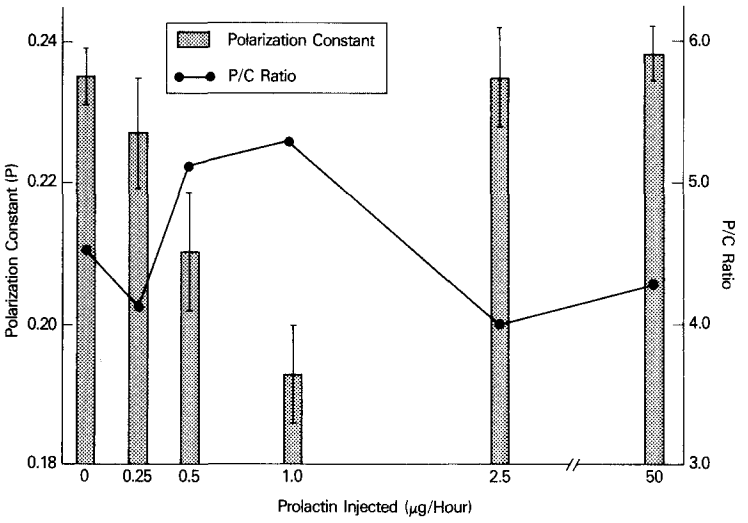

Figure 1. Hourly in vivo injections of graded amounts of oPRL into PMSG + hCG-treated hypophysectomized, immature, female Sprague-Dawley rats resulted in a dose-related changes in the fluorescence polarization (with 1 μM 1,6-diphenyl-hexatriene in phosphate-buffered saline) measured at 24° C and phospholipids / cholesterol (P/C) ratios of a 100,000 x g liver membrane preparation. Each point represents data from 3-5 observations. The values for 0.5 and 1.0 μg oPRL groups were significantly different from control group at $p < 0.01$ and $p < 0.001$ for P/C ratio and $p < 0.001$ for fluorescence polarization. Vertical bars = 1 S.D.M.

an increase in the lipid fluidity of membranes in 0.5 and 1.0 μg oPRL-treated animals followed by a decrease when the amount of oPRL injected was increased.

There was no significant specific ^{125}I-oPRL binding detectable in any of the groups probably due to the fact that these animals were sacrificed 1 hr after receiving the last oPRL injection and the binding sites were occupied with exogenously injected prolactin. According to a recent report (11), a short (5 min) exposure of membrane preparations to 4 M $MgCl_2$ results in 91-97% dissociation of bound prolactin. After membranes obtained from various groups were treated with 4 M $MgCl_2$ for 5 min, a significant increase in prolactin binding was observed in the animals from 0.5 and 1.0 μg oPRL groups. The ^{125}I-oPRL specific binding were 80, 273, 521, 70 and 75% of control values in the 0.25, 0.5, 1.0, 2.5 and 50 μg oPRL groups, respectively. Treatment with $MgCl_2$ resulted in 30-40% losses of membrane protein (Fig. 2). These data indicate that the level of circulating prolactin influences the liver microsomal membrane fluidity and detectable prolactin receptors.

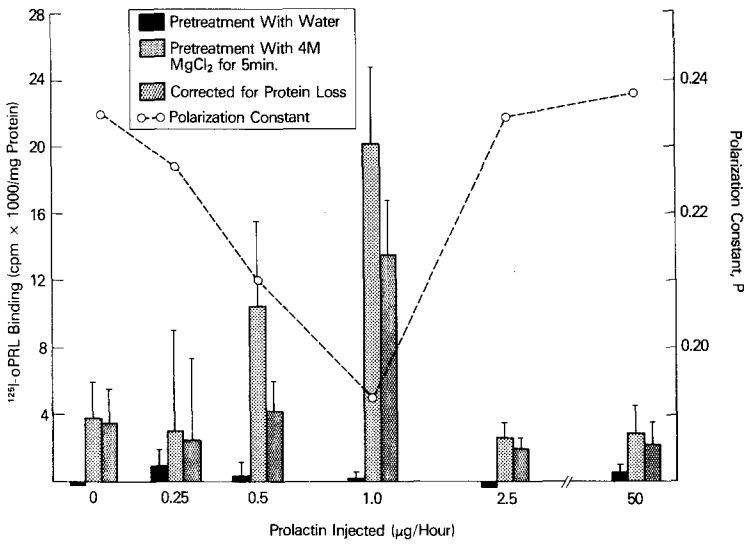

Figure 2. Effects of hourly in vivo injections of graded amounts of oPRL into PMSG + hCG-treated animals on induction of specific ^{125}I-oPRL binding and fluorescence polarization (from Fig. 1) (with 1 μM DPH in phosphate-buffered saline) measured at 24°C of a 100,000 x g liver membrane preparations. Pretreatment of membranes with 4 M $MgCl_2$ for 5 min resulted in 25-35% loss in protein due to solubilization, the third bar at each doses of oPRL represents specifiecd binding of oPRL after correcting the protein loss. Each point represents data from 3-5 observations. The values for 0.5 and 1.0 μg oPRL groups after $MgCl_2$ treatment were significantly different from control group at $p < 0.01$ and $p < 0.001$. Vertical bars = 1 S.D.M.

DISCUSSION

The functional characteristics of cell membranes reflect many biophysical and biochemical factors active within such a biological system. Thus, it is now well accepted that membranes undergo many functional changes when subjected to various intrinsic and extrinsic stimuli. One such function is the ability to bind a variety of antigens or hormones.

In a recent study carried out in this laboratory (3), it was observed that when PMSG/hCG/DES-treated hypophysectomized female rats were injected with oPRL graded in quantity from 0 to 50 μg, their granulosa cells exhibited a dose-related modification in the synthesis of both PGE and PGF 2α. This suggested that alterations within the prostaglandin cascade might be the mechanism through which prolactin exerted physiologic and pathophysiologic effects in the ovary and, perhaps, other organ systems. Another series of experiments

(2) demonstrated that prostacyclin increased both the fluidity and oPRL-binding capacity of mouse liver microsomal membrane preparations in vitro.

The present study shows that administration of ovine prolactin to immature hypophysectomized female rats resulted in changes in the fluidity of liver microsomal membranes, being increased when small amounts of prolactin are injected but decreased when hyperprolactinemic states are achieved.

Such changes may be due to alterations in local prostaglandin levels that affect a biochemical event which particpates in maintaining the proper membrane lipid composition. This may result in the changes in the membrane phospholipid/ cholesterol ratio as reported herein. Such a role has already been demonstrated, PRL being known to influence the amount of cholesterol substrate available for steroid synthesis (12,13) and increase the free and esterified cholesterol levels in the corpora lutea of hypophysectomized rats (12). These data suggest that prolactin modifies the metabolism of cholesterol in the corpus luteum, favoring its storage in the absence of other hypophyseal factors (12,14).

Since the P/C ratio is one of the important determinants of normal lipid fluidity in membranes, a similar situation can be presumed to occur in the liver. The changes observed in the prolactin binding to the liver membranes of the 0.5 and 1.0 µg oPRL-treated animals of the present study can, therefore, be a result of a change in membrane fluidity.

The data presented herein demonstrate that prolactin modifies the lipid fluidity of hepatic microsomal membranes in a dose-related fashion, being increased at low to physiological levels of prolactin and suppressed at pathologically high prolactin levels. These changes in fluidity are probably a direct result of changes in the P/C ratios. Furthermore, prolactin also modulates its own receptors, again, in a dose-related fashion that corresponds to the fluidity of the membrane preparations. These changes in both receptor levels and fluidity viewed against the background of previous studies suggest that circulating levels of prolactin may be of prime importance in modulating

the fluidity of target cell membranes, modifying membrane-associated receptors and thus affecting the ability of the cells to respond to various hormonal or antigenic stimuli.

REFERENCES

1. Horrobin, D.F. (1978) in Prolactin, ed. Horrobin, D.F. (Eden, Montreal), 6, 16-28.
2. Dave, J.R. and Knazek, R.A. (1980) Proc. Natl. Acad. Sci. USA, 77, 6597-6600.
3. Knazek, R.A., Christy, R.J., Watson, K.C., Lim, M.F., Van Gorder, P.N., Dave, J.R., Richardson, L.L. and Liu, S.C. Endocrinology (Communicated).
4. Knazek, R.A., Liu, S.C. and Gullino, P.M. (1977) Endocrinology, 101, 50-58.
5. Louvet, J.P., Harman, S.M. and Ross, G.T. (1975) Endocrinology, 96, 1179-1186.
6. Lowry, O.H. and Rosebrough, N.J. (1951) J. Biol. Chem. 193, 265-275.
7. Chen, R.E., Edelhoch, H. and Steiner, R.J. (1969) in Physical principles and techniques of protein chemistry. ed. Leach, S.J. (Academic Press, New York) part A, 171-173.
8. Folch, H., Less, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem., 226, 497-509.
9. Bartlett, G.R.(1959) J. Biol. Chem. 234, 466-468.
10. Bowman, R.E. and Wolf, R.C. (1962) Clin. Chem., 8, 302-309.
11. Kelly, P.A., Leblanc, G. and Djiane, J. (1979) Endocrinology, 104, 1631-1638.
12. Armstrong, D.T., Knudsen, K.A. and Miller, L.S. (1970) Endocrinology, 86, 634-641.
13. Behrman, H.R., MacDonald, G.J. and Greep, R.O. (1971) Lipids, 6, 791-796.
14. Armstrong, D.T., Miller, L.S. and Knudsen, K.A. (1969) Endocrinology, 85, 393-398.