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STIMULATORY EFFECTS OF PROLACTIN AND ANTI-PROLACTIN RECEPTOR SERUM ON PROLACTIN BINDING SITES IN RAT LIVER CELLS IN SUSPENSION CULTURE

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The effects of prolactin and a serum containing anti-prolactin receptor antibodies on prolactin binding sites were investigated in a suspension culture of rat liver cells. In this model, prolactin binding sites decline rapidly with time, with 90% of the sites lost at 24-48 h of culture. The inclusion of 10 to 100 nM ovine prolactin in the incubation medium, results in a 6-fold increase in prolactin binding compared to control cultures. Anti-prolactin receptor serum is capable of preventing this PRL-induced increase in its receptors. However, when incubated alone, these antibodies at lower concentrations (0.5 to 5%) mimic the up-regulatory effect of prolactin on its own binding site. These findings suggest that in rat liver cells, as has been observed for rabbit mammary gland, that the prolactin molecule is not required beyond the initial binding to its receptors for its action to be attained.

INTRODUCTION

The interaction of prolactin (PRL) with the surface receptors of its target cells is recognized to be an initial and important step on the mechanism of action of prolactin. Prolactin binding sites are found in high concentrations in the mammary gland and liver (1,2). In vivo, a long-term up-regulation (3,4) and a brief and reversible down-regulation at higher prolactin concentrations have been observed (5). Using in vitro models, only down-regulation of prolactin receptors has been observed (6).

In the mammary gland, one of the major effects of prolactin is the stimulation of casein synthesis (7). Prolactin-stimulated

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casein synthesis can be blocked by a serum containing anti-prolactin receptors (8) confirming studies initially reported by Shiu and Friesen (9). In mammary explants, Djiane et al. (8) has recently reported that anti-prolactin receptor serum when utilized at lower concentrations surprisingly elicited a stimulatory effect on the casein synthesis by enhancing casein mRNA and DNA synthesis. It was therefore of interest to compare the effects of prolactin and antiprolactin receptor antibodies in another *in vitro* model for which the up-regulation of prolactin receptors can be examined, namely rat liver cells in suspension culture.

MATERIAL AND METHODS

Hepatocytes dissociation and culture

Female rats pretreated with 17β-estradiol (5 µg) twice a day for seven days were given three injections of CB-154 (500 µg) every 12 h starting 24 h prior to sacrifice. Liver cell isolation was performed by perfusion of Hepes buffer (pH 7.4) via the hepatic portal vein. The superior vein cava was cut and 700 ml buffer at 37°C was pumped through the liver in 10 min. Dissociation was initiated by perfusing 300 ml of collagenase (Sigma, specific use, 30000 IU/rat) in 15 min. Following washing with 100 ml of Hepes, the liver was removed and placed in a Petri dish containing Minimum Essential Medium (MEM). From this point, all solutions utilized were at pH 7.4 and all procedures were made under sterile conditions in a laminar flow hood. After removal of the liver capsule the cells were dissociated by gentle agitation of the liver in MEM solution (Flow Laboratories). Two centrifugations were performed (700 g for 5 min each) and after the first, the pellet was resuspended in L-15 Medium (Flow Laboratories). Cells were counted in a hemacytometer and diluted to 3.3×10^6 cells/ml of L-15 (the incubation medium). Appropriate volumes of L-15 containing isolated liver cells were placed in 50 ml Erlenmayer flasks and incubated at 37°C under continuous shaking under an atmosphere of air. Samples were removed at various times, indicated in the figures and frozen at -20°C.

Prolactin receptor assay

Cell solutions were centrifuged at 1000 g for 15 minutes and the pellet resuspended in Tris buffer 25 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$) and homogenized in a glass teflon homogenizer at medium speed for 10 sec. Since prolactin does not appear to dissociate from its receptor during membrane preparation, it was necessary to desaturate prolactin receptors prior their assay. This technique involves an *in vitro* incubation with 3M $MgCl_2$ as has been described in detail (10).

Three hundred µl of liver cell homogenates were incubated with approximately 10^5 cpm/min of ^{125}I-hGH in the presence or absence of excess of ovine prolactin (1 µg). Human GH which has been shown to have lactogenic activity in the mammary gland (11) was iodinated with a modified Chloramine T method (10) and has been utilized to identify prolactin receptor in a number of target organs (1).

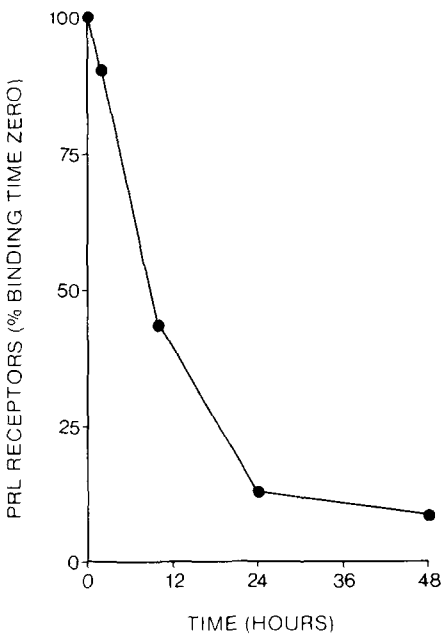

Fig. 1. Effect of incubation time on prolactin binding expressed as a % of the value observed at time zero (before incubation, which was 38.0% specific binding per 10^6 cells) in rat liver cell in suspension culture.

Final volumes were adjusted to 0.5 ml with Tris buffer containing 0.1% BSA. The reaction was allowed to proceed at room temperature for 18 h and stopped with 3 ml of cold buffer Tris containing BSA. Tubes were centrifuged at 2300 g for 15 min and the pellets were counted in a LKB counter with a counting efficiency of 66.5%.

Production of antiprolactin receptor antibodies

Partially purified receptor was prepared (8,12) and injected (50 µg) monthly into male sheep, mixed with complete Freund's adjuvant. Animal were bled 7 to 10 days after each booster immunization. Serum containing antiprolactin receptor antibody inhibits the binding of [^{125}I] prolactin to the receptor in rabbit mammary gland membranes (8) as well as in a number of tissues containing prolactin receptors (Kelly, Djiane, Leblanc, and Katoh, manuscript submitted).

RESULTS

Prolactin receptor levels in hepatocyte cultures shows a rapid decline with the time. At 10 h of incubation, levels are only 50% of the levels measured at the time zero (just prior to incubation), and continue to decline until 48 h when only 10% of the initial prolactin binding sites are present (Figure 1). This loss of binding sites is similar to that observed in vivo in hypophysectomized animals (13, 14). However, when liver cells are

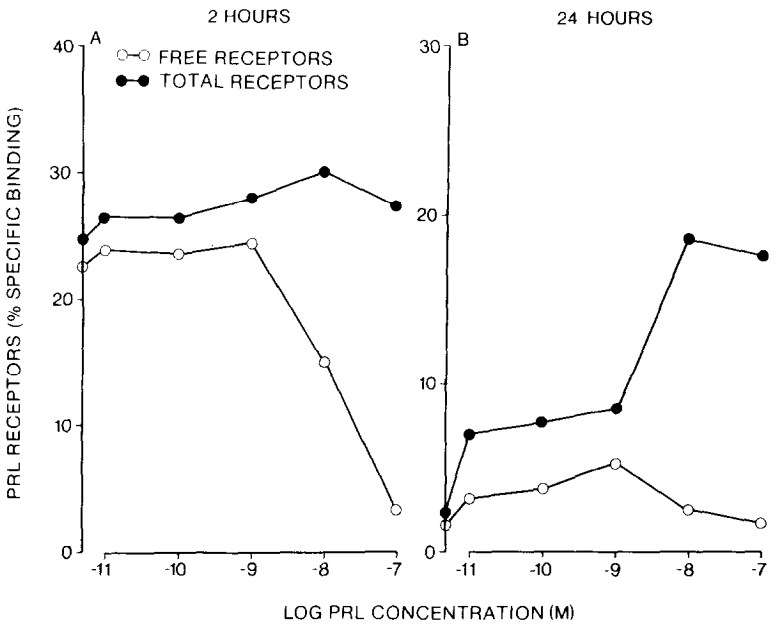

Fig. 2. The effect of increasing concentrations of ovine prolactin on free and total prolactin receptor levels in rat liver cells at 2h (panel A) and 24h (panel B) of suspension culture.

cultured in monolayer, the decline is much more rapid (results not shown).

The effect of increasing concentrations of ovine prolactin on free and total prolactin binding sites in rat liver cells in culture is shown in Fig. 2. Cells cultured for 2 h at 37°C show an occupation of free receptors beginning at 10 nM and which is nearly complete at 100 nM. There is very little effect on total receptor levels, which are only 20% lower than for the time 0 control values (see Fig. 1). When cells are allowed to incubate for 24 hours, there is a marked decline in binding in the absence of prolactin. However, very low doses (10^{-11} to 10^{-9}M) of prolactin, which have little effect on occupation of free receptors, result in an increase in total receptor levels. At concentrations of 10 and 100 nM, the stimulatory effect is much more apparent with levels similar to those observed at 2 h of culture.

Prolactin receptor levels in rat liver cells incubated for 48 h with antiprolactin receptor serum in increasing concentration

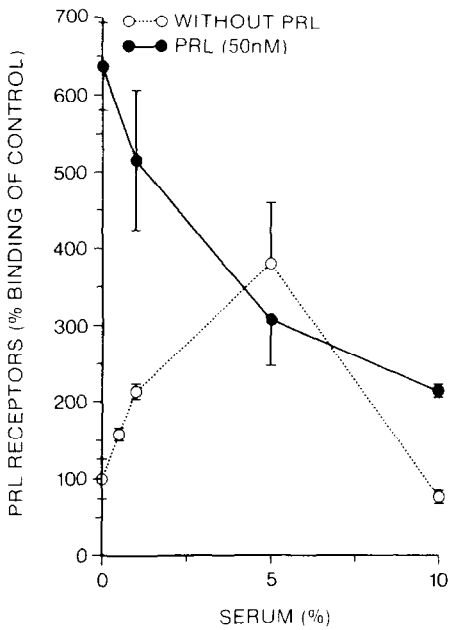

Fig. 3. Effect of a serum containing prolactin receptor antibodies, utilized in increasing concentration (%), on prolactin binding sites of hepatocytes cultured in continuous suspension in the presence or absence of prolactin for 48 h. Values are expressed as a % of control (cells cultured in the absence of prolactin) at 48h of culture (4.6 ± 0.7% specific binding). Non-immune serum from a sheep had no stimulatory or inhibitory effect on prolactin binding.

(%) in the presence and absence of prolactin (50 nM) is shown in Figure 3. A 6-fold, up regulatory effect of prolactin alone is observed ($p < 0.01$). This up-regulation is inhibited by increasing concentrations of antiprolactin receptor serum, which inhibits other prolactin actions in the mammary gland (8, 9). When incubated in the absence of prolactin, however, prolactin receptor antibodies are able to significantly increase prolactin binding sites at a serum concentration of 1% ($p < 0.01$) with a maximum at 5% serum resulting in a 3- to 4- fold stimulation. The stimulatory effect of the antiserum is seen as early as 24 h of incubation, when doses of 1 or 5% are equally effective in increasing prolactin binding levels ($p < 0.05$, data not shown). Non-immune serum from a sheep, at the concentrations shown for antiprolactin recep-

tor serum, had no effect on prolactin receptors levels (data not shown).

DISCUSSION

Prolactin receptor antibodies have recently been utilized to study the link between prolactin receptors and the biological activity of prolactin (8, 9). In rat liver cells in suspension culture, prolactin has an up-regulatory effect that can be inhibited by the addition of prolactin receptor antibodies. When utilized alone, at lower concentrations, this serum mimics the up-regulatory effect of prolactin as has been shown in rabbit mammary explants (8). Similar stimulatory actions have been reported for insulin receptor antibodies on glucose uptake and oxidation (15, 16) anti-thyroid receptor that mimics TSH action on cAMP formation (17) and more recently for anti-β-adrenergic receptors on adenylate cyclase in turkey erythrocytes (18).

The up-regulation of prolactin receptors by prolactin or anti-receptor antibodies could be related either to a reduction in the rate of degradation of receptors or to a stimulation of receptor synthesis or both (manuscript in preparation).

These studies demonstrate the first <u>in vitro</u> model where the prolactin-induced up-regulation of its own receptors can be evaluated. The fact that antibodies against the prolactin receptors also mimic the action of prolactin suggests that the prolactin molecule itself is not required beyond its initial binding to receptors for its effects to be observed.

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REFERENCES

1. Djiane, J., Durand, p. and Kelly, P.A. (1977) Endocrinology 100, 1348-1356.
2. Posner, B.I., Kelly, P.A., Shiu, R.P.C. and Friesen, H.G. (1974) Endocrinology 96, 521-531.
3. Djiane, J. and Durand, P. (1977) Nature 266, 641-643.
4. Posner, B.I., Kelly, P.A. and Friesen, H.G. (1975) Science 188, 57-59.
5. Djiane, J., Clauser, H. and Kelly, P.A. (1979) Biochem. Biophys. Res. Commun. 1371-1378.
6. Djiane, J., Delouis, C. and Kelly, P.A. (1979) Proc. Soc. Exp. Biol. Med. 162, 342-345.
7. Houdebine, L.M. and Gaye, P. (1975) Mol. Cell. Endocr. 3, 37-55.
8. Djiane, J., Houdebine, L.M. and Kelly, P.A. (1981) Proc. Natl. Acad. Sci. USA 78, 7445-7448.
9. Shiu, R.P.C. and Friesen, H.G. (1978) Science 192, 259-261.
10. Kelly, P.A., Leblanc, G. and Djiane, J. (1979) Endocrinology 104, 1631-1638.
11. Forsyth, I.A. (1971) J. Dairy Res. 38, 419-442.
12. Shiu, R.P.C. and Friesen, H.G. (1974) J. Biol. Chem. 249, 7902-7911.
13. Posner, B.I., Kelly, P.A. and Friesen, H.G. (1974) Proc. Natl. Acad. USA 71, 2407-2411.
14. Kelly, P.A., Posner, B.I. and Friesen, H.G. (1975) Endocrinology 97: 1408-1415.
15. Jarret, D.B., Roth, J., Kahn, C.R. and Flier, J.S. (1976) Proc. Natl. Acad. Sci. USA 73, 4115-4119.
16. Baldwin, D., Terris, S. and Steiner, D.F. (1980) J. Biol. Chem. 225, 4028-4034.
17. Mutoh, H., Totsuka, Y., Chou, M.C.Y. and Field, J.B. (1980) Endocrinology 107, 707-713.
18. Couraud, P.O., Delavier-Klutchko, C., Durieu-Trautmann, O. and Strosberg, A.D. (1981) Biochem. Biophys. Res. Commun. 99, 1295-1302.