Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7445–7448, December 1981
Biochemistry

Prolactin-like activity of anti-prolactin receptor antibodies on casein and DNA synthesis in the mammary gland

(prolactin receptors/antisera/casein mRNA/organ culture)

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Communicated by J. Edward Rall, September 2, 1981

ABSTRACT **Prolactin receptors were partially purified from rabbit mammary gland membranes by using an affinity chromatography technique. Antibodies against this prolactin receptor preparation were obtained in guinea pig and sheep. Both antisera were able to inhibit the binding of ^{125}I-labeled ovine prolactin to rabbit mammary gland membranes. When added to culture media of rabbit mammary explants, the anti-prolactin receptor antiserum inhibited the capacity of prolactin to initiate casein synthesis and casein mRNA accumulation as a function of the antiserum concentration. However, in the absence of prolactin, both antisera (guinea pig and sheep) at moderate concentrations were capable of mimicking prolactin action on casein gene expression and on DNA synthesis. At higher concentrations, the anti-prolactin receptor antibodies inhibited their own actions. Several characteristics of the prolactin effect were also observed with the anti-prolactin receptor antibody: the stimulatory effect of the antibody was amplified by glucocorticoids; colchicine, which was capable of blocking prolactin action, also prevented the induction by the antibody. Lysosomotropic agents, which do not interfere with prolactin action, did not alter the response observed with the antibody. These results indicate that an anti-prolactin receptor antibody can mimic two major actions of prolactin obtained in mammary explant culture and suggests that the prolactin molecule is not required beyond the initial binding to its receptor.**

Prolactin is a major hormone controlling the growth of the mammary gland and the differentiation of the rabbit mammary cell. One of the most striking events of this differentiation is the activation of casein gene transcription. Prolactin injected into pseudopregnant rabbits (1) or added to culture medium of mammary explants induces casein synthesis (2, 3). This effect is supported by a simultaneous accumulation of casein mRNAs (1, 2, 4, 5) and a stimulation of their translation (6). The accumulation of casein mRNA results from an activation of casein gene transcription and from an enhancement of casein mRNA stability (7). These effects are modulated by glucocorticoids and by progesterone (1, 2, 8, 9).

The action of prolactin on the mammary cell is mediated by a specific receptor located in membrane components of the cell. This receptor has been well characterized and has been shown to respond to both a down- and an up-regulation by the hormone (10, 11). An antibody against the partially purified receptor was obtained several years ago (12). This antibody was demonstrated to inhibit the capacity of prolactin to support the synthesis of casein and the uptake of α-isoaminobutyric acid by rabbit mammary explants in culture (13) and to attenuate the action of prolactin on mammary gland and ovary when injected into rat (14). The experiments depicted in the present report were carried out to investigate the possible stimulatory effect of an anti-prolactin receptor antibody on casein and DNA synthesis in the rabbit mammary cell.

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MATERIALS AND METHODS

Purification of Prolactin Receptors. Prolactin receptors were partially purified from crude microsomal fractions of lactating rabbit mammary glands by using human growth hormone (hGH, NIH-GH-HS19340; 2.6 units/mg), a lactogenic hormone equipotent to ovine prolactin in rabbit mammary gland, bound to Affi-Gel 10 (Bio-Rad) essentially as described by Shiu and Friesen (15).

New Zealand rabbits between day 6 and 10 of lactation were pretreated with CB-154 (Sandoz, Basel, Switzerland) for 36 hr to increase the content of prolactin receptors (16). Crude microsomes were prepared from 15 rabbits and the pellets were solubilized in 1% Triton X-100 for 2 hr at room temperature. The solubilized extract was centrifuged for 2 hr at 40,000 × *g*, and the supernatant was diluted with 2 vol of 25 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$. This reduced the Triton X-100 concentration to 0.33%, a concentration that allowed the binding reaction between the hormone and receptor to occur.

The solubilized extract was passed over a column of hGH-Affi-Gel 10 at room temperature at a flow rate of 50 ml/hr, slightly more than the bed volume of the column. After the extract was passed through the column (2 days), the column was washed successively with 1 liter of 0.1 M borate buffer, pH 7.4/0.1% Triton X-100, followed by alternate washings with 8 M urea and 4 M guanidine·HCl. The receptor was eluted with 5 M $MgCl_2$, followed by 1 vol of borate buffer. The partially purified receptor was passed over a column of Sephadex G-100 (300 ml) to remove the $MgCl_2$. The fraction eluting in the void volume was concentrated approximately 15-fold with an Amicon UM-10 filter, retaining molecular weights larger than 10,000.

Production of Anti-Prolactin Receptor Antibodies. The partially purified receptor fraction was injected at three monthly intervals into male guinea pigs and sheep at a concentration of 50 μg of antigen per injection in Freund's complete adjuvant. Animals were bled at monthly intervals 7–10 days after the booster immunization.

Binding of Prolactin to Its Receptor. The presence of receptors and the activity of the antibodies were assayed by measuring the specific binding of ^{125}I-labeled hGH or ^{125}I-labeled ovine prolactin essentially as described (10, 11, 16). The total number of binding sites were determined by Scatchard analysis to characterize the purity of the receptor preparation after the various steps in the purification. The starting material corresponded to the microsomal membranes prepared as described (15).

Abbreviation: hGH, human growth hormone.

Casein and DNA Synthesis. Mammary fragments (≈2 mm^3) from pseudopregnant rabbits were cultured for 24 hr in medium 199 in the presence of insulin (5 μg/ml) and cortisol (500 ng/ml) with or without ovine prolactin (100 ng/ml) and antibodies as specified in the figure legends. Casein synthesis was evaluated by immunoprecipitation with an anti-casein antibody after proteins were labeled with ^{14}C-labeled amino acids for 3 hr. DNA synthesis was calculated by the amount of [^{14}C]thymidine incorporated into DNA during the last 2 hr of the culture. These techniques are essentially as described (2, 17, 18). Casein mRNA concentration was measured by a hybridization with a cDNA probe obtained by a reverse transcription of partially purified β-casein mRNA (7). The anti-ovine prolactin added to the culture medium was prepared in horse, and the Ig fraction was donated by G. Kann.

RESULTS

Purification of Prolactin Receptors. Treating lactating animals with CB-154 prior to sacrifice markedly increased prolactin receptor concentrations in mammary gland to levels of ≈100 fmol/mg of protein in the homogenate, as determined by Scatchard analysis. Solubilization of the crude microsomal fraction resulted in a 3-fold increase in receptor affinity. After affinity chromatography and gel filtration on Sephadex G-100, the binding capacity of the receptor preparation was increased to 180 pmol/mg of protein, with a recovery of 4%. This represents an approximate 2000-fold increase in purity of the prolactin receptors in rabbit mammary glands.

Action of the Anti-Receptor on the Binding of Prolactin to Membranes. Antisera of animals injected with the partially purified receptor were assayed for their capacity to inhibit the binding of ^{125}I-labeled ovine prolactin to the receptors in rabbit mammary gland membranes. The antisera of immunized guinea pig and sheep were capable of preventing the binding of prolactin to its receptor (Fig. 1). Both antisera started to act at the dilution of 1:10,000; however, the sheep antibody was slightly more potent. The antisera of two other guinea pigs and of one other sheep were also active (data not shown). Sera of nonimmunized animals did not significantly alter the formation of the prolactin–receptor complex.

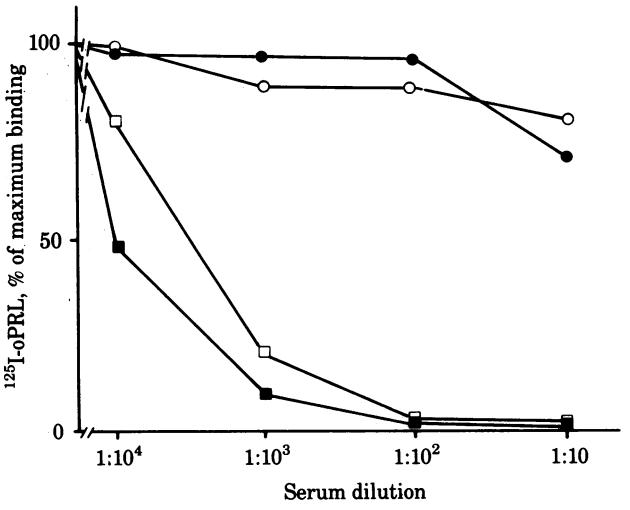

FIG. 1. Action of anti-prolactin receptor antiserum on the binding of ^{125}I-labeled ovine prolactin (^{125}I-oPRL) to mammary membranes. About 100,000 cpm of the hormone was incubated for 18 hr at 20°C with rabbit mammary membranes (200 μg of protein per incubate), in the presence of various concentrations of antiserum. ○ and ●, Control sera; □ and ■, anti-receptor-containing sera; ○ and □, guinea pig; ● and ■, sheep.

Action of Anti-Receptor on Casein Synthesis. The anti-receptor antibodies which prevented the binding of prolactin to its receptor were expected to also prevent the biological activity of the hormone. This was indeed the case: guinea pig antiserum inhibited the initiation of casein synthesis as a function of the antiserum concentrations in the medium (Fig. 2*A*). More surprising is the fact that in the absence of prolactin, the anti-prolactin receptor-containing anti-serum stimulated casein synthesis, thus mimicking prolactin action. This effect was also dose dependent: at the lowest concentrations, the antiserum was inactive, whereas at the highest concentrations, it inhibited its own action. At none of the concentrations tested did the antiserum exhibit any significant toxic effect as judged by the incorporation of ^{14}C-labeled amino acids into proteins in the explant cultures; therefore, the inhibitory effect of the antiserum at high concentrations may be considered as specific.

An examination of the activity of the sheep antiserum containing anti-prolactin receptor antibodies revealed that it also was able to mimic prolactin action on casein synthesis in a dose-dependent manner (Fig. 2*B*). This antiserum was more active in stimulating casein synthesis at low concentrations but less potent in inhibiting prolactin action at high concentrations than was the guinea pig antiserum. Control serum incubated even at high concentrations failed to inhibit the action of prolactin. Interestingly, the sheep anti-receptor antiserum at low concentrations was even slightly but significantly capable of stimulating casein synthesis when added to prolactin at 100 ng/ml (a prolactin concentration that gives the near-maximum response in this system). This fact was observed repeatedly in other experiments not shown here. It is conceivable that the stimulation, or at least part of it, by the anti-receptor antiserum is due to prolactin present in the antiserum. This hypothesis is not tenable in the case of sheep antiserum because all of the cultures were performed in the presence of anti-ovine-prolactin present

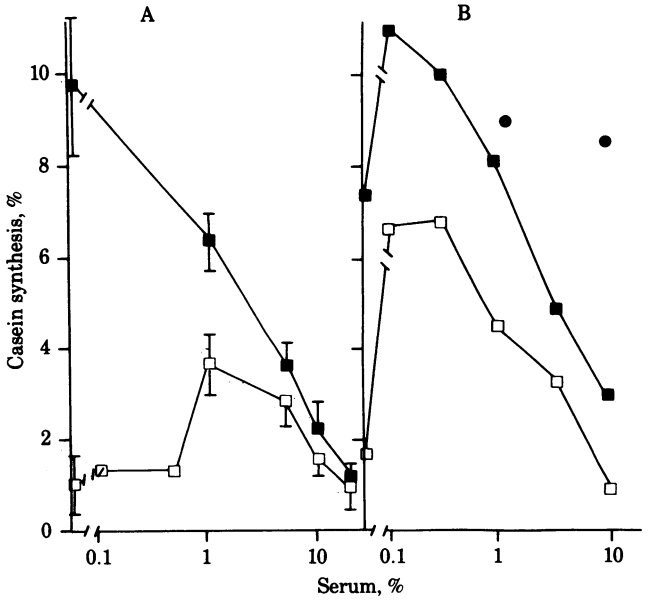

FIG. 2. Action of anti-prolactin receptor on the induction of casein synthesis. Cultures of rabbit mammary explants were carried out in all cases in the presence of insulin and cortisol with or without ovine prolactin and serum. Results are expressed as the percentage of the labeled mammary proteins precipitated by the anti-casein antibody as a function of the antiserum concentration in the medium. □, Without prolactin; ■, with prolactin; ●, with prolactin and control serum. (*A*) Results with guinea pig antiserum are the mean of four independent cultures. (*B*) Results with sheep antiserum are the mean of two experiments.

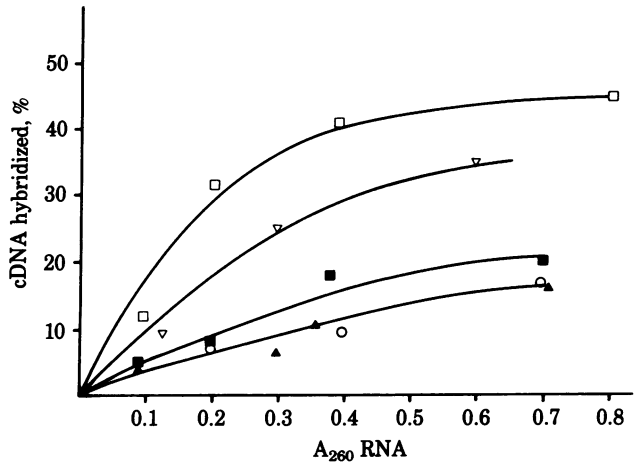

FIG. 3. Action of anti-prolactin receptor on the accumulation of β-casein mRNA. Results are expressed as the percentage of the β-casein cDNA probe in hybrid as a function of the amount of total mammary nucleic acids extracted from explants at the end of the culture and added to the hybridization medium. ■, Insulin plus cortisol; □, insulin plus cortisol and prolactin; ▽, insulin plus cortisol and 1% guinea pig anti-receptor antiserum; ○, insulin plus cortisol and 10% antiserum; ▲, insulin plus cortisol and prolactin and 10% antiserum.

in the culture medium in sufficient amount to suppress the effect of 1 μg of prolactin per ml.

Action of the Anti-Receptor on the Accumulation of β-Casein mRNA. The initiation of casein synthesis in organ culture by prolactin has been shown to be accompanied in all cases by a parallel accumulation of the corresponding mRNA (2). This effect of prolactin was also blocked by a high concentration of guinea-pig anti-receptor antiserum (Fig. 3). Similarly, this antiserum was able to mimic prolactin action at moderate concentrations and to suppress its own effect at high concentrations. Under all conditions examined, there was an excellent agreement between the rate of casein synthesis and the content in β-casein mRNA.

Glucocorticoids are known to favor prolactin action on casein mRNA accumulation and to a lower degree on casein synthesis, although being inactive alone (2–4, 8, 9, 19, 20). Casein synthesis in the presence of cortisol was 18% and 20% above the control culture in the absence of the glucocorticoid when stimulation was obtained with prolactin and anti-receptor, respectively (not shown). β-Casein mRNA was accumulated also more readily when cortisol was added with the anti-receptor antiserum (Fig. 4). The sheep anti-receptor antiserum was also capable of stimulating lactose synthetase activity in mammary explants, and this effect was also amplified by cortisol (data not shown).

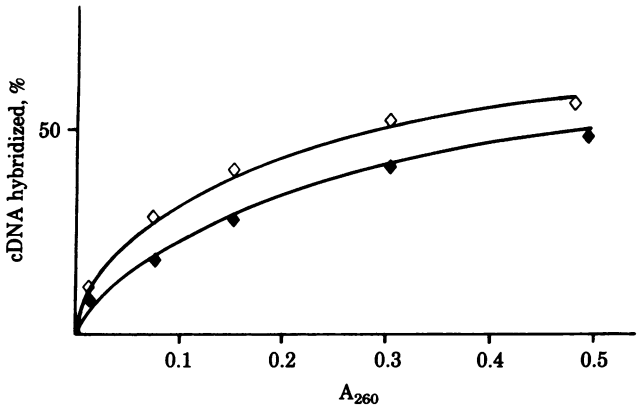

FIG. 4. Action of cortisol on the accumulation of β-casein mRNA. Culture was carried out in the presence of 0.5% sheep anti-prolactin receptor with cortisol (◇) and without cortisol (◆).

Table 1. Effect of various drugs on the initiation of casein synthesis by anti-receptor antiserum

Culture medium	Casein synthesis, %
IC	2.7
IPC	13.2
IC + 0.5% control serum	3.7
IC + 0.5% anti-receptor antiserum	10.0
+1 μM colchicine	3.5
+10 mM NH_4Cl	10.7
+100 μM chloroquine	10.5

Results are the mean of two independent cultures. I, insulin (5 μg/ml); P, prolactin (100 ng/ml); C, cortisol (500 ng/ml). Sera used in this study were obtained from a nonimmunized sheep (control) or a sheep which received three successive immunizations with a partially purified prolactin receptor preparation.

Effects of Various Drugs on the Initiation of Casein Synthesis by Anti-Receptor Antiserum. Results in our laboratory have pointed out that the prolactin action on casein and DNA synthesis is strongly blocked by colchicine (17, 18, 21) but unaffected by lysosomotropic agents (17). Attempts were made to determine whether the stimulation by the anti-receptor shares these properties of the stimulation by prolactin. Colchicine totally prevented the stimulation of casein synthesis by the anti-receptor (Table 1). Two lysosomotropic agents (NH_4Cl and chloroquine) were essentially ineffective.

Action of the Anti-Receptor on DNA Synthesis. Prolactin added to culture medium is able to enhance the incorporation of [^{14}C]thymidine into DNA (18). In three experiments not depicted here in detail, prolactin stimulated DNA synthesis by 340 ± 82%, whereas the sheep anti-receptor antiserum at the concentration of 0.5% stimulated DNA synthesis by 213 ± 43% above controls observed with serum of a nonimmunized sheep. This property of the anti-receptor was further examined with the immunoglobulin fraction of guinea pig anti-receptor antiserum. This fraction, which was free of endogenous prolactin,

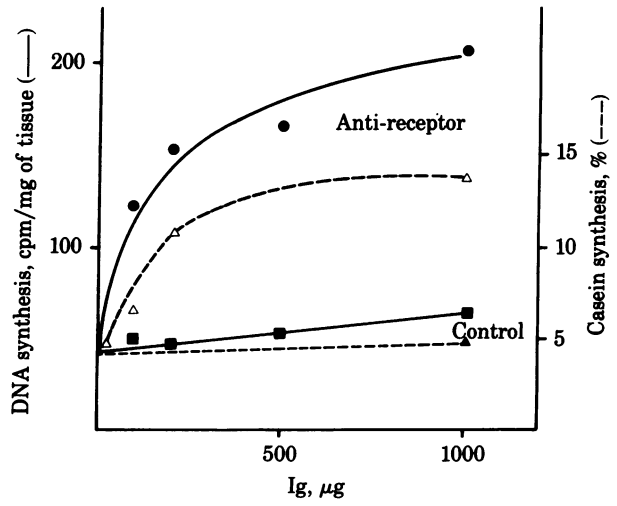

FIG. 5. Action of Ig fraction of guinea pig anti-prolactin receptor antiserum on casein and on DNA synthesis. Results are expressed as the percentage of labeled proteins precipitated by the anti-casein antibody and as cpm of [^{14}C]thymidine incorporated into DNA as a function of the amount of Ig added to the culture medium. ▲, ■, Ig of a nonimmunized guinea pig; △, ●, Ig of a guinea pig immunized with prolactin receptor. The dotted lines are casein synthesis.

exhibited a strong capacity to stimulate the incorporation of [^{14}C]thymidine into DNA, whereas a similar fraction prepared from a nonimmunized guinea pig was essentially devoid of activity (Fig. 5).

DISCUSSION

The anti-prolactin receptor antibody is a potent inhibitor of at least one prolactin action on the mammary cell: the initiation of casein synthesis. This fact is in good agreement with earlier observations of Shiu and Friesen who reported other inhibitory actions of anti-prolactin receptor on the mammary gland (13) and on ovary (14). The data of the present paper indicate that at low concentrations, the antibody can also mimic two prolactin actions. This result is reminiscent of the action of anti-insulin receptor found in the serum of some patients or obtained from purified receptors that can mimic insulin action on the uptake and oxidation of glucose (22–28). However, it should be noted that these effects of insulin or anti-insulin receptor antibodies do not necessarily involve specific gene activation, although a late insulin-like effect mediated by the anti-insulin receptor has been reported (29). By contrast, the action of prolactin and anti-prolactin receptor on casein and DNA synthesis requires the transfer of a message from the receptor to the genome. A comparison of the prolactin and anti-prolactin receptor actions indicates that they share common properties as far as the effects of cortisol and various drugs are concerned, suggesting that the antibody has been able to generate a large part of the prolactin messages normally delivered in the mammary cell. It is possible that an internalized receptor may evoke the responses. However, we feel that these observations are in agreement with the hypothesis that the binding of prolactin to its receptor provokes changes in the conformation of the receptor and probably of the surrounding membrane components leading to the formation of second messengers eliciting the hormone action in the cell. The anti-prolactin receptor might act by inducing such modifications of the receptor. However, it should be kept in mind that the receptor fraction used as antigen and, thus, the anti-receptor antibody were not pure entities. Therefore, the action of the antibody may have been mediated through binding to membrane components present in the antigen but not in the receptor. In this respect, it is worth noting that the insulin-like activity of antibodies to adipocyte plasma membrane antigens appears to be elicited neither by direct interaction with the insulin receptor nor with the glucose transport system (30, 31); that insulin action can be mimicked by lectines (32), diamide, H_2O_2 (33, 34), and vitamin K_5 (33); and also that an anti-thyroid membrane antibody can mimic thyrotropin action to generate the formation of cAMP (35). The results of the present report do not support a recent hypothesis attributing to different prolactin fragments the capacity to specifically and selectively activate the various parameters in the mammary cell (36–38). Whatever mechanism is involved in the anti-prolactin receptor action on the mammary gland, the major conclusion that can be drawn from our data is that the prolactin molecule does not appear strictly necessary beyond its bindi: g to the receptor for the transmission of the hormonal message and that the receptor plays a role of transducer probably by generating a second messenger carrying the hormonal information to the genome.

The authors express their gratitude for the helpful suggestions concerning the purification of prolactin receptor provided by Dr. R. P. C. Shiu, Winnipeg, Manitoba; to the Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases, for providing hGH and ovine prolactin; to Prof. Hubert Clauser for helpful discussions; and to Dr. G. Kann, Institut National de la Recherche Agronomique, Jouy-en-Josas, for kindly donating the anti-ovine prolactin Ig fraction. These studies were supported in part by grants from the Medical Research Council of Canada, National Cancer Institute (Canada), the Centre National de la Recherche Scientifique, the Institut National pour la Santé et la Recherche Médicale, and the Délégation à la Recherche Scientifique et Technique. The technical assistance of Mr. G. Leblanc, Mrs. Lucette Bélair, and Claudine Puissant is greatly appreciated.

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