Receptors, antibodies
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Prolactin and growth hormone receptors

H. G. FRIESEN, R. P. C. SHIU, H. ELSHOLTZ, S. SIMPSON and J. HUGHES

Department of Physiology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Canada R3E OW3

Abstract The two hormones prolactin and growth hormone exhibit considerable structural homology as well as exerting similar biological effects, especially the primate hormones. One effect of prolactin that deserves greater attention is its action on the immune system including the stimulation of growth of experimental lymphomas, both *in vivo* and *in vitro*. One cultured lymphoma cell line has proved to be a very useful model system in which to examine prolactin receptor synthesis and turnover as well as post-receptor mechanisms of action.

Prolactin and growth hormone receptors from rabbit mammary gland and liver respectively have been partially purified and characterized. Polyclonal antibodies to prolactin and growth hormone receptors have been generated. The antibodies have been shown to cross-react with prolactin or growth hormone receptors from a number of species, indicating structural homology among receptors as well as hormones. The polyclonal antisera inhibit the action of prolactin *in vivo* as well as *in vitro*. In addition, several of the same antisera also mimic the action of prolactin. As yet the presence of autoantibodies to prolactin or growth hormone receptors in human serum samples has not been recognized.

The extensive structural homology between the two pituitary hormones, growth hormone (GH) and prolactin, as well as their overlapping spectrum of biological effects, prompted us to examine the receptors for these two hormones. The ontogeny, distribution, regulation, purification and characterization of the two receptors has been one focus of our research for almost a decade. In this symposium selected aspects of our work will be reviewed with particular emphasis on the characterization of the receptors and the use of antibody probes to examine the two receptors. Despite the accumulation of considerable information about these two receptors, their precise role in mediating hormone action and the mechanisms involved remain obscure. An emerging concept that deserves more scrutiny is that prolactin itself may have an immunoregulatory role. The data on this last point are derived from a

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series of isolated and unrelated reports which taken together provide compelling evidence for this point of view.

Prolactin and the immune system

In keeping with the symposium's title of 'Receptors, Antibodies and Disease', we wish first to address the role of prolactin in immunoregulation. Prolactin is a fascinating hormone with many and varied actions ranging from stimulation of milk-protein synthesis to action as a luteotropic agent to the promotion of growth. In one review more than 80 biological effects of prolactin were documented but no mention was made of any action on lymphoid tissue or immune mechanisms (Nicoll 1974). The first example of the effect of prolactin on lymphoid tissues relates to the mammary gland and IgA-secreting cells. Weisz-Carrington et al (1978) reported that the migration of IgA immunoblasts into the mammary gland is a hormonally mediated event, with prolactin greatly enhancing the entry of IgA-secreting plasma cells. An immunoregulatory role for prolactin has also been suggested by experiments by our colleagues at the University of Manitoba; and this is the second example we wish to cite. Berczi & Nagy (1981) have shown that antibody production, delayed hypersensitivity to dinitrochlorobenzene, rejection of skin grafts and the development of adjuvant arthritis are all impaired either after hypophysectomy or after the pharmacological suppression of prolactin secretion with bromocriptine.

The third example derives from the remarkable and specific growth-stimulating effect of prolactin on an experimental rat lymphoma (Noble et al 1980). This tumour has proved to be of considerable interest, since the lymphoma cells derived from the tumour begin to proliferate in a dose-dependent manner when prolactin is added to the culture medium. These cells form the basis of an exquisitely sensitive bioassay for prolactin (pg/ml), have receptors for prolactin and are useful for examining the mechanism of action of prolactin (Tanaka et al 1980). The growth rate of lymphomas *in vivo* is accelerated in proportion to the elevation of serum prolactin levels. Thus during pregnancy the growth of tumour to a 10 g size takes only 10 days compared to 38 days in a control rat. Parenthetically, it is interesting that when Burkitt's lymphoma occurs during pregnancy it often presents with extensive mammary gland infiltration. This association raises the question whether prolactin in some manner influences the proliferation of lymphoma cells at this site. Of some interest is the fact that several human lymphomas that we have examined have prolactin and growth hormone receptors and, perhaps of even greater interest, appear to concentrate prolactin and human growth hormone (hGH) in their cytosol (Table 1). The growth of leukaemic

TABLE 1 Prolactin and growth hormone-like activity in extracts of human lymphomas

	Nb2 cell count $\times 10^3$				
	Tissue	*Extract +*	*Extract +*	*PRL(ng/g)*	
Specimen	*Extract*	*anti-hPRL*	*anti-hGH*	*Bioassay*	*RIA*
1	41.4	32.1	27.0	7.5	7.5
2	104.0	61.0	50.5	55.0	10.0
3	58.6	42.5	34.9	15.0	15.0

The lymph nodes from three patients were homogenized in 0.3 M-sucrose in ratio of 1 to 5 (w/v) and centrifuged at 100 000 × ***g***. The supernatant (50 μl added to 2 ml culture media) was assayed by Nb2 cell bioassay. These assays were repeated in the presence of either anti-hPRL or anti-hGH. The cell count in the control in the absence of prolactin was 21.1 × 10^3 cells. It is apparent that all three lymphomas stimulate growth of the Nb2 cells. Not all the mitogenic activity is neutralized by anti-hPRL or anti-hGH. In the case of lymphoma 2 the bioassay estimate of PRL is five-fold greater than the radioimmunoassay (RIA) estimates.

cells and an increase in colony-forming units under the influence of hGH also has been reported (Desai et al 1973, Golde et al 1980).

Prolactin and the Nb2 lymphoma cell

The Nb2 cell is a poorly differentiated lymphoblastic cell with a high mitotic index. Electron microscopy indicates the absence of phagocytic vacuoles, suggesting that this tumour is not of macrophage origin. Immunocytochemical studies have revealed an absence of both surface and cytoplasmic IgG. It is worth noting that most (perhaps as many as 75% of) human lymphoblastic lymphomas are of B cell origin, yet some tumours may express surface immunoglobulins poorly. No detectable α-naphthyl-acetate esterase activity, a marker for T cells, was found on the Nb2 cells. Further characterization with antibodies to IgA, IgM and IgE and antibodies to T cell helper and non-helper cells is under way.

Fig. 1 shows the dose–response curve and the specificity of Nb2 node lymphoma cells in culture. The mitogenic response appears to be specific for lactogenic hormones. In addition, as shown in Fig. 2, prolactin receptors on the Nb2 cell exhibit the same specificity and affinity as was previously reported for the rabbit mammary epithelial cell (Shiu et al 1973). In an experiment examining the prolactin concentration required for half-maximal receptor occupancy and half-maximal stimulation of lymphoma cell growth it was shown that 5.8 pM prolactin concentrations stimulated growth to 50% of maximum while 50% receptor occupancy occurred only at 75 pM. Similarly, the minimal effective concentration to inhibit prolactin binding was 10- to 15-fold greater than required to stimulate cell growth, with a maximal effect on growth observed at 30% receptor occupancy. These results are very

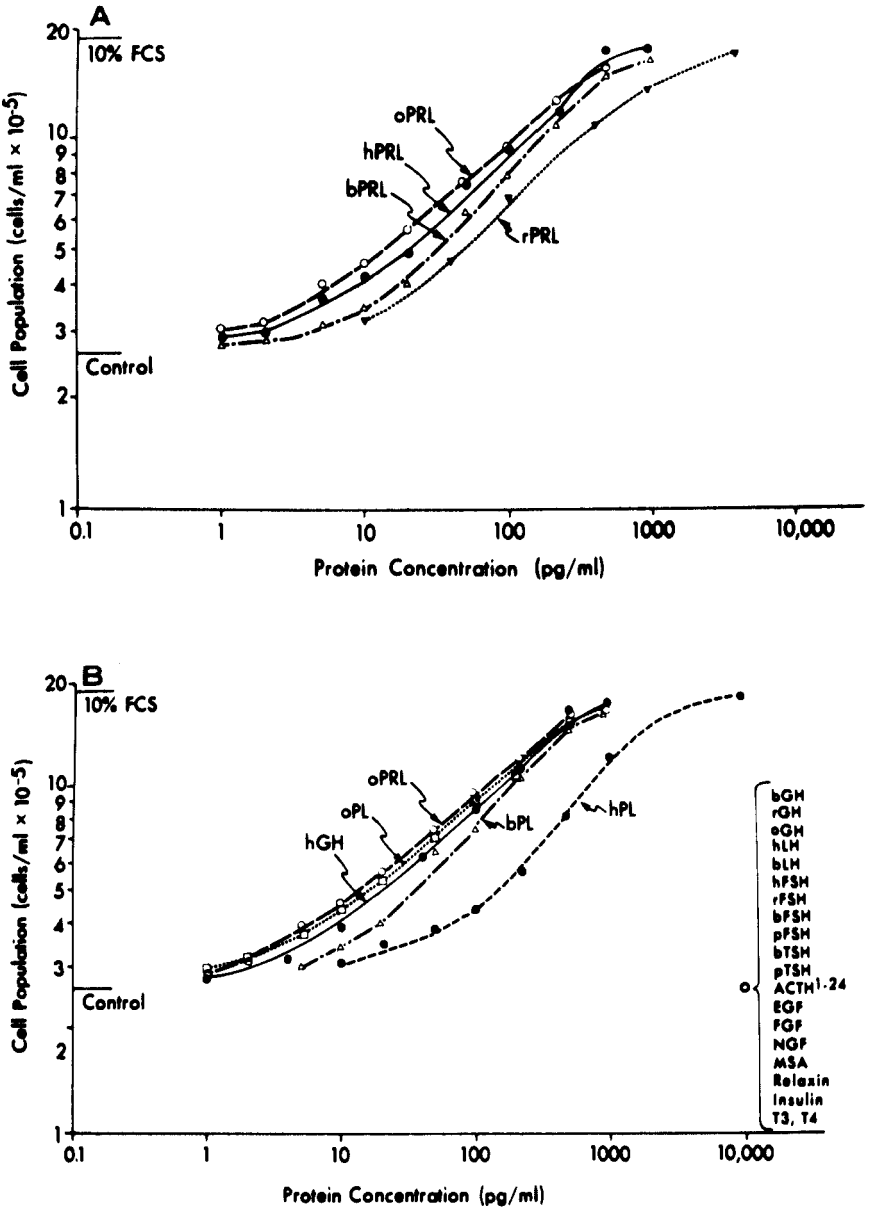

FIG. 1. The increase in lymphoma cell number over a three-day incubation period. A. The effect of purified prolactin from four species (ovine, bovine, rat, human). B. Lactogenic hormones, including primate growth hormone, cause an increase in cell number whereas other peptides and hormones do not.

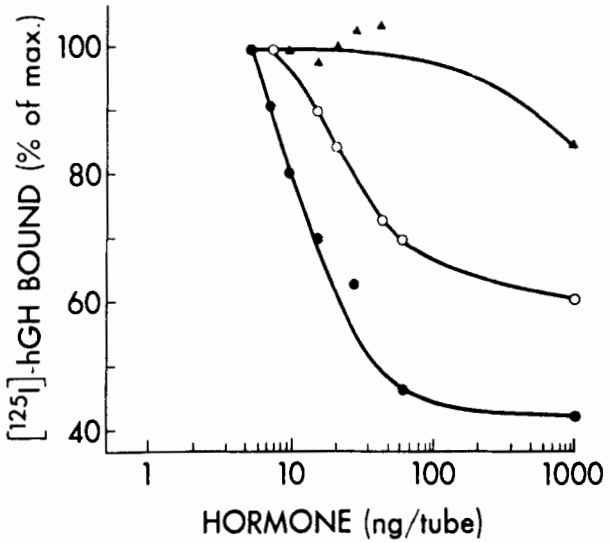

FIG. 2. The inhibition of binding of ^{125}I-labelled hGH to lymphoma cells by human prolactin (●—●) and ovine prolactin (○—○). Ovine growth hormone (▲—▲) or other non-primate growth hormones do not inhibit the binding of ^{125}I-hGH to receptors. Thus ^{125}I-labelled hGH is binding to prolactin receptors. Note that only primate growth hormones show both lactogenic and somatogenic activity.

similar to those reported for other hormones and receptors where a biological response is elicited by a concentration of hormone which is at least an order of magnitude less than that which leads to measurable occupancy (Dufau & Catt 1976).

When incubated in calf serum the lymphoma cells continue to proliferate, as a result of the mitogenic effect of endogenous prolactin and placental lactogen. Under these circumstances down-regulation of receptors occurs. When Nb2 cells are incubated in the presence of horse serum the cell number remains constant but the number of receptors increases at least twofold (Fig. 3). This increase in receptor number is abolished when the culture medium contains 100 ng/ml cycloheximide, and as little as 10 ng/ml partially prevents the increase. After binding of ^{125}-I-labelled prolactin to the surface of Nb2 cells, the tracer is internalized and degraded. Maximal binding of ^{125}I-prolactin is achieved about one hour after incubation at 37 °C, after which time the quantity of cell-bound radioactivity decreases with incubation time.

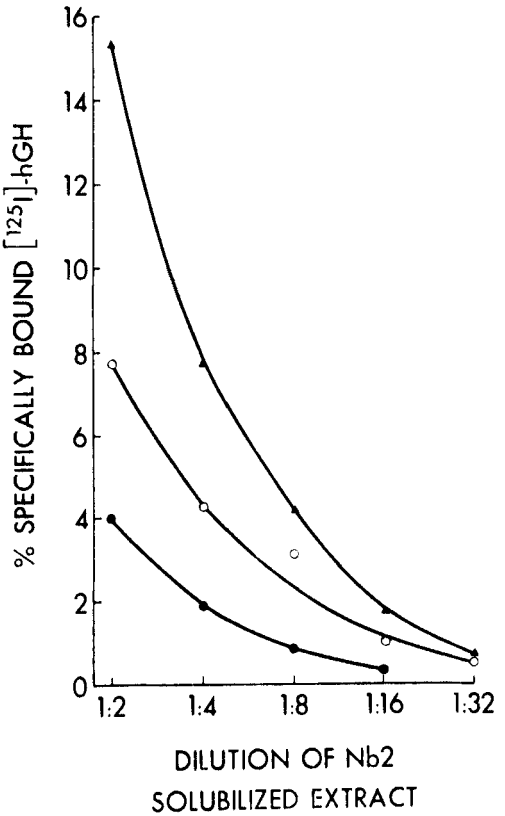

FIG. 3. When lymphoma cells are incubated for extended periods in 10% horse serum (stationary growth phase) there is a gradual increase in binding of ^{125}I-labelled hGH to receptors. ●—●, 4 h in horse serum. ○—○, 8 h in horse serum. ▲—▲, 24 h in horse serum. The increase in binding is presumed to be a return to a basal state after down-regulation of receptors by exposure to prolactin present in calf serum.

Biosynthesis and turnover of receptors

The biosynthesis and turnover of prolactin receptors in Nb2 cells has been examined using density-labelling methods. Lymphoma cells were incubated with ^{2}H- and ^{13}C-labelled amino acids. After 12 hours the cells were solubilized and the extracts were subjected to density equilibrium ultracentrifugation using caesium chloride gradients. Fractions of the gradients were assayed for prolactin receptors. Newly synthesized prolactin receptors were recognized by a density shift in the receptors in caesium chloride gradients. The refractive index at which the prolactin receptor banded when cells were

in the usual culture media was 1.3575 while the ^{2}H-, ^{13}C-labelled receptor had a refractive index of 1.361—a downward shift of 0.004 to 0.005 ηD units.

After 12 hours of exposure to heavy amino acids, virtually all the prolactin receptor was density-labelled, implying the fairly rapid turnover of these receptors. This approach to the study of the synthesis of the prolactin receptor circumvents the methodological difficulty inherent in studies using radioactive isotopes which demand the purification and/or specific identification of labelled receptors. It also makes it feasible to investigate the regulation of synthesis, transport to the cell surface and degradation of prolactin receptors in cultured prolactin-responsive cells.

Characterization of prolactin and growth hormone receptors

The purification and characterization of rabbit mammary gland prolactin receptors has been reported (Shiu & Friesen 1974). Rabbit mammary glands were homogenized and centrifuged and a 100 000 × *g* pellet was obtained containing the bulk of prolactin receptors. The particulate membrane fraction was solubilized with Triton X-100 and prolactin receptors were purified by affinity chromatography. An approximate 2000-fold purification was achieved. Isoelectric focusing of purified receptor protein reveals several bands with maximal binding in eluants with a pH of 6 to 7. Complete purification of membrane receptors is complicated by the presence of detergents which retain contaminating membrane lipids and proteins in micelles along with the receptor.

Similar approaches have been used to purify and characterize the growth hormone receptors from rabbit liver (Waters & Friesen 1979a, b). Instead of using 5 M-$MgCl_2$ to elute GH receptors we used 2.5 to 4 M-urea. Under these circumstances GH receptors but not prolactin receptors, which are also present in rabbit liver, were eluted. Subsequently the latter could be eluted with 5 M-$MgCl_2$. Table 2 summarizes some of the known characteristics of prolactin and GH receptors.

Antibodies to receptors

The purified GH or prolactin receptors were used for immunizing guinea-pigs to generate antibodies to receptors. The antibodies were detected by the inhibition of binding of ^{125}I-prolactin or GH to particulate membrane fractions containing receptors for prolactin and GH (Shiu & Friesen 1976b, Waters & Friesen 1979b). Antibodies to rabbit prolactin receptors inhibit the binding of this hormone to rabbit mammary gland receptors, and, to varying

TABLE 2 Properties of rabbit mammary gland prolactin and rabbit liver growth hormone receptors

	Prolactin	*Growth hormone*
Composition	Glycoprotein	Sialoglycoprotein
Relative molecular mass (M_r) in Triton	220 000	300 000
Subunit size (M_r)	64 000 and 75 000	70 000 tetramer
pI	6.5	4.7
K_a	3.16×10^9 M^{-1}	2×10^9 M^{-1}
Common antigenic sites	rabbit, rat, human	rabbit, rat, mouse, sheep, human

degrees, to prolactin receptors located on target tissues from several different species. Thus not only are the amino acid sequences of hormones conserved among species, but homology of receptors for the same hormones in different species is also evident.

The antisera have been shown not only to inhibit binding of these hormones to receptors but also, in the case of prolactin, to block hormone-mediated events *in vitro* and *in vivo*. In mammary gland explants one of the guinea-pig antisera inhibits prolactin stimulation of casein synthesis and aminoisobutyric acid transport while not interfering with glucose utilization by the explants (Shiu & Friesen 1976a). When antisera to the prolactin receptor was administered to lactating rats, milk production was inhibited, as judged by a decreased weight gain of pups suckling dams that were injected daily with antisera to the prolactin receptor (Bohnet et al 1978).

Antibodies to the GH receptor do not inhibit the binding of prolactin to the mammary gland prolactin receptor and vice versa (Fig. 4). As yet, antibodies to the GH receptor have not been shown to inhibit any biological effects stimulated by growth hormone. One attempt to inhibit the weight gain in GH-treated hypophysectomized rats by anti-GH receptor antisera proved unsuccessful.

More recently, we have examined the effects of antisera to prolactin receptors on the stimulation of Nb2 cell growth. A number of antisera inhibit the full expression of the mitogenic effect of prolactin on cell growth, while themselves exerting a modest proliferative effect on the growth of Nb2 cells (Fig. 5). The IgG fraction as well as the bivalent $F(ab')_2$ fragments derived from the IgG of these antisera cause these effects while the univalent Fab fragments fail to stimulate growth of the Nb2 cell. Thus the cross-linking and clustering of surface prolactin receptors appears to be a prerequisite for triggering hormone-mediated events, which is similar to the effects of other hormones. In addition, these experiments, together with comparable experi-

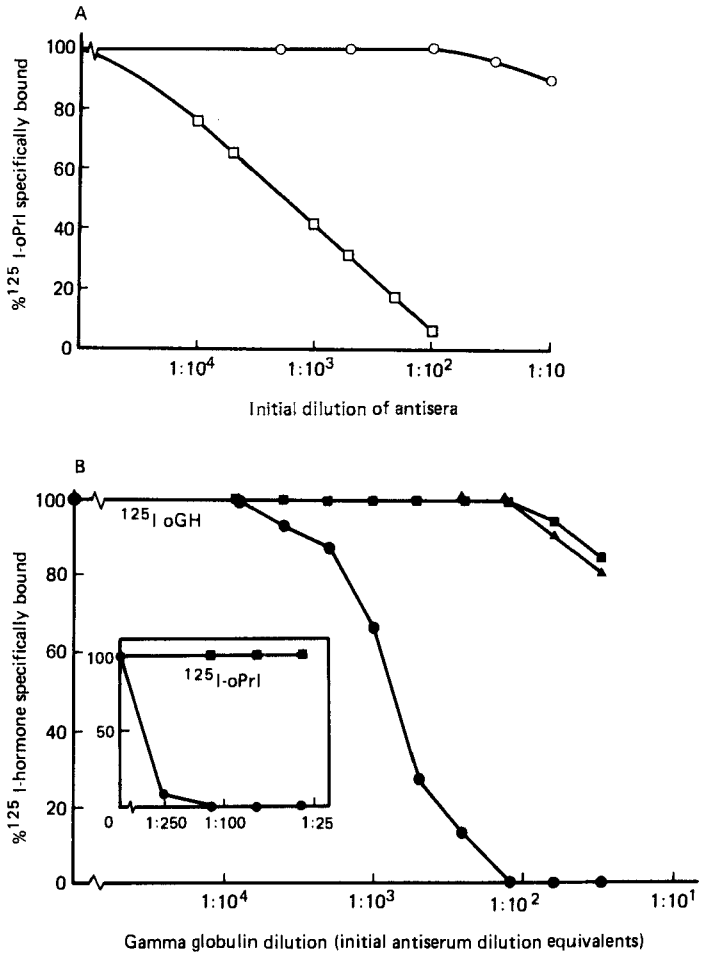

FIG. 4. A. Antibodies to the prolactin receptor inhibit binding of ^{125}I-labelled ovine prolactin (^{125}I-oPrl) to rabbit mammary gland receptors (□—□) but antibodies to GH receptor do not (○—○). B. Effect of anti-receptor IgG fraction on binding of ^{125}I-oGH to GH receptor purified by affinity chromatography (urea fraction). ●—●, anti-GH receptor. ■—■, anti-prolactin receptor IgG. ▲—▲, normal guinea-pig IgG fraction. The inset shows the effect of the IgG on binding of ^{125}I-oPrl to the same purified receptor. Anti-GH receptor but not anti-prolactin receptor antibodies inhibit binding of ^{125}I-oPrl. Therefore, we conclude that ^{125}I-oPrl does bind to GH receptor.

ments for other hormones, provide compelling evidence that the protein sequence embraced by the prolactin molecule is not a mandatory structural requirement for initiating and mediating the action of prolactin.

We have begun to generate monoclonal antibodies to prolactin and growth

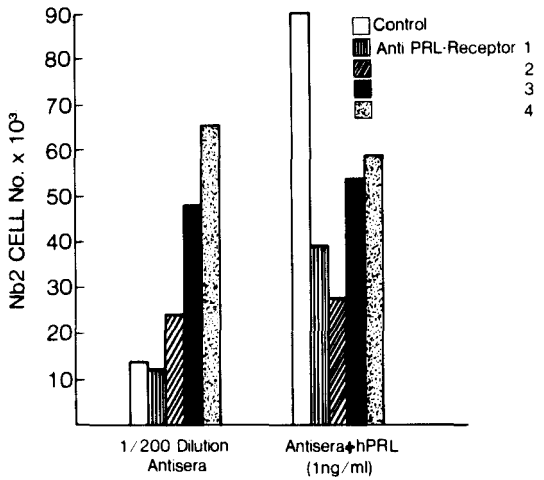

FIG. 5. Effect of antisera to prolactin receptors on the growth *in vitro* of Nb2 cells. (The antisera were generated in goats by Dr P. Kelly, Laval University.) When Nb2 cells were incubated with four different antisera a significant stimulation of cell growth was evident with three of them (left-hand columns). Similar results were obtained with guinea-pig antisera to prolactin receptors (results not shown). In the presence of 1 ng/ml human prolactin (hPRL), all antisera at 1/200 dilution inhibited the proliferative response of the Nb2 cells to prolactin, whereas control sera did not (right-hand columns).

hormone receptors using the techniques described by Köhler & Milstein (1975). Antibodies are readily generated in mice against growth hormone receptors. The detection of low titre antibodies to hormone receptors in culture media from hybridomas, however, does present some problems. The presence of fetal calf sera in the media inhibits binding to receptors to a considerable extent because of the endogenous lactogenic hormones. Thus, we have covalently coupled ^{125}I-hGH to rabbit liver receptors for growth hormone using disuccinimidyl suberate. The conjugate was partially purified to remove free hGH and test antisera were examined for their ability to precipitate the ^{125}I-hGH–GH receptor complex (Table 3). Subsequently we have been successful in propagating several hybridomas that secrete antibodies which inhibit the binding of ^{125}I-hGH to particulate receptors from rabbit liver. We are now in the process of characterizing the specificity and IgG class of the antibody produced.

Conclusion

The objective of studies on growth hormone and prolactin receptors has been to gain an understanding of the mechanism of action of each of these

TABLE 3 Immunoprecipitation of ^{125}I-hGH–GH receptor complex

	Radioactivity in immunoprecipitate (c.p.m.) (antiserum dilution)		
	1:100	*1:200*	*1:500*
Control	865 ± 47[a]		
Antiserum 1	1039 ± 8	1070 ± 115	1090
Antiserum 2	1676 ± 18	1418 ± 61	1143

Rabbit liver was solubilized in 1% Tris-Triton, pH 7.6. A fraction was incubated overnight with ^{125}I-hGH and the bifunctional cross-linking reagent disuccinimidyl suberate (0.25 mM) was added for 30 min at room temperature the next morning. The mixture was added to a Con A column and the unbound ^{125}I-hGH removed. The eluted ^{125}I-hGH–GH receptor was then used as label to assess the presence of antibodies in mouse sera: 50 μl of serum at 1:100, 1:200 and 1:500 dilution was incubated with 25 μl of label (^{125}I-hGH coupled to GH receptor) and 50 μl of buffer or normal mouse serum to give a 1 100 serum concentration and 430 μl of buffer (1% bovine serum albumin in phosphate-buffered saline, pH 7.5). Normal mouse serum served as a control. After incubation at room temperature for 3 h, 100 μl of goat anti-mouse IgG at a dilution of 1:2 was added to precipitate mouse IgG. This mixture was left overnight at 4 °C. The mixture was centrifuged, the supernatant decanted and the pellet counted.
[a]Mean ± SD.

hormones. Some progress on this path has been achieved but much remains to be discovered. The isolation and complete characterization of the receptors must be accomplished, and the mediators of hormone action that are generated after the hormone–receptor interaction remain to be identified. It is our view that the lymphoma cell offers an excellent model system in which to study these questions.

Acknowledgements

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DISCUSSION

Kahn: Thinking of screening human diseases for anti-prolactin receptor antibodies, there are many patients with idiopathic galactorrhoea, gynaecomastia, and some women with macromastia which can be unilateral, sometimes almost mimicking autonomous growth. One wonders if any of these breast diseases could be associated with anti-prolactin receptor antibodies.

Friesen: We have looked at selected examples of some of these conditions, particularly patients with hyperprolactinaemia and galactorrhoea. So far we have not found any patients with autoantibodies to the prolactin receptor.

Bitensky: Have you looked at patients with prolactin-producing microadenomata? Could these pituitary tumours be driven by a prolactin receptor antibody?

Friesen: Yes; we looked at 25 or 30 of these patients, and haven't found any prolactin receptor antibodies.

Jacobs: Does prolactin have any effect on the turnover of its receptor?

Friesen: Density-labelling studies, currently under way, should provide an answer to that question.

Harrison: What is the evidence that this growth factor, which is not prolactin itself, because it is not inhibited by antibodies to prolactin, is not an activated prolactin receptor, by analogy with the effects of steroids in activating their receptors? You are looking at a growth effect, which presumably is mediated inside the target cell by an action at the nucleus. Is there any evidence that it is 'activated' receptor that you are looking at, or a piece of it?

Friesen: We have no direct evidence that the putative mediator is or is not part of the prolactin receptor. If it were the receptor, or a component of it, it would have to be released from the liver membranes which are co-cultured with the lymphoma cells, enter these lymphoma cells and trigger the mitogenic response in them.

Harrison: The 'factor' would have to do that anyway?

Friesen: Yes, but very little receptor is shed from particulate cell membranes into culture media, so on that basis the factor is unlikely to be part of the receptor. But of course it could be a small fragment derived from it.

Mitchison: It would be of considerable interest if any of your antisera had an agonist action. Have you looked for this effect at high concentrations of the antibody? Do you have an assay which would pick up a growth hormone agonist effect?

Friesen: We have tested our anti-growth hormone receptor antisera to see whether they block the effect of growth hormone in a hypophysectomized rat. In that study, they had no effect; but perhaps we didn't give enough antiserum. We didn't see any stimulating effect of the growth hormone receptor antisera on growth in this model.

Drachman: In your studies where bromocriptine (which suppresses prolactin secretion) altered immune responses, did it abolish an existing response or did it prevent the subsequent development of immune responses?

Friesen: The drug was given concurrently with the immunization. We haven't yet looked to see whether bromocriptine abolishes an established immune response.

Mitchison: Have you looked at the antisera to the prolactin receptor for immunosuppressive activity? The prediction from your results is that these antisera should be immunosuppressive.

Friesen: Provided that the antibody titre generated passively is of sufficient magnitude, yes. We haven't done this yet.

Roitt: Could bromocriptine or its analogues be given to patients with lymphoma?

Friesen: Bromocriptine is primarily used clinically to reduce prolactin

secretion in patients with hyperprolactinaemia. We haven't used it in lymphoma patients or as an immunosuppressant in other patients.

Harrison: In the prolactin adenoma patients you find no antibodies to the prolactin receptor, but antibodies to pituitary tissue have been reported by Franco Bottazzo and Deborah Doniach in such patients. Do these antibodies stimulate the pituitary to make prolactin? If there are such antibodies, is their production being inhibited by bromocriptine?

Friesen: That hypothesis has not escaped us, but I don't know the answer.

Bitensky: In patients treated with bromocriptine for microadenomata, there is no clinical evidence for immunosuppression, so far as I know.

Friesen: No, but to my knowledge no one has looked for this.

Hall: It is an interesting idea that prolactin might be involved in attracting immune cells to the breast so that these cells can produce the specific secretory immunoglobulins (IgA). Has anyone looked at this, in animals or in patients, where there is hyperprolactinaemia or bromocriptine suppression of prolactin, and measured the specific immunoglobulin content of the milk?

Friesen: Not to my knowledge.

Mitchison: These experiments could be interpreted entirely in terms of changes in the breast tissue rather than in the plasma cells. Is there evidence, in the experiment on the accumulation of plasma cells in the breast tissue, of an effect on the plasma cell rather than on the breast tissue?

Friesen: The data of Weisz-Carrington et al (1978) indicate an increase in the number of IgA-secreting plasma cells and in the amount of IgA in the mammary gland of virgin mice treated with prolactin. These increases appear to be due to the enhanced capacity of the gland to attract or retain precursors of IgA plasma cells derived from gut-associated lymphoid tissue.

Vincent: Have you compared the prolactin receptor on lymphocytes with the receptor you isolate from rabbit mammary glands?

Friesen: The prolactin receptor on the Nb2 rat lymphoma cells cross-reacts with antibodies generated against rabbit mammary gland prolactin receptors. Antigenically therefore they appear to be the same.

Jacobs: Is there any increased incidence of lymphomas in patients with hyperprolactinaemia?

Friesen: Not that I'm aware of, but I am also unaware of anyone who has examined that question.

Davies: How about prolactin receptors on normal lymphocytes? Have you had any luck with isolating those?

Friesen: The concentrations that we have found are very low.

Kahn: The lymphocyte lines that we have haven't been screened systematically for prolactin receptors, only for growth hormone receptors. The hGH receptors are present in varying amounts on different cultured human lymphoblastoid cells.

Davies: In the normal human, do you then think there are high affinity prolactin receptors in low concentrations, on the lymphocytes?

Friesen: Yes.

Roitt: It would be interesting to look at blast cells, induced by mitogen stimulation of lymphocytes. Often the blast cells express surface molecules that are not abundant on the resting lymphocyte, because it is not very interested in doing anything at that stage.

Harrison: Could this growth stimulation be a heterologous effect of prolactin—an indirect effect mediated by another receptor for a growth factor in the medium in which you grow the lymphoma cells? In other words, could prolactin up-regulate receptors for other growth factors in the medium?

Friesen: There are no data to suggest that possibility. However, the mechanism of action of prolactin on casein synthesis by the mammary gland has been clarified by Djiane et al (1982). They have done similar studies to ours; namely, incubating prolactin with plasma membranes from target tissues to determine whether a mediator of the action of prolactin is generated. They report that only plasma membranes from target tissues of prolactin release a mediator. The signal generated from the plasma membrane appears to be a small molecular weight peptide. They postulate that this peptide activates the nuclear mechanisms leading to an increase in the transcription of casein gene mRNA.

Mitchison: Are antisera against prolactin itself also powerful immunosuppressive agents *in vivo*?

Friesen: They haven't been examined for this effect but from the results I presented, one would predict that they should be.

Bottazzo: Are patients treated with bromocriptine more susceptible to viral infections than normal?

Hall: No, but this is at a crude clinical level. There could be more subtle evidence of immune deficiency.

Friesen: It is also important to recognize that in the clinical setting when bromocriptine is used, circulating levels of prolactin are never decreased to zero. In most conditions for which bromocriptine is given, prolactin levels are reduced from 100–1000 ng/ml to concentrations of 10–20 ng/ml or even somewhat greater. These levels are well into the normal range and hardly a state of prolactin deficiency. Moreover, serum human growth hormone levels are unaltered after bromocriptine treatment. In human subjects, hGH is a potent lactogenic as well as somatogenic hormone. Thus patients on bromocriptine are rarely if ever seriously prolactin-deficient.

Davies: One might even predict lymphomas in patients on chronic bromocriptine therapy?

Hall: There has been no evidence of neoplasms in patients treated with bromocriptine.

Mitchison: The hypothesis that prolactin is a powerful immunoregulatory agent would be greatly strengthened by antisera experiments. The same point was made about interferon, and when antisera were tested *in vivo* they did in fact confirm the prediction.

Vincent: What happens to the animals in which you raise the antibody to prolactin receptor? Do they develop any immunodeficiency?

Friesen: This hasn't been tested.

Kahn: Thinking of the possible clinical conditions in which one might look for antibodies to growth hormone receptors, there are patients with immunological disease and growth retardation. Have you screened for the possibility that growth deficiency in these diseases is due to antibodies to the growth hormone receptor?

Friesen: No.

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