Prolactin and Testicular Leydig Cell Function: Characterization of Prolactin Receptors in the Murine MA-10 Testicular Leydig Cell Line (43752)

RONNIE J. BARKEY,*,[1] ESTHER WEISS-MESSER,* HAIM HACHAM,* Sara Herscovich,* ROSALIE BER,† AND TAMAR AMIT*

Department of Pharmacology, and Department of Cell and Tumor Biology,† The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel*

Abstract. **The direct role of prolactin (PRL) in testicular function is still unclear, mostly because of lack of a suitable *in vitro* model. To establish the suitability of the MA-10 murine tumor Leydig cell line for the study of PRL receptors (PRLR) and effects on steroidogenesis, we initially characterized PRLR on cultured MA-10 cells. The specific binding (B_s) of [^{125}I]human growth hormone (hGH) depends on time, temperature, and Mg^{2+} ion and protein concentrations, with absolute specificity for the lactogenic hormones hGH and ovine PRL. B_s is saturable and is to a single class of high-affinity ($K_a = 3.6 \times 10^9\ M^{-1}$) low-capacity ($B_{max}$ = 19.5 fmol/mg protein) binding sites. The molecular weight of PRLR, determined by cross-linking to [^{125}I]hGH, SDS-PAGE and autoradiography, is 35 kDa for the free receptor, suggesting that the short-form PRLR protein, previously described in liver and mammary glands, is that primarily found in MA-10 cells. Thus, the demonstration of specific PRL binding sites on MA-10 Leydig cells, with characteristics similar to primary Leydig cell PRLR, suggests that this cell line can serve as a good model for both the study of PRLR mechanism of action and the role of PRL in Leydig cell function.** [P.S.E.B.M. 1994, Vol 206]

The direct effects of prolactin (PRL) and its mechanism of action on testicular Leydig cell steroidogenesis have not been broadly studied and remain poorly defined, mostly for lack of a reliable and practical *in vitro* experimental model. *In vivo* studies in animals and man suggest that low PRL levels are essential for normal testicular function, whereas elevated levels are deleterious and that these effects are exerted at all levels of the hypothalamic-pituitary-gonadal axis (reviewed in 1).

An early step in characterization of a model to study hormone action at the cellular level must be the demonstration and characterization of specific receptors. Binding sites for PRL are widely distributed in mammalian tissues and have been demonstrated in crude membrane preparations of rat Leydig cells (2–5), where they could mediate PRL's direct actions on testicular steroidogenesis. The identification of cDNAs encoding PRL receptors (PRLR) (6) and GH receptors (GHR) (7), has led to the discovery that these receptors, like the hormones themselves, are part of a gene family which was expanded to include receptors for various cytokines, all making up the cytokine/GH/PRL receptor superfamily (for review, see 8). The sequence of mature rat liver PRLR deduced from cDNA, contains 291 amino acids (aa) (6). A long form of PRLR cDNA, identified in the rabbit mammary gland (9) encodes a mature receptor of 592 aa, with an extracellular and transmembrane region very similar to that of rat liver PRLR, but with a much longer cytoplasmic domain. Cross-linking of [^{125}I]labeled human GH (hGH) to solubilized rat testicular Leydig cell membranes and SDS-PAGE revealed the presence of both the long (mol wt 81,000–91,000) and short (mol wt 31,000–37,000) forms of PRLR (4).

There have been very few broad studies of the *in*

[1] To whom requests for reprints should be addressed at Department of Pharmacology, The Bruce Rappaport Faculty of Medicine, Technion—Israel Institute of Technology, P.O. Box 9649, Haifa 31096, Israel.

0037-9727/94/2063-0243$10.50/0

vitro effects of PRL on well defined and functional Leydig cell cultures. Welsh *et al.* (10) reported on the biphasic modulation of hCG-stimulated androgen biosynthesis by PRL, using primary cultures of dispersed mixed testicular cells from adult hypophysectomized rats. Using primary cultures of Percoll gradient-purified adult rat Leydig cells, we previously demonstrated the inhibitory effects of PRL and of an antiidiotypic anti-PRL antibody, on both basal and human chorionic gonadotropin-(hCG)-induced steroidogenesis (5). Paracrine regulatory mechanisms are most likely at play in both systems and both systems suffer from the limitations of primary cell cultures, namely repeated killing of laboratory animals, tedious preparation of cells for each experiment, and batch to batch variability.

The MA-10 murine cell line (11) provides a well defined cell culture system for the study of Leydig cell functions and steroidogenesis, offering all the advantages of an immortalized cell line. In order to establish the suitability of these cells as a model to study the direct role of PRL and its receptor in Leydig cell function, we initially demonstrate here and characterize for the first time, the MA-10 cell PRLR.

Materials and Methods

Materials. Ovine PRL (NIADDK-oPRL-16, 30.5 IU/mg) was kindly provided by the National Hormone and Pituitary Program (NIADDK, NIH, Bethesda, MD). Recombinant hGH, bovine GH (bGH), and rat GH (rGH) were a generous gift from BioTechnology General Ltd. (Rehovot, Israel). Sephadex G-100 was purchased from Pharmacia (Uppsala, Sweden). Na[^{125}I] was purchased from the Nuclear Research Center (Negev, Beersheva, Israel). Sterile culture media and reagents were from Biological Industries Ltd. (Kibbutz Bet Haemek, Israel). All other reagents were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO), or from local commercial sources. Dr. Mario Ascoli (Iowa University, Iowa City, IA) kindly provided the MA-10 Leydig cell line.

MA-10 Cell Culture. The MA-10 cells were grown in RPMI 1640 medium supplemented with 15% horse serum and 20 m*M* hepes buffer. The cells were plated in 24-well dishes at a density of 150,000–200,00 cells/ml/well, in the serum-containing medium, to allow adhesion. After 24 hr, and again after 48 hr, the medium was changed to serum-free culture medium composed of DMEM + F–12 (1:1) supplemented with 15 m*M* hepes buffer, 2.4 g/L $NaHCO_3$, insulin (0.1 U/ml), transferrin (5 μg/ml), glutamine (0.1 mg/ml), and gentamycin (50 μg/ml) for 24 hr. The cells were then washed and used for PRL binding assays.

PRL Binding Assays. Lactogenic binding was determined with [^{125}I]hGH (radiolabeled as described in 12) as a ligand. MA-10 cell monolayers were incubated in 210 μl of 10 m*M* PO_4 buffer containing 50 m*M* Mg^{2+} and 1% BSA, pH 7.4, with 1–2 ng/well of [^{125}I]hGH and with or without 1–2 μg/well oPRL, for 90 min, at 30°C. The dishes were then placed on ice, radioactive medium quickly aspirated and the cells washed twice with ice cold PBS + 0.1% BSA (washing buffer). The cells were then dissolved in 0.5 ml of 0.5 *N* NaOH (2 hr at 37°C), counted for ^{125}I and an aliquot used to determine protein (13). For internalization studies, solubilization of cells was preceded by a 1 min wash with 500 μl of either washing buffer containing 50 m*M* HCl, to remove surface-bound [^{125}I]hGH (14), or washing buffer only. Thus, in acid-washed cells, only internalized hormone was detected, while in the buffer-washed cells, both membrane-bound and internalized hormone were detected.

Cross-linking of [^{125}I]hGH to MA-10 Cell Lactogenic Receptor. 10^6 MA-10 cells/ml of ice cold 10 m*M* tris buffer containing 0.3 *M* sucrose and 1 m*M* KCl, 10 m*M* $MgCl_2$ and 0.1% NaN_3, were homogenized with an Ystral ×10/20 laboratory disperser (3 × 15 sec; speed 6) and centrifuged at 40,000*g* at 4°C for 1 hr; the resultant pellet was defined as the total particulate fraction (TPF). [^{125}I]hGH (500,000 cpm) was incubated with MA-10 TPF (1 mg protein) and with or without excess unlabeled hGH (20 μg), in a final volume of 150 μl of 50 m*M* PO_4 buffer, pH 7.4, containing 0.1% BSA at 4°C for 20 hr. Cross-linking was achieved by addition of fresh ethylene glycol-bis (succinic acid-N-hydroxysuccinimide) (EGS) in DMSO (final concentration 1 m*M*) for 15 min at 22°C, followed by centrifugation at 5000*g* for 5 min at 4°C. The resuspended pellet was mixed with an equal volume of 2× concentrated electrophoresis sample buffer and boiled for 5 min. Samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (15) followed by autoradiography.

Statistics. The LIGAND program (16) was used to evaluate the linearity of the Scatchard analysis (17) of the binding data (single class versus two classes of binding sites). Values shown are mean ± SE of 3–4 replicates from representative experiments which were repeated 2–8 times.

Results

Kinetics of [^{125}I]hGH Binding to MA-10 Cells. The first step in the characterization of MA-10 cell PRLR was the determination of the optimal time and temperature of incubation for measuring [^{125}I]hGH specific binding. As shown in Figure 1, at 30°C, binding initially increased rapidly, peaked at 90 min and then decreased. At 5°C however, binding increased much more slowly and was consistently lower than at 30°C. Since [^{125}I]hGH binding to whole cells reflects

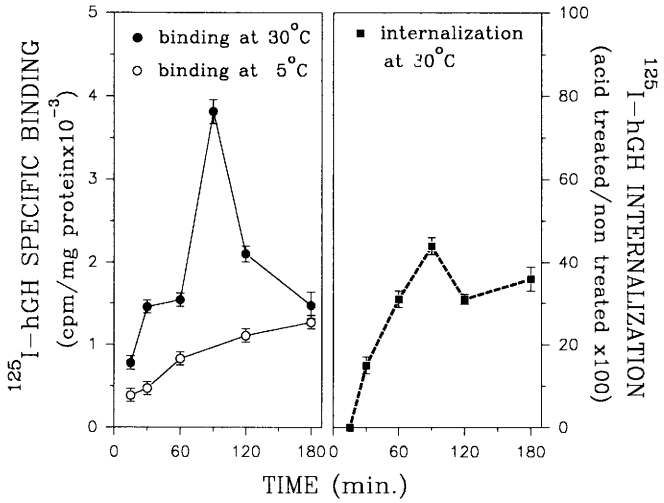


Figure 1. Effects of time and temperature on [^{125}I]hGH binding and internalization. 200,000 MA-10 cells were plated and cultured as described in Methods, were washed and incubated with 2 ng of [^{125}I]hGH and with or without 2 μg unlabeled oPRL at 30°C or 5°C for various times. After washing to remove unbound hormone, specific PRL binding and protein concentrations were determined in the NaOH solubilized cells of each well (left panel). For internalization studies, half of the wells were washed with 500 μl of washing buffer and the other half with washing buffer containing 50 m*M* HCl, for 1 min, to remove surface-bound [^{125}I]hGH. Thus in the acid-washed cells, only internalized hormone was measured, while in buffer-washed cells, both membrane-bound and internalized hormone were measured. The % internalization shown was obtained from the ratio of those two values (right panel). Each point is the mean ± SE of four determinations.

both surface membrane-bound hormone as well as internalized hormone-receptor complexes, the degree of internalization was estimated by measuring the cell-associated radioactivity remaining after subjecting the cells to a short acid treatment, which was shown to remove surface-bound tracer (14). At 30°C, internalization reached a maximum of 44% (of the total tracer specifically bound) at 90 min, while only very low internalization was observed at 5°C (data not shown), as would be expected from such an energy-demanding process. Since the maximum cell-associated (surface-bound + internalized) [^{125}I]hGH was measured after 90 min at 30°C, these conditions were adopted for subsequent binding studies. Under those conditions, [^{125}I]hGH binding was highly (r = 0.998) and linearly dependent upon MA-10 cell protein content (measured ~72 hr after plating), within the range of 50–200 μg/well (resulting from plating densities of 100,000–400,000 cells/well, respectively; data not shown). Higher plating densities were consistently associated with overfilling of wells and detachment of cells. Thus, plating densities of 150,000–200,000 cells/well were used in these studies.

Effects of Mg^{2+} Concentration. In view of the importance of cations for lactogenic binding (12, 18–20), we evaluated the role of Mg^{2+} in PRL binding to MA-10 Leydig cells. The addition of increasing concentrations of $MgCl_2$ considerably raised specific [^{125}I]hGH binding, with a maximal three-fold increase observed with 50 m*M* Mg^{2+} (Fig. 2). Thus this Mg^{2+} concentration was used for the binding assays in this study.

Hormonal Specificity. The lactogenic hormones, oPRL and hGH were equipotent in inhibiting [^{125}I]hGH binding to cultured MA-10 cells (IC_{50} = 1.35 ng/well), while the purely somatogenic recombinant bGH and the irrelevant hormone insulin, were ineffective (Fig. 3). Furthermore, no specific binding of the somatogenic [^{125}I]labeled recombinant rGH to MA-10 Leydig cells could be demonstrated, even in the absence of added $MgCl_2$ or with added 2 m*M* EDTA, to chelate any endogenous Mg^{2+} in the system (data not shown).

Saturation Analysis and Scatchard Plot. [^{125}I]hGH binding to cultured MA-10 cells was saturable, reaching a plateau between 4–6 ng/well (Fig. 4). A Scatchard plot (17) of the saturation data (Fig. 5, inset) revealed the presence of a single class of high-affinity, low-capacity binding sites. Summarizing eight such experiments gave the following values: K_a: 3.46 ± 0.4 × 10^9 M^{-1} and B_{max}: 19.5 ± 2.3 fmol/mg protein (mean ± SE).

Evaluation of Molecular Weight of PRLR in MA-10 Cells. Covalent cross-linking of [^{125}I]hGH to MA-10 cell membranes, followed by SDS-PAGE and autoradiography, revealed a single clearly labeled band with a molecular weight of 57,000 for the complex ([^{125}I]hGH-PRLR); this band was not observed when

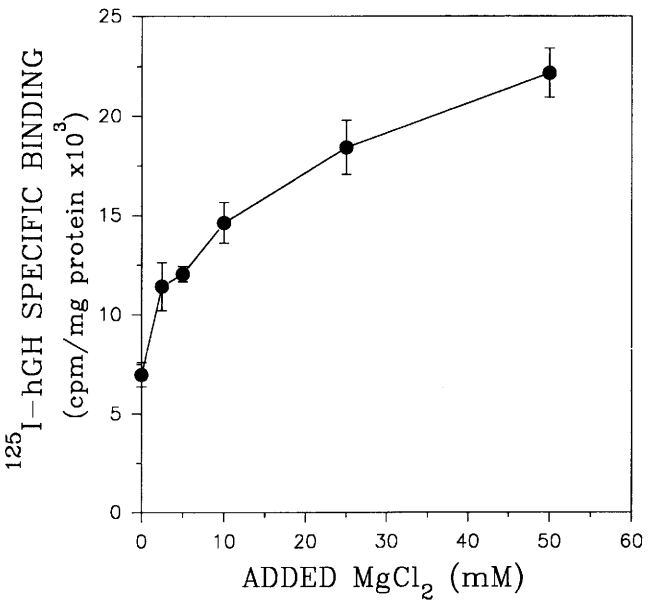


Figure 2. Dose response effect of Mg^{2+} on specific [^{125}I]hGH binding. [^{125}I]hGH binding to MA-10 cells was determined at 30°C for 90 min as described under Figure 1, except that incubations were performed in the presence of increasing concentrations of added $MgCl_2$ (0–50 m*M*). Values are the mean ± SE of four replicates.

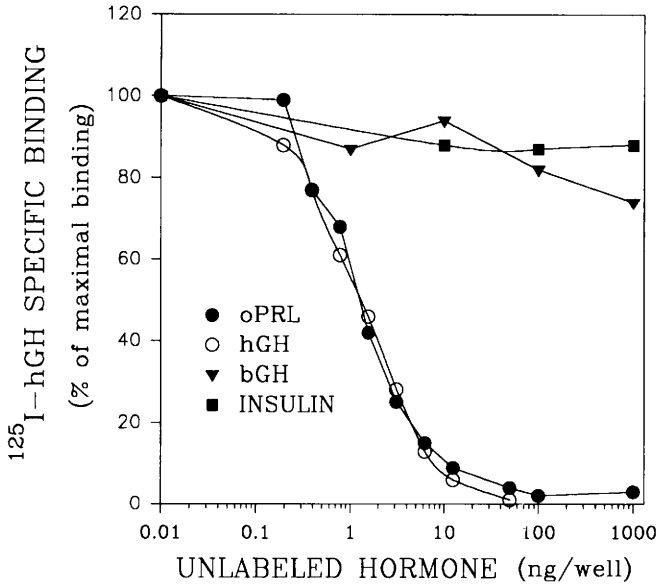


Figure 3. Hormonal specificity of [^{125}I]hGH binding. Binding was determined as described under Figure 1, except that incubations were performed in the presence of increasing concentrations of unlabeled oPRL, hGH, bGH, and insulin. Maximal binding (100%) in the absence of unlabeled hormone was 2650 cpm/well. Nonspecific binding in the presence of excess oPRL was 400 cpm/well. Values are the mean of triplicate determinations; SE for all points was <10%.

the binding experiment was carried out in the presence of excess unlabeled oPRL or hGH (Fig. 5). Subtraction of the molecular weight of hGH (22,000) gave a molecular weight of 35,000 for the MA-10 cell PRLR.

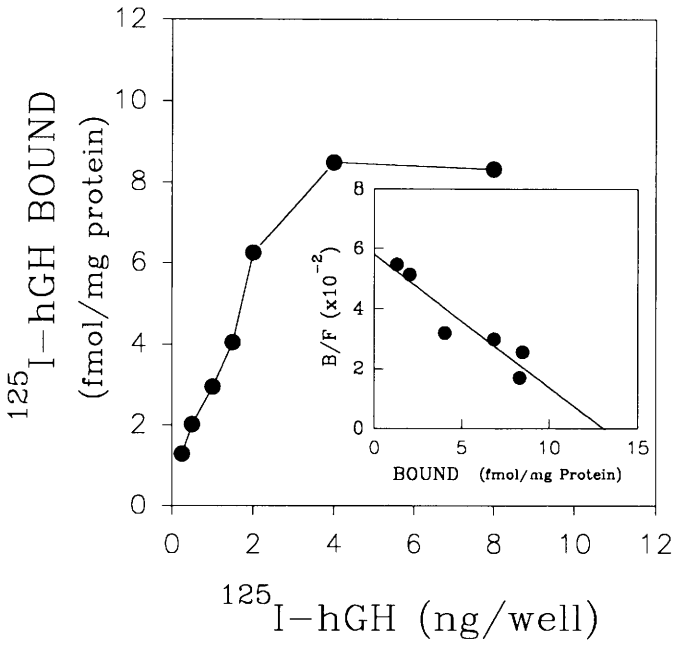
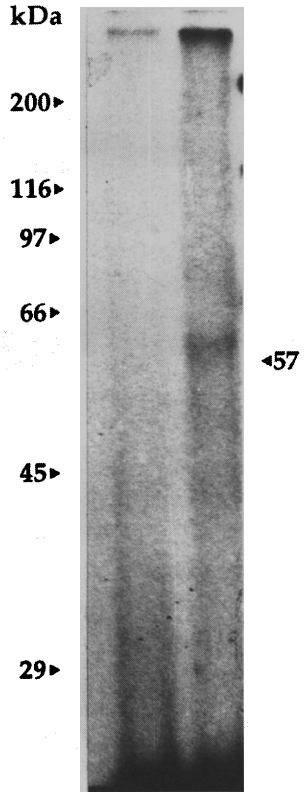


Figure 4. Saturation plot of [^{125}I]hGH binding. Binding was determined as described under Figure 1, except that incubations were with increasing concentrations of [^{125}I]hGH (0.25–8.0 ng/well) and with or without excess unlabeled oPRL. Values are the mean of triplicates. Inset: Scatchard plot of the data: K_a was 3.76 × 10^9 M^{-1} (r = 0.94).



[^{125}I]hGH + +
hGH + -

Figure 5. Cross-linking of [^{125}I]hGH to MA-10 cell crude membranes. MA-10 cell TPF (1 mg protein) was incubated with [^{125}I]hGH (10 ng) and with or without unlabeled hGH (20 μg), as described in Materials and Methods. Cross-linking was achieved by addition of 1 m*M* EGS in DMSO. Samples containing equal amounts of protein were subjected to 10% SDS-PAGE and autoradiography. Right arrow—the molecular weight estimate for the radioactive band that appeared in the absence, but not in the presence, of excess unlabeled oPRL. Left arrow—the position of molecular weight standards. Three such gels have given similar results.

Discussion

In this study, we demonstrate and characterize for the first time PRLRs on the MA-10 murine Leydig tumor cell line. This cell line is a useful model to study Leydig cell function and gonadotropin-generated signal transduction (for review see 21). In spite of qualitative differences in the steroidogenic pathway of MA-10 cells compared with normal Leydig cells, both respond to LH/hCG with increased steroid production. The use of a cell line offers some obvious advantages compared with primary cells, namely immortality (and thus dispensation of the need to kill animals), homogeneity of the cell population, and all the practical advantages associated with the use of anchored, cultured cells. These same characteristics suggest that the MA-10 cell line could also provide a suitable model system to study effects of PRL on a differentiated function, as well as the mechanism of PRL action,

signal transduction and regulation of PRLR in testicular Leydig cells.

The first step necessary to establish the validity of the MA-10 cell line as a model to study PRL effects, was to demonstrate the specific binding of PRL to cell surface receptors on intact MA-10 cells. Binding studies performed with [^{125}I]hGH confirmed the presence of specific, lactogenic binding sites on Leydig cell monolayers. The ligand-receptor interaction was time and temperature dependent and showed a pattern similar to that reported for hCG binding to its receptors on MA-10 cells (11) and for GH binding to GHR on mouse fibroblasts (22). Internalization was studied after removal of surface-bound [^{125}I]hGH with a mild acid treatment and revealed a pattern which essentially paralleled the binding kinetics at 30°C, over the first 90 min. Since the binding affinity of [^{125}I]hGH to lactogenic receptors is very high, it seems likely that the decrease with time in cell-associated radioactivity is not due to simple dissociation from the receptor, but rather is due to degradation, as was shown for GH binding to fibroblasts (22). In cultured rat adipocytes, most of the more rapidly (20 min) internalized [^{125}I]hGH was intact, degradation increasing with time (14).

The cation dependence of the binding of lactogenic hormones has been variously studied. Detailed reports showed cation dependence of binding of the mixed lactogenic/somatogenic hGH to rabbit liver membrane GHR (23), to GH-binding protein (GHBP) in rabbit liver cytosol and serum (24, 25), to sera of several mammalian species (12), and of binding of the purely lactogenic oPRL to chicken kidney membranes (26). In contrast, binding of the somatogenic rGH, bGH and oGH to rabbit liver membranes, cytosol (18, 19) or to serum GHBP (12), is cation independent. Thus, at least part of the Mg^{2+} effect appears to reside in the hormone and allows distinction between lactogenic and somatogenic binding.

A substantial increase in binding of [^{125}I]hGH to cultured MA-10 cells was found with increasing Mg^{2+} concentrations, up to 50 m*M*, whereas no specific binding of [^{125}I]rGH was observed, whether in the presence or absence of Mg^{2+}. Indeed, we have shown that high Mg^{2+} concentrations (>35 m*M*) might inhibit binding of somatogenic hormones to their receptors (12) and here we added EDTA to chelate any remnant Mg^{2+} ions in the incubation. These findings support the specificity studies, since high concentrations of recombinant bGH did not inhibit hGH binding, indicating the strict lactogenic nature of the binding to MA-10 cells. They also support various reports of the lactogenic nature of hGH/PRL specific binding to rat testicular homogenates or Leydig cell membranes (2–5).

Saturability is also an important criterion for demonstrating specific receptors and indeed was shown for PRL binding to MA-10 cells. Scatchard analysis (17) of the data revealed the presence of a single class of binding sites with high-affinity ($\sim 3 \times 10^9\ M^{-1}$) and low-capacity (13.4 fmol/mg protein ≡ 96.4 fmol/mg DNA or 3693 receptors/cell). Similar affinities were reported for PRL binding to various preparations of adult rat Leydig cells (4, 5, 27), while differences in binding capacities reflect the different species and degrees of purity of Leydig cells used in each of these studies, the MA-10 cells being the only strictly homogeneous system of those studied, albeit their tumor origin.

The molecular weight characteristics of PRLR can also be indicative of the type and possibly the function of PRLR in a given tissue. The two major forms of PRLR, the long (~88 kDa) and the short (40 kDa) form found in most mammalian tissues, and the intermediate length PRLR of rat Nb2 lymphoma cells, differ only in the sequence encoding the intracellular domains, a domain postulated to be responsible for transduction of hormone signals in target cells (for review see 8). It has been suggested that, although those major forms are able to bind PRL with equal affinity, they are clearly involved in different biological functions, since only the long form was able to stimulate transcription of milk protein genes (28). The biological effects specifically associated with the short form PRLR remain to be identified, although its structural arrangement is reminiscent of several receptors acting primarily as transporters (transferrin, LDL, IGF-II receptors).

Using cross-linking techniques, we demonstrated that in the mouse MA-10 Leydig cells, the size of the major PRLR protein is 35 kDa, corresponding to the short form PRLR, although we cannot yet exclude the presence of other minor forms. Whether this reflects on a possible role of PRLR as a transporter in MA-10 cells remains to be determined, although we have indications of a biphasic effect of PRL on MA-10 Leydig cell steroidogenic response to hCG (29). These results agree partly with findings of two short forms (31–37 kDa) of PRLR in rat Leydig cells, although solubilization of the membranes also allowed detection of two high molecular weight PRLRs (81–91 kDa) (4).

In conclusion, this is the first demonstration and characterization of PRLR in MA-10 murine tumor Leydig cells. The kinetic properties of these sites, their hormonal specificity and their molecular weight characteristics all suggest that these cells provide a valid and useful model to study the effects and mechanism of action of PRL on Leydig cell function.

The technical assistance of Sarah Dickbuch-Zieger is gracefully acknowledged. This study is in partial fulfillment of the requirements of the Technion-Israel Institute of Technology, for a DSc degree by E. W.-M. H.H. was supported by the Center for Absorption in Science, Israel Ministry for Absorption of Immigration. We

thank the Chief Scientist's Office of the Israel Ministry of Health and the Technion Vice President for Research for their financial support (to R.J.B. and H.H.).

1. Evans WS, Cronin MJ, Thorner MO. Hypogonadism in hyperprolactinemia—proposed mechanisms. In: Ganong WF, Martini L, Eds. Frontiers in Neuroendocrinology. New York: Raven Press, Vol 7:p77, 1982.
2. Barkey RJ, Shani J, Amit T, Barzilai D. Specific binding of prolactin to seminal vesicle, prostate and testicular homogenates of immature, mature and aged rats. J Endocrinol **74:**163–173, 1977.
3. Charreau EH, Attramadal A, Torjesen PA, Purvis K, Calandra R, Hansson V. Prolactin binding in rat testis: Specific receptors in interstitial cells. Mol Cell Endocrinol **6:**303–308, 1977.
4. Bonifacino JS, Dufau ML. Lactogen receptors in rat Leydig cells: Analysis of their structure with bifunctional crosslinking reagents. Endocrinology **116:**1610–1614, 1985.
5. Barkey RJ, Weiss-Messer E, Mandel S, Gahnem F, Amit T. Prolactin and antiprolactin receptor antibody inhibit steroidogenesis by purified rat Leydig cells in culture. Mol Cell Endocrinol **52:**71–80, 1987.
6. Boutin JM, Jolicoeur C, Okamura H, Gagnon J, Edery M, Shirota M, Banville D, Dusanter-Fourt I, Djiane J, Kelly PA. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell **53:**69–77, 1988.
7. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature **330:**537–543, 1987.
8. Kelly PA, Djiane J, Postel-Vinay M-C, Edery M. The prolactin/growth hormone receptor family. Endocr Rev **12:**235–251, 1991.
9. Edery M, Jolicoeur C, Levi-Meyrueis C, Dusanter-Fourt I, Petridou B, Boutin J-M, Lesueur L, Kelly PA, Djiane J. Identification and sequence analysis of a second form of prolactin receptor by molecular cloning of complementary DNA from rabbit mammary gland. Proc Natl Acad Sci USA **86:**2112–2116, 1989.
10. Welsh TH Jr., Kasson BG, Hsueh AJW. Direct biphasic modulation of gonadotropin-stimulated testicular androgen biosynthesis by prolactin. Biol Reprod **34:**796–804, 1986.
11. Ascoli M. Characterization of several clonal lines of cultured Leydig tumor cells: Gonadotropin receptors and steroidogenic responses. Endocrinology **108:**88–95, 1981.
12. Amit T, Hochberg Z, Barkey RJ. Influence of Mg^{2+} on detection of somatogenic and lactogenic components of growth-hormone-binding protein in mammalian sera. Biochem J **293:**345–349, 1993.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the Folin phenol reagent. J Biol Chem **193:**265–275, 1951.
14. Roupas P, Herington AC. Receptor-mediated endocytosis and degradative processing of growth hormone by rat adipocytes in primary culture. Endocrinology **120:**2158–2165, 1987.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685, 1970.
16. Munson PJ, Rodbard D. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. Anal Biochem **107:**220–239, 1980.
17. Scatchard G. The attraction of proteins for small molecules and ions. Ann NY Acad Sci **51:**660–672, 1949.
18. Fix JA, Leppert P, Moore WV. Growth hormone and prolactin binding to rabbit liver plasma membranes. Horm Metab Res **13:**508–515, 1981.
19. Barnard R, Waters MJ. Serum and liver cytosolic growth hormone binding proteins are antigenically identical with liver membrane "receptor" types 1 and 2. Biochem J **237:**885–892, 1986.
20. Barnard R, Waters MJ. Use of calcium dependence as a means to study the interaction between growth hormones and their binding proteins in rabbit liver. Biochem J **250:**533–538, 1988.
21. Ascoli M. Functions and regulation of cell surface receptors in culture Leydig Tumor cells. In: Conn PM, Ed. The Receptors. New York: Academic Press, Vol **2:**pp368–402, 1985.
22. Murphy LJ, Lazarus L. The mouse fibroblast growth hormone receptor: Ligand processing and receptor modulation and turnover. Endocrinology **115:**1625–1632, 1984.
23. Tsushima T, Sasaki N, Imai Y, Matsuzaki F, Friesen HG. Characteristics of solubilized human-somatotropin-binding protein from the liver of pregnant rabbits. Biochem J **187:**479–492, 1980.
24. Ymer SI, Herington AC. Water-Soluble hepatic growth hormone receptors: Structural studies using gel chromatography and chemical cross-linking. Endocrinology **114:**1732–1739, 1984.
25. Ymer SI, Herington AC. Evidence for specific binding of growth hormone to a receptor-like protein in rabbit serum. Mol Cell Endocrinol **41:**153–161, 1985.
26. Krishman KA, Proudman JA, Bahr JM. Radioligand receptor assay for prolactin using chicken and turkey kidney membranes. Comp Biochem Physiol **100B:**769–774, 1991.
27. Shiu RPC, Paz G, Faiman C. Prolactin receptors in interstitial cells of testes from rats at different stages of development. Int J Androl **5:**525–533, 1982.
28. Lesueur L, Edery M, Ali S, Paly J, Kelly PA. Comparison of long and short forms of the prolactin receptor on prolactin-induced milk protein gene transcription. Proc Natl Acad Sci USA **88:**824–828, 1991.
29. Barkey RJ, Weiss-Messer E, Hacham H, Amit T. Characterization of prolactin effects and receptors in MA-10 testicular Leydig cells. VI International Prolactin Congress, Paris, p42, Abstract H-12, 1993.