

Development of a Homologous Radioimmunoassay for Secreted Hamster Prolactin (41574)

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Abstract. A specific and sensitive homologous radioimmunoassay has been developed for secreted hamster prolactin. Hamster serum and pituitary extracts showed parallel dilution-response curves with hamster prolactin. The sensitivity of the assay ranged from 0.5 to 1.0 ng/ml, and the intra- and inter-assay coefficients of variation were 6 and 10%, respectively. Additionally we have demonstrated that the rat prolactin radioimmunoassay kit distributed by the National Institute of Arthritis, Metabolism, and Digestive Diseases is an inadequate method for the measurement of hamster prolactin.

A number of laboratories have measured prolactin in hamster blood (1-5) using the rat prolactin radioimmunoassay kit (rPRL RIA) distributed by the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). The secreted form of hamster prolactin (haPRL) has recently been purified (6) but its activity in the rPRL RIA has not been evaluated. In this study we demonstrate that the NIAMDD rPRL RIA lacks the specificity and sensitivity required for the valid determination of haPRL and describe the development of a homologous haPRL RIA.

Materials and Methods. *Hormone preparations.* Hamster PRL was isolated and purified according to procedures previously described by this laboratory (6). Rat PRL used for radioiodination (NIAMDD-rPRL-I-5) and as a reference standard (NIAMDD-rPRL-B-3) were obtained from the NIAMDD.

Antisera. Purified haPRL was dissolved in 5 mM glycinate, pH 9.0, at 250 µg/ml and mixed with 2 ml of Freund's complete adjuvant. This mixture was injected subcutaneously (sc) into five sites in the neck region of each of two New Zealand White rabbits. One month later, each rabbit was injected with a mixture of 200 µg of haPRL in 1 ml of 5 mM sodium glycinate, pH 9.0, and 2 ml of Freund's incomplete adjuvant. Two weeks later each rabbit was injected with 50 µg of haPRL in 0.5 ml sodium glycinate in 0.8 ml of Freund's incomplete adjuvant. The two booster injections were administered sc in

several sites over the intrascapular region. Blood was collected from each rabbit by cardiac puncture 12 days after the last injection.

Rabbit antiserum to rPRL (NIAMDD-anti-rPRL-S-8) was obtained from the NIAMDD.

Radioiodination of haPRL and rPRL. Purified haPRL and rPRL were radioiodinated by a modification of the lactoperoxidase method (7). Five micrograms of hormone dissolved in 10 µl of 50 mM sodium phosphate, pH 7.5, 0.5 µg of lactoperoxidase in 20 µl of 0.5 M sodium phosphate, pH 7.5, and 3.3 ng of H₂O₂ in 20 µl of distilled water were added to 1 mCi of carrier-free Na ¹²⁵I (Amersham-Searle) and allowed to react for 3 min at room temperature, after which the reaction was diluted with 0.3 ml of 25 mM Tris-HCl. Radiolabeled haPRL and rPRL were purified from the reaction mixture as previously described (8).

Assay procedure. A double-antibody RIA for haPRL was developed similar to those previously described for mPRL (9) and mouse placental lactogen (10). All dilutions of samples and assay standards were made with assay buffer (0.01 M sodium phosphate, 0.15 M EDTA, 0.15 M NaCl, 0.1% sodium azide, and 1.0% bovine serum albumin, pH 7.6). Working dilutions of antisera were made with 0.01 M sodium phosphate-buffered saline, pH 7.6, containing 0.15 M EDTA, 0.15 M NaCl, 0.1% sodium azide, and 3.0% normal rabbit serum. For the assay, 100 µl of standard or unknown were incubated with 100 µl of a 1:30,000 dilution of haPRL antiserum (No. 47) for 24 hr, followed by the addition of 10,000 cpm (100 µl) of ¹²⁵I-haPRL. After a 24-hr incu-

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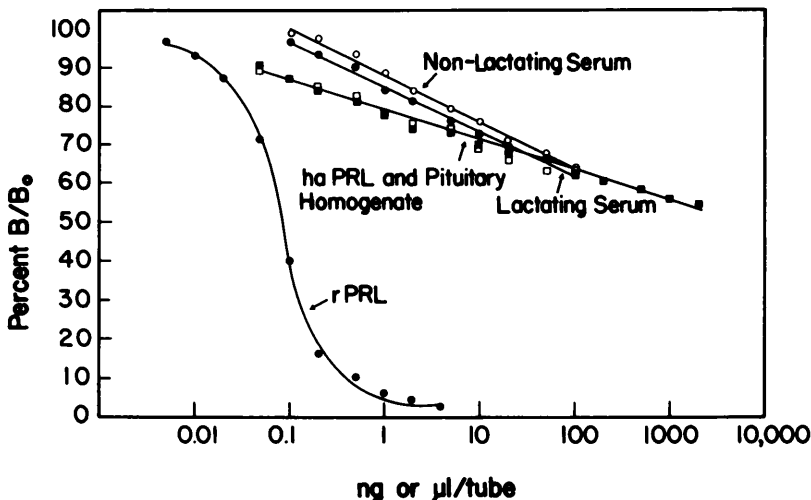


FIG. 1. Displacement curves for rPRL, haPRL, hamster pituitary homogenate, and hamster sera in the NIAMDD rPRL RIA. Each point represents the mean of five determinations.

bation with the radiolabeled hormone, separation of bound and free hormones was accomplished by incubating the reaction mixture with 100 μ l of goat anti-rabbit gamma globulin (1:16) (Antibodies Inc., Davis, Calif.). The assay was terminated 24 hr after the addition of the second antibody by the addition of 1 ml of cold assay buffer and a 20-min centrifugation at 3000g. The assay tubes were decanted and the pellet counted in a Beckman Gamma 8000 spectrometer. All incubations were conducted at room temperature and the centrifugation performed at 4°C. The dilution of the anti-haPRL provided 50% specific

binding of radioiodinated haPRL and less than 5% nonspecific binding. Reference standards ranged from 5 to 2000 pg of purified haPRL per tube. The rPRL-RIA was conducted as recommended by the NIAMDD.

Evaluation of the rPRL and haPRL RIAs. Displacement curves for rPRL, haPRL, a hamster pituitary homogenate, and hamster sera were determined for the NIAMDD rPRL-RIA. The haPRL RIA was evaluated by generating displacement curves for haPRL, a hamster pituitary homogenate, and hamster sera. The pituitaries were obtained from adult female hamsters and homogenized in assay buffer (1 pituitary/ml). Hamster sera were obtained by decapitation from lactating female hamsters, cycling female hamsters, and 2-Br- α -ergocryptine (CB-154; kindly provided by Sandoz Inc.) treated female hamsters (1 mg/animal 3 hr prior to decapitation).

Results. As shown in Fig. 1, displacement curves generated from serial dilutions of the hamster pituitary homogenate, sera, and purified haPRL were not parallel to the rPRL standard in the NIAMDD rPRL-RIA. Additionally, serial dilutions of hamster sera were not parallel to serial dilutions of haPRL and the pituitary homogenate. These findings have been independently confirmed by Dr. Gary Campbell of the Department of Physiology, University of Nebraska (personal communication).

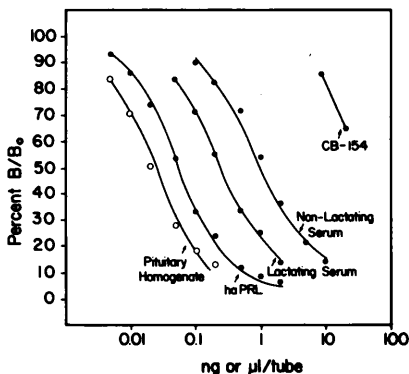


FIG. 2. Displacement curves for haPRL, hamster pituitary homogenate, and hamster sera in the haPRL RIA. Each point represents the mean of five determinations.

In contrast, displacement curves generated from serial dilutions of the hamster pituitary homogenate and sera were parallel with the purified haPRL standard in the homologous haPRL RIA (Fig. 2). PRL levels were higher in serum from lactating females than in cycling females and very low in CB-154 treated females. The sensitivity of the assay (90% of buffer control) ranged from 0.5 to 1.0 ng/ml (10 to 20 pg/tube), and intra- and inter-assay coefficients of variation were 6% and 10%, respectively (determined from 10 assays).

Discussion. We have developed a sensitive and homologous radioimmunoassay for haPRL and offer unequivocal evidence that the NIAMDD rPRL-RIA is an inadequate method for the measurement of haPRL. In addition to the relative insensitivity of the NIAMDD rPRL-RIA as a method for detecting haPRL it also appears that serum factors other than haPRL cause displacement in the rPRL-RIA. The application of RIAs developed for peptide hormones from one species to be used in other species has been a valuable tool in endocrine research, especially in understanding the comparative physiology of the gonadotropins; however, this methodology should not be used without some knowledge of species differences in the hormones being studied and only after rigorous physiological validation.

During the preparation of this manuscript the development of an RIA for the stored form of haPRL was reported (11).

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