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Simultaneous Radioimmunoassay for Luteinizing Hormone and Prolactin[1] (42070)

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Abstract. A combined radioimmunoassay (RIA) for the measurement of the anterior pituitary proteins luteinizing hormone (LH) and prolactin (PRL) is described and compared with individual RIAs for these hormones. The standard curves and the sample values for LH and PRL were identical when determined in a combined or in an individual RIA. This technique may prove useful to a number of laboratories where it is desirable to determine levels of more than one hormone in limited sample volumes.

Since the initial description of the technique (1), radioimmunoassays (RIAs) have been used routinely to measure a variety of hormones in the circulation or in tissue. Usually, a sufficient amount of plasma or supernatant from a tissue extraction is available to perform a number of individual RIAs. However, some *in vivo* experimental protocols in small animals involve the withdrawal of blood samples in volumes (i.e., 50 μl) such that multiple, individual RIAs are not possible (2, 3). In an attempt to maximize the amount of data obtained from small sample volumes, we have developed a combined RIA for the measurement of luteinizing hormone (LH) and prolactin (PRL) levels within the same plasma sample, using different isotopes to label the antigens. The feasibility of a simultaneous RIA has been demonstrated previously for a number of substances in the blood, i.e., angiotensin I and angiotensin II (4), secretin and gastrin (5), and insulin and growth hormone ((6) see also (7–10)). Until the present time, however, a simultaneous RIA for the anterior pituitary hormones LH and PRL has not been described. Blood levels of these hormones are often intimately linked regarding reproductive physiology (11, 12). It would be useful to have a RIA whereby changes in plasma levels of both LH and PRL can be determined simultaneously in the small plasma volumes obtained from rats. The present paper compares the binding, the standard curves, and the concentrations of LH and PRL in plasma samples determined in individual and combined RIAs. These data demonstrate that both LH and PRL levels can be accurately determined in a single, combined RIA.

Materials and Methods. Hormones and antibodies were obtained from the National Hormone and Pituitary Program of the NIADDK.

For the LH RIA, the standard curve ranged from 0.025 to 0.5 ng/tube using the rRP-2 standard. The rLH antiserum (S-7, in 0.1 *M* phosphate buffered saline, PBS, pH 7.0) containing 0.05 *M* EDTA and 1.0% normal rabbit serum (NRS) was used at a final dilution of 1:60,000. Rat LH (I-5, 2.5 μg) was radioiodinated with 0.5 mCi of carrier free ^{125}NaI (Amersham, IMS-30) using the chloramine-T method. The reaction mixture was fractionated on a column (0.7 × 20 cm, Bio-Rad Labs, Richmond, Calif.) of Sephadex G-50. The radioiodinated hormone in the tube containing the greatest number of counts was used in the assay and diluted with PBS–0.1% gelatin so that a fixed amount ranging from 10,000 to 15,000 cpm was added to each assay tube. Approximately 30% of the ^{125}I was incorporated into the hormone during the radioiodination.

For the PRL RIA, the standard curve ranged from 0.1 to 2.0 ng/tube using the rRP-3 standard. The rPRL antiserum (S-9 in EDTA-PBS with 1.0% NRS) was used at a final dilution of 1:12,000. Rat PRL (I-5, 2.5 μg) was radioiodinated as described above with 0.2 mCi of carrier free ^{131}NaI, (Amersham, IBS-30). Each assay tube contained a fixed amount of tracer ranging from 15,000

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to 20,000 cpm. Approximately 25% of the ^{131}I was incorporated into the rPRL by the radioiodination.

Concentrations of LH and PRL were determined in serial dilutions of plasma samples obtained from female rats following decapitation at random stages of the estrous cycle ($n = 8$) and, from animals ovariectomized for 1 week, either steroid-free ($n = 8$) or injected subcutaneously with estradiol benzoate (Sigma, 20 g in 0.2 ml corn oil) 2 days prior to blood collection ($n = 8$).

For both the individual and combined RIAs, standards and plasma samples were combined with varying amounts of buffer (PBS–0.1% gelatin) to achieve a final volume of 200 μl. In the individual assays, the LH and PRL antisera were added in a volume of 200 μl (LH:1:15,000; PRL 1:3,000). In the combined assay, the LH and PRL antisera were added together in a total volume of 200 μl (100 μl each; LH 1:7500; PRL 1:1500). After incubation for 24 hr at room temperature, radioiodinated LH or PRL was added to the individual RIAs in a volume of 100 μl and to the combined assay in volumes of 50 μl each. Following further incubation at room temperature for 24 hr, 200 μl of goat antiserum to rabbit γ-globulin (1:40, P-4, Antibodies Inc., Davis, Calif.) was added to each tube in the individual and combined assays. The incubation was continued for 18 hr at room temperature and then 2.0 ml of cold water was added to each tube.

The tubes were centrifuged at 3000g for 40 min at 40°C, the supernatant was decanted, and the tubes were allowed to drain. The tubes were counted for 1.0 min in two gamma counters: a single-well Nuclear Chicago Automatic Gamma Counter (Model 1185) with two analyzers to detect either ^{125}I (window setting: 15 to 100 KeV) or ^{131}I (window setting: 320 to 400 KeV) and in an IsoData 20/20 (20-well) Gamma Counter with windows set to detect ^{125}I (15 to 65 KeV) and ^{131}I (90 to 700 KeV). Two machines were used in order to compare the data acquired from a single well and a multi-well machine. The use of a multi-well gamma counter shortens the time required for counting the tubes of an RIA; however, counting high energy sources such as ^{131}I could be a problem due to the "cross-talk" between the various detectors. The data management software available with this counter automatically corrected for this phenomenon.

The standard curves for the individual ^{125}LH and ^{131}PRL RIAs were calculated in the following manner: the total counts bound by the first antibody in the absence of antigen were designated as B_0, and the counts bound in the remaining tubes in the presence of increasing amounts of antigen were expressed as a ratio of that number (B/B_0). The curves were plotted on log (dose)/logit (B/B_0) display and hormone concentrations in the unknowns were determined by comparison of their counts with those of the standard curve.

In the combined RIA, the standard curves and the unknowns were calculated as described above, with the exception that the counts registered in the ^{125}I channel were corrected for the "spillover" from the ^{131}I channel. In the Nuclear Chicago gamma counter, the percentage spillover was calculated from the individual ^{131}PRL RIA where the counts recorded in the ^{125}I channel were 27.4 ± 0.3% ($n = 100$) of those in the ^{131}I channel. Therefore, in the combined RIA, "true" ^{125}LH counts were determined by subtracting 27.4% of the ^{131}I counts from the total ^{125}I counts.

In the IsoData 20/20 gamma counter, the accompanying data management/reduction software automatically corrected for spillover of ^{131}I into the ^{125}I window, after initial calibration with an ^{131}I source.

Results. The total counts bound (B_0) in the individual versus the combined RIAs differed by 3.07 ± 0.99% ($n = 6$ assays). A typical set of standard curves for either LH or PRL alone or in combination is presented in Fig. 1. Plasma hormone concentrations of LH and PRL, determined either in an individual or in a combined RIA, are shown in Fig. 2. Values calculated from the combined RIA were highly correlated with those from the individual assays (LH: $r^2 = 0.98$; PRL: $r^2 = 0.98$). Hormone levels could be determined in the same volume of plasma from ovariectomized animals (25 μl) and intact females (75 μl). In plasma samples from ovariectomized rats treated with estrogen, however, LH concentrations were too low and PRL levels too high to be determined in the same sample volume.

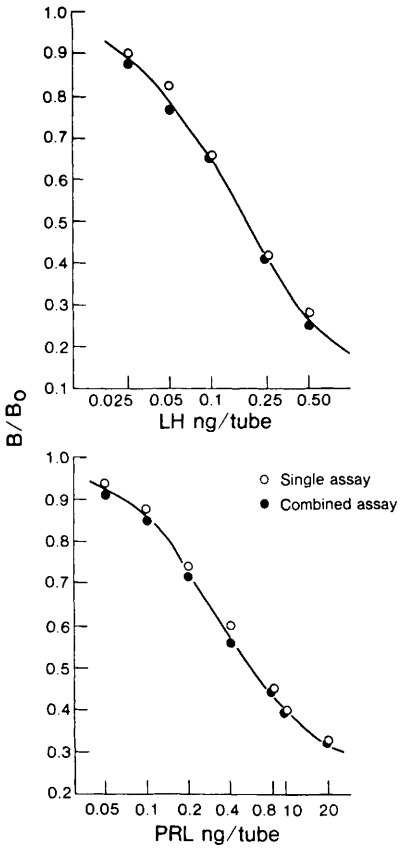

FIG. 1. Standard curves for LH (top) and PRL (bottom) determined in either a single (open circles) or a combined (closed circles) RIA. The B/B_0 ratio is plotted versus the log of the dose of the hormone. For the calculation of the assays, a log/logit plot was used.

Hormone levels determined by manual calculation of the data were the same as those derived from the data reduction software available with the IsoData gamma counter (LH manual vs LH IsoData: $r^2 = 0.95$; PRL manual vs PRL IsoData: $r^2 = 0.96$).

Discussion. The present data demonstrate that concentrations of LH and PRL can be accurately and simultaneously determined in the same sample by a combined RIA. The antigens and their respective antibodies do not cross-react, and the labeling isotopes (^{125}I and ^{131}I) can be separated by appropriate window settings on a gamma counter. The standard curves and hormone values of the samples determined in the combined assay are the same as those calculated from an individual RIA. In addition, sample concentrations were the same when calculated manually or by an automatic data reduction program, which further facilitates data management.

The combined RIA is most appropriate when the levels of LH and PRL are in an intermediate range (i.e., from ovariectomized or intact female rats). When the hormone concentrations are at two extremes, (i.e., in ovariectomized animals pretreated with estradiol benzoate where LH levels are very low and PRL concentrations are elevated), levels cannot be determined in a single sample volume with the method described here. Under these conditions, however, individual RIAs can be performed since a small sample volume is sufficient to determine the elevated hormone levels. The impetus for the development of the combined RIA for LH and PRL was provided by the desire to determine plasma concentrations of more than one hormone in a limited sample volume, i.e., multiple bleeding from conscious rats or portal blood collections from anesthetized animals. This procedure is applicable and efficient for larger sample volumes also, since

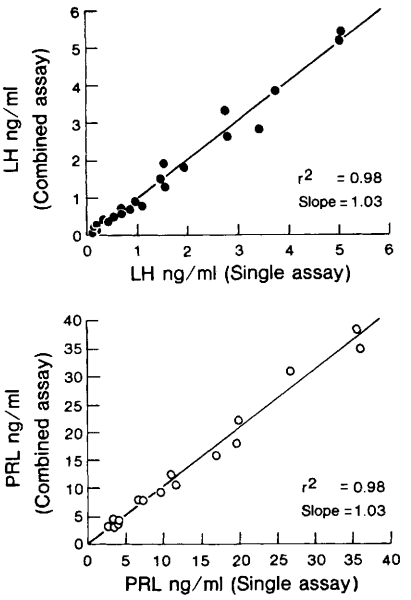

FIG. 2. Comparison of hormone values for LH (top) and PRL (bottom) determined in either a single, individual RIA (horizontal axis) or in a combined RIA (vertical axis).

only one rather than two RIAs need to be performed. Furthermore, the reagents used in this RIA are readily available from NIADDK. Based on the cross-reactivity data available from NIADDK, a combined RIA for any two of the anterior pituitary hormones should be feasible.

Therefore, based on the ease of radiolabeling these hormones with iodine, the ability of a variety of gamma counters to separate ^{125}I from ^{131}I and the efficiency of performing one rather than two RIAs, a combined RIA for measurement of anterior pituitary proteins is a reasonable option for many research laboratories.

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