Modification of the Choriogonadotropin Beta-Subunit Radioimmunoassay for Determination of Urinary Choriogonadotropin

Jill McCready, Glenn D. Braunstein, Douglas Helm, and Maclyn E. Wade

The choriogonadotropin beta-subunit radioimmunoassay has been used extensively to measure human choriogonadotropin in the sera of pregnant women and individuals with trophoblastic and nontrophoblastic tumors. Unmodified, this method cannot be used to measure choriogonadotropin in urine because of interfering substances. We circumvented the non-parallelism between the standards and serial dilutions of urine containing choriogonadotropin by adding pooled urine from men to the standard tubes and limiting the volume of urine to 100 μl. This modified assay has a sensitivity of 3 int. units/liter of urine and is specific for choriogonadotropin concentrations of 40 int. units/liter of urine. Analytical recovery of choriogonadotropin added to urine ranged from 96 to 105%.

We described here a modification of the hCG beta-subunit radioimmunoassay that circumvents this non-specific interference and allows the detection and quantification of choriogonadotropin in urine.

Materials and Methods

Reagents

Purified preparations of urinary hCG (CR 117 and CR 119), containing 5200 int. units/mg by radioimmunoassay, relative to the Second International Standard for hCG, were obtained from the Hormone Distribution Office, NIAMDD, NIH, Bethesda, Md. These preparations gave identical dose-response curves in the hCG radioimmunoassay and therefore were used interchangeably as reference standards and for iodination. Rabbit antisera (SB-6) against the beta subunit of hCG (same source) was used for both the double antibody and dioxane radioimmunoassays. The specificity of this antisera has been previously described (3).

Additional Keyphrases: peptide hormones · diagnosis of pregnancy · stability of samples · concentrations in serum and urine · intermethod comparison · diagnosis of testicular tumor, trophoblastic disease

In 1927, Aschheim and Zondek reported the appearance of choriogonadotropin (hCG) in the urine of pregnant women (1). Subsequently, numerous bioassays, immunoassays, and, more recently, radioreceptor assays, have been developed to measure this substance in body fluids of pregnant women.

The most widely used methods for detecting hCG are the 2-min slide and 2-h tube tests, based on the principle of inhibition of hemagglutination or latex-agglutination (2). The antisera used in these tests also react with pituitary lutropin (hLH). Therefore, the sensitivity of these tests was purposely decreased to the point where physiologic quantities of hLH are not detected, thereby minimizing the false-positive diagnoses of pregnancy. Although these tests may detect pregnancy as early as 31 days after the last menstrual period, they are not 98% accurate until 42 or more days after the last menses (2). Similarly, radioimmunoassays that have been developed to measure hCG and that utilize antisera generated against the intact hCG molecule do not distinguish between hLH and hCG and therefore are of limited usefulness. In 1972, Vaitukaitis et al. (3) described a radioimmunoassay for hCG in serum that eliminated the interference from physiologic amounts of hLH by the use of an antiserum raised against the beta subunit of the hCG molecule. This assay as originally described and recently modified (4) cannot be used to measure hCG in urine because of interfering substances (5).

We described here a modification of the hCG beta-subunit radioimmunoassay that circumvents this non-specific interference and allows the detection and quantification of choriogonadotropin in urine.

Lodination of hCG

hCG was labeled with Na125I (Amersham/Searle, Arlington Hts., Ill. 60005) by the Chloramine T method (4). Specific activities ranged from 50 to 100 Ci/g and the immunoprecip-
Immunoprecipitability without dioactivity was maximum when the specific activity of Na\textsuperscript{125}I was less than 300 Ci/liter.

Radioimmunoassay Procedures

Add reagents to 12 × 75 mm glass tubes in the following sequence: 100 µl of unknown urine sample or standard dissolved in 100 µl of pooled urine from men, 200 µl of suitably diluted antiserum to hCG-beta subunit, 100 µl of 0.1 mol/liter (ethylenedinitriilo)tetraacetic acid in distilled water, pH 7.6, and 400 µl of buffer. Vortex mix the reagents and equilibrate for 16 h at 4 °C; then add goat anti-rabbit serum in a quantity sufficient to produce a bound/total ratio of 33% in the tubes without added hCG. After a further 6-h equilibration, centrifuge the tubes, discard the supernate, and count the radioactivity in the precipitate in a gamma counter.

We performed the rapid modification of the hCG-beta subunit radioimmunoassay in which dioxane is used in place of goat anti-rabbit serum, by the technique previously described (4), with all standards dissolved in 100 µl of pooled urine from men.

Samples

Simultaneously collected untimed specimens of urine and blood were obtained from 312 menstruating women, ages 21–39, 71 women who were one to six days postpartum, 44 men, and five postmenopausal women. Sixteen untimed urine specimens were also collected serially from postmenopausal women in order to examine the variables of urine concentration, time of collection, and possible interference in the radioimmunoassays by the high hLH concentrations present in the urine of these women.

Serum was frozen at −20 °C until assayed. The urine samples were apportioned and frozen at −20 °C. At the time of assay the urines were thawed to room temperature, mixed, and any sediment present was allowed to settle before the supernatant fluid was assayed.

Statistical Treatment of Data

We used a modification of the statistical methods of Rodbard and Frazier for dose interpolation and determination of inter-assay and intra-assay coefficients of variation (5). Student's t-test, linear regression analysis, and Pearson product-moment correlations were done.

Figure 1. Dose-response curves for purified hormone preparations and serial dilutions of urine in the hCG beta-subunit radioimmunoassay

B represents counts bound in the presence of labeled and unlabeled ligand, B\textsubscript{0} counts bound in the presence of labeled ligand alone, and logit (B/B\textsubscript{0}) equals log\textsubscript{e} [(B/B\textsubscript{0})/(1 − B/B\textsubscript{0})]. Coordinates are logit-normalized percentage counts precipitated ([B/B\textsubscript{0}] × 100) on the ordinate and log of mass of choriogonadotropin standards and purified hormone preparations or volume of urine added per tube on the abscissa. U, urine; PMU, urine from postmenopausal women; MU, urine from men

Results

Assay Validation

Figure 1 illustrates the effect of adding 100-, 200-, 300-, and 500-µl samples of urine from a normal menstruating woman to the standards used to prepare the standard curve in the double antibody hCG beta-subunit radioimmunoassay. The 100-µl sample curve was nearly identical to that obtained with buffer, but addition of the larger volumes of urine (300 and 500 µl) resulted in deviation from parallelism and a decrease in the sensitivity. Urine from men gave similar results, although not as marked. Therefore, 100 µl of urine from men was used in tubes for the standard curves, and all dilutions of unknown urines were made in pooled urine from men to give a final urine volume of 100 µl. Serial dilutions of urine from pregnant and postmenopausal women, and hLH (LER 907) gave data paralleling the standard curve (Figure 1).

The sensitivity of the assay for choriogonadotropin was 3 int. units/liter of urine. The specificity of the assay was determined by examining the displacement of the unlabeled ligand by urine from postmenopausal and menstruating women. The hLH present in urine samples from postmenopausal women (mean concentration = 250 ± 140 int. units/liter; n = 21) was detected as 20 ± 10 int. units/liter (range, <3–40 int. units/liter) in the hCG beta-subunit assay. As expected, the lower concentrations of hLH appearing in urine from menstruating women (mean concentration, 120 ± 80 int. units of hLH activity per liter; n = 275) showed a smaller displacement in the hCG beta-subunit assay, accounting for 10 ± 8 int. units/liter (range <3–30 int. units/liter). Therefore, concentrations >40 int. units of hCG per liter in the hCG beta-subunit radioimmunoassay were considered to be due to immunoreactive hCG and not to the cross reaction from the high physiologic quantities of hLH present in postmenopausal women and in normal menstruating women during the midcycle peak of hLH secretion. The quantities of immunoreactive materials (hLH and hCG) in urine of postpartum, postmenopausal, and normally menstruating women detected in the nonspecific hLH/hCG radioimmunoassay and in the hCG beta-subunit radioimmunoassay correlated well (r = 0.92, P < 0.001) (Figure 2).

No significant differences were found in the quantities of cross-reacting material in serially diluted samples of urine collected at various times from the same postmenopausal women when assayed in the hCG beta-subunit radioimmunoassay.
Correlation of Serum and Urinary hCG Concentrations

Simultaneously collected blood and urine samples were obtained from 71 postpartum women and 47 women early in the first trimester of their pregnancy. The hCG concentrations in these samples, assayed in the respective double-antibody hCG beta-subunit radioimmunoassays, were highly correlated ($r = 0.76, P < 0.001$) (Figure 3).

The serum and urine samples obtained from the 275 menstruating women were also tested in both assays. No immunoreactive hCG was detected in any of these serum samples. Fourteen (5.1%) of the urines had immunoreactive material in concentrations of 10 to 40 int. units/liter, again indicating that only values greater than 40 int. units/liter should be considered to represent hCG in the urinary hCG beta-subunit radioimmunoassays.

Precision and Reproducibility

The between-assay coefficient of variation was 11.8% and the within-assay coefficient of variation was 7.6% for urine samples containing 120 int. units of hCG per liter.

After 5, 10, and 20 ng of purified hCG was added to concanavalin A-stripped urine, 96, 105, and 101%, respectively, could be analytically accounted for.

Stability of hCG in Urine

To determine the stability of the hCG concentrations in the urine samples, we collected urine from five pregnant women and divided it into portions, which were either frozen at −20 °C without delay, allowed to set at room temperature for one to seven days before freezing, or refrigerated at 4 °C for one to seven days before freezing. Another portion of each sample was repeatedly frozen and thawed, as many as nine times. We determined immunoreactive hCG in all portions concurrently, to eliminate between-assay variation. hCG activity significantly decreased in the samples of urine maintained at room temperature or at 4 °C as compared to those frozen immediately ($P < 0.01$). Repeated freezing and thawing had no effect on the concentration of immunoreactive hCG.

Comparison of Double-antibody and Dioxane hCG Beta-subunit Radioimmunoassays

Urine samples with various hCG concentrations from 26 pregnant women, and from eight postmenopausal women and 25 menstruating women were analyzed for hCG with both the double-antibody and the rapid dioxane hCG beta-subunit radioimmunoassay. The resulting values showed no significant difference between assays ($r = 0.96, P < 0.001, n = 55$). Figure 4 depicts the comparison for the 26 pregnant women.

Discussion

Our studies indicate that the hCG beta-subunit radioimmunoassay, as modified, can specifically measure hCG in the urine of pregnant women when the hormone is present in concentrations exceeding 40 int. units/liter. At these concentrations the high concentrations of hLH in the urine of postmenopausal women and in the urine of women with mid-cycle peaks in LH excretion do not interfere. Neither the urine volume nor time of voiding had an substantial relation to urinary hCG concentration. These results are compatible with those of other investigators who demonstrated that measurements of pituitary gonadotropins in either single first (morning) voidings or a 3-h urine sample validly reflect total 24-h excretion of the hormone in urine (7, 8). Our results also demonstrate the close correlation between serum and urinary hCG, again indicating that measurement
of hCG concentrations in casual (i.e., not strictly timed) urine samples provides a reliable means of indirectly monitoring serum hCG concentrations.

If one considers our previously published data on the time course of the appearance of immunoreactive hCG in the serum after fertilization (9), the minimum sensitivity of 40 int. units of hCG per liter of urine would allow a diagnosis of pregnancy as early as 10 days after conception or 25 days after the last menstrual period, considerably more sensitive than the urinary slide or tube hemagglutination or latex-agglutination pregnancy tests, which require that 750 to 3500 int. units hCG be present per liter of urine before the reaction is positive (2). The radioreceptor assay described by Saxena et al. (10) is quite sensitive to small amounts of hCG, but the receptor does not discriminate between hCG and LH and therefore would have to exceed 78 μg of hCG per liter before one could reliably state that hCG was detected in an untimed urine sample (10). Because the radioreceptor assay generally measures two- to threefold more hormone in the same specimen than does the hCG beta-subunit radioimmunoassay, this would indicate that the hCG concentration in the urine must be between 312 and 468 int. units/liter in terms of immunological activity before the radioreceptor assay would become specific for hCG.

The virtually identical values obtained with the double-antibody and rapid dioxane radioimmunoassays indicates that as little as 40 int. units of hCG per liter of urine can be reliably measured in an untimed sample of urine within 3 h. The relative ease of collection and the decreased sample preparation time are some of the advantages of measuring hCG in the urine rather than in the serum. Because the currently available commercial slide and tube pregnancy tests are relatively insensitive and are unable to detect hCG in about half of patients with ectopic pregnancies (4), the use of this assay may provide a rapid confirmation of pregnancy. Similarly, this test could be used to detect the low amounts of hCG excreted by a large proportion of patients with germ cell tumors of the testes and in monitoring the therapy of gestational trophoblastic disease (11).

The authors wish to express their grateful appreciation to Ms. Joan Rasor for helpful comments and Ms. Helene Zauderer for secretarial assistance. This work was supported by USPHS Contract N01-HD-2826.

References