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Measuring Arginine Vasopressin in Children and Babies, G. A. Crawford1 and A. Z. Györy2 (1Dept. of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW 2065, and 2Dept. of Medicine, University of Sydney, NSW 2066, Australia)

Because of the low concentrations of arginine vasopressin (AVP) in plasma and the presence of nonspecific interfering substances, at least 5 mL of blood is required for the assay of AVP (1). To measure AVP in children and babies, we have modified the method to allow accurate determinations in 1 mL of blood.

We acidified 0.4–0.5 mL of plasma with 40–50 µL of 1 mol/L HCl and applied this to a Sep-Pak C18 column (Waters Associates, Milford, MA) that had been washed once with 5 mL of methanol, then twice with 10 mL of water. We then rinsed the column plus sample twice with 10 mL of acetic acid (0.67 mol/L) and finally eluted the AVP with 2 mL, followed by 1 mL, of an equilibrium solution of acetonitrile and water. The 3 mL of effluent was dried at room temperature under a stream of air.

For radioimmunoassay, we dissolved each extract in 225 µL of 0.05 mol/L phosphate buffer (pH 7.5) containing, per liter, 1 mmol of EDTA, 0.15 mol of NaCl, 1 g of neomycin sulfate, and 2.5 g of bovine serum albumin; 100 µL of this was added to tubes in duplicate and mixed with 100 µL of antisera to rabbit AVP from Calbiochem (San Diego, CA; lot nos. 393079 and 597006) or Amersham (Amersham International, Bucks, U.K.; lot nos. 9 and 10) to give a total binding of 30–36% or 42–48%, respectively. After incubating the sample at 5 °C for 26–30 h, we added 100 µL of 125I-labeled AVP (NEN; Du Pont, Wilmington, DE), specific activity 2200 kCi/mol, equivalent to 4000 counts/min, and incubated for a further 16 h at 5 °C. Sac-Cel (Wellcome Reagents, Beckenham, U.K.), an anti-rabbit antibody-coated cellulose suspension, was used to precipitate the antibody.

With this method we could detect as little AVP as 0.1 pg/tube (0.45 ng/L, or 0.42 pmol/L). Analytical recoveries were 95.3% for 125I-labeled AVP and 77.2% for 5 pg of added AVP. The within-run CV was 11.6% at 3–10 ng/L, and the between-run CV was 7.6–11.3%. With Calbiochem antisera, the correlation between the current assay of AVP (z) and the assay of AVP extracted from 1 mL of plasma in duplicate with acetone (2) (y) yielded y = 0.79x + 1.71 (r = 0.86, n = 27).

We tested two commercial anti-vasopressin antibody preparations at different concentrations to give maximum sensitivity with the percentage of total binding shown above. Similar sensitivity was achieved with either product in the assay. Furthermore, the modified extraction procedure removed bilirubin, which we have found to interfere in the assay when present at >100 µmol/L.

Reference

Discordant Results for Lutropin among Immunoassays in Two Cases of Male Hypogonadism, Giuseppe Banfi, Marcello Marinelli, Michelangelo Murone, and Pierangelo Bonini (Lab. Analisi, Istituto Scientifico S. Raffaele, Via Olgettina 60, 20132 Milano, Italy)

Provocative testing with luteinizing (GnRH) is an important tool for diagnosing hypogonadism and for discriminating pituitary or hypothalamic defect. Measurement of pulsatile nocturnal release of lutropin (LH) could elucidate the approaching pubertal spurt.

In two cases of male hypogonadism we found discrepancies among results by three immunoassays, all involving monoclonal antibodies and a "sandwich" methodology: two assays were nonisotopic ("Delfia," Pharmacia, Bromma, Sweden, and "AELIA," Novo Labs, London, U.K.), the other one isotopic (IRMA LH; Becton Dickinson, Rutherford, NJ). All the kits are standardized against WHO International Reference Preparation 68/40.

The first case was a 16-year-old boy presenting with delayed secondary sexual characteristics, mobile testis, and 46XY/46XX mosaicism. Results of the GnRH test (100 µg intravenously, with blood drawn 30 min before injection; at injection; and 15, 30, 45, 60, and 90 min later (sampling times 1–7, respectively)) showed an appropriate pubertal LH release by the Delfia kit, whereas the other two showed a prepubertal negative curve (Figure 1). Follitropin (FSH) values were absolutely discordant among the kits; results for human chorionadotropin (hCG) were negative.

The second case was a 20-year-old man presenting with hypogonadism, gynecomastia, and eunuchoidal somatic characteristics. Results of the GnRH test (samples taken 0, 30, and 60 min after injection) and evidence of nocturnal rhythm (10 analyses with 15-min sampling, starting at 2400 h) showed negative LH release (from 0.05 to 1.5 int. units/L) by two kits, whereas the Delfia kit values were rather higher (from 1.8 to 2.5 int. units/L), with three peaks of LH production. Negative FSH release was confirmed by all the methods; results for hCG were also negative.

In 32 other provocative tests performed on infertile and hyperprolactinemic women and hypopituitary or hypo- somic children, no discrepancies in LH results have been found. Discordant LH results can greatly influence the
diagnosis and prognosis of hypogonadic subjects, particularly in the phase of pubertal spurt. Sources of discordance have been ascribed to ultra-sensitivity of methods (1), attributable to the use of monoclonal antibodies. Their high specificity could emphasize heterogeneity of LH molecular species, particularly if the proportions of the various forms differ in particular pathological states (2).

References

Radiolmmunoassay for Pepsinogen C, Mario Plebani, Maurizio Masiero, Francesco Di Mario,\textsuperscript{a} Antonio Boniolo,\textsuperscript{a} and Angelo Burlina (Depts. of Clin. Biochem. and \textsuperscript{a}Gastroenterology, Univ. of Padova, 35128 Padova, Italy; \textsuperscript{b}Sorin Biomedica, Saluggia, Italy)

Human gastric mucosa contains four aspartic proteinases: pepsinogen group I (PG A or PG I), pepsinogen group II (PG C or PG II), cathepsin E (formerly termed slow-moving proteinase), and cathepsin D (1). Because gastric and duodenal ulcer patients have increased concentrations of PG A in serum (2), the determination of this enzyme is of value in clinical practice (3). The cellular origin of PG C differs from that of PG A; their combined determination (PG A/PG C ratio) may be a sensitive and specific indicator of increased cancer risk (4).

We describe a new radioimmunoassay for PG C in serum, a modified version of the original assay of Samloff (5). Antiserum to human PG C prepared in rabbits was kindly supplied by Dr. I. M. Samloff, who also purified the antigen (5).

We radiiodinated PG C according to the method of Hunter and Greenwood (6), to a specific activity of about 14.7 Ci/g. The diluent buffer was 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol of NaCl, 2 g of bovine serum albumin, and 1 g of sodium azide per liter. We diluted \textsuperscript{125}I-labeled PG C in working buffer (prepared by mixing diluent buffer containing, per liter, 2.5 g of normal rabbit serum with 0.1 mol of EDTA in a volume ratio of 6:1) to provide about 10,000 counts/min in 100 μL; anti-PG C was diluted in the working buffer 1:100,000. The standard curve consists of serial dilutions of unlabeled PG C standards ranging from 80 to 2.5 μg/L. After incubating 100 μL of standards or test sera with 100 μL of \textsuperscript{125}I-labeled PG C for 18–24 h at room temperature, we added 1 mL of the second antibody (goat anti-rabbit gamma globulins in 0.01 mol/L phosphate buffer, pH 7.4, containing polyethylene glycol, 80 g/L) to precipitate the immunocomplex. The samples were then centrifuged at 2500 × g at 4°C for 20 min, the supernates decanted, and the radioactivity in the precipitates counted for 3 min with an automatic gamma counter. Non-specific binding was about 2%, and binding in the absence of added antigen was about 50%.

Intra-assay CV (n = 15) was 3.4% at 18.3 μg/L and 5.3% at 44.2 μg/L. Interassay CV (n = 11) at the same concentrations was 4.3% and 6.0%, respectively. Analytical recovery of PG C from serum supplemented with PG C standard in concentrations ranging from 5.5 to 62.1 μg/L ranged from 98.3% to 109.4% (mean = 104.3%). Serial dilutions of three samples showed a good linear response to dilution. The detection limit, corresponding to 3 SD minus the mean counts bound in five zero standards in duplicate, is 2.1 μg/L. There was no cross-reactivity with PG A at 500 μg/L.

The mean serum concentration of PG C in 40 healthy subjects was 14.07 (SD 8.7) μg/L. No statistically significant differences were found between values for males and those for females.

In conclusion, the modified assay is accurate and reliable. Its greatest advantage is its shorter incubation time (18–24 h vs 96 h); it also better separates bound from free fractions than the original method.

Further studies are in progress to evaluate the clinical usefulness of the PG A/PG C ratio in gastric cancer and precancerous conditions.

References

Simplified Fluorometric Determination of Salicylate in Plasma, Ram N. Gupta and Mohebullah Zamananei (Dept. of Lab. Med., St. Joseph’s Hospital, Hamilton, Ontario, Canada L8N 4A6)

For the last few years immunassay reagents (1, 2) and enzyme reagents (3) have been available for the emergency