Extensive Experience and Validation of Polyethylene Glycol Precipitation as a Screening Method for Macroprolactinemia, José Gilberto H. Vieira, Teresinha T. Tachibana, Leda H. Obaya, and Rui M.B. Maciel (Endocrinology Section, Laboratório Fleury, Rua Cincinato Braga 282, 01333-910 São Paulo, SP, Brazil; * author for correspondence: fax 55-11-287-2482, e-mail jose.vieira@fleury.com.br)

Serum human prolactin (PRL) is heterogeneous in molecular size, with the 23-kDa monomer being the predominant form in healthy subjects and patients with prolactinomas. From the point of view of molecular size, other circulating forms include the 50-kDa dimer (big-PRL) and the 150- to 170-kDa form (big-big-PRL, or macroprolactin) (1). Recent publications have associated asymptomatic hyperprolactinemia with a predominance of macroprolactin in the circulation; this occurrence appears to be more common than previously thought (2–4) and can have obvious practical implications. The finding of a predominance of macroprolactinemia can change the focus of the evaluation of a patient, with the possible avoidance of more sophisticated and expensive imaging studies.

We evaluated the polyethylene glycol (PEG) precipitation method to screen for the presence of macroprolactinemia in a large series of clinical samples. Serum PRL was measured by immunofluorometric assay (IFMA; reference range, 2–15 μg/L; Delfia, Wallac Oy), and samples with values ≥30 μg/L were studied. The value of 30 μg/L or higher, considered as overtly abnormal, was arbitrarily selected. To 250 μL of serum, we added 250 μL of a 250 g/L PEG 6000 solution (in water, kept at 4 °C), mixed them for 1 min with a vortex mixer, and centrifuged them (9500g for 5 min at room temperature). PRL was determined in the supernatant, using the same IFMA and the recovery calculated on the basis of the original serum value. Reproducibility of the PEG precipitation process was evaluated in four different serum samples, studied seven times each, on different days and in different assays. The following values were obtained: for a sample with PRL value of 32 μg/L and mean recovery of 63%, the CV was 15%; for a sample with PRL value of 45 μg/L and mean recovery of 83%, the CV was 7%; for a sample with PRL of 68 μg/L and mean recovery of 47%, the CV was 28%; and for a sample with PRL of 71 μg/L and mean recovery of 5%, the CV was 20%.

The gel filtration used Sephacryl S-200 (Pharmacia) packed in a 0.9 × 30 cm column previously calibrated with monomeric125I PRL (New England Nuclear); 0.5 mL of serum was applied and eluted with sodium phosphate buffer (0.05 mol/L, pH 7.4), and 0.7-mL (0.1 mL/min) aliquots were collected and analyzed for PRL. The samples were classified as having a predominance of monomeric PRL forms when the analysis of the area under the curve corresponding to monomeric PRL represented >50% of the total area of PRL elution. Among 19,228 consecutive clinical samples studied in 12 months, PRL was ≥30 μg/L in 1279 (6.7%). Of these, 1220 were submitted to PEG precipitation, and 171 samples were submitted to the chromatographic study. They were selected to cover the whole range of recovery. The values of the percentage of recovery plotted against the percentage of the area under the curve that was classified as high molecular weight PRL (macroprolactin) are depicted in Fig. 1. On the basis of the data presented in Fig. 1, recoveries of ≥65% were classified as predominantly monomeric, and recoveries of ≤30% as predominantly high molecular weight forms. Values between 30% and 65% were classified as indeterminate and submitted to chromatography. The data also showed negative correlation (r = −0.84) between the percentage of recovery and the percentage of high molecular forms. Of the 1220 samples studied, recoveries were ≤30% in 441 (36%), ≥65% in 607 (50%), and 30–65% in 172 (14%). The chromatographic study of these 172 samples showed 72 (42%) with a predominance of high molecular weight forms.

Our data support the findings of Bjoro et al. (5), who also reported a large proportion of macroprolactinemia in their clinical population. One of the reasons for our surprisingly high incidence of macroprolactinemia may be the kind of samples we receive. As a reference laboratory, we receive samples for confirmation of an unexpected high value detected elsewhere. The PEG precipitation technique that we use is a convenient and simple procedure to screen for the presence of macroprolactinemia. Recoveries >65% are indicative of the absence of, and those <30% indicative of the presence of, macroprolactinemia. Samples with recoveries between 30% and 65% need gel filtration chromatography to characterize the predominant molecular form. Our data, in spite of the special characteristics of the samples studied, stress the necessity that laboratories offering PRL assay service should include, in all reported increased PRL results, some form of study for the presence of macroprolactinemia, as already suggested by Lindstedt (6).

Fig. 1. Correlation between percentage of recovery after PEG precipitation and percentage of the area under the curve, obtained after Sephacryl S-200 gel chromatography, corresponding to PRL of high molecular weight.

Data from 171 samples are depicted, as well as the 30% and 65% recovery limits and the 50% limit for the area under the curve.
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References


Effect of Urine pH, Storage Time, and Temperature on Stability of Catecholamines, Cortisol, and Creatinine, *Keiichi Miki* and Ayako Sudo (National Institute of Industrial Health, 21-1, Nagao 6-chome, Tama-ku, Kawasaki 214, Japan; * author for correspondence: fax 81-44-865-8116, e-mail miki@nih.go.jp)

In studies of stress in humans and animals, urinary excretion of catecholamines and cortisol is widely used as a stress index (1, 2). In most studies that have measured urinary catecholamines, hydrochloric acid or some antioxidant has been added to the urine samples immediately after voiding. This is probably because classic methodology demands preservation of urine specimens to prevent catecholamine degradation (3–5). On the other hand, urine is not usually acidified when glucocorticoids are to be analyzed (2, 6). Because of the difference in urine treatment required for studies of catecholamines and glucocorticoids, it has been necessary to prepare two kinds of urine samples in parallel, i.e., acidified and unpreserved. Clearly it would be preferable if a single urine treatment could be used for analysis of both stress-related hormones. However, to our knowledge, little is known about the stability of these compounds in urine kept under different conditions. In the present study, we examined changes in catecholamine and cortisol concentrations in human urine samples stored at various pH values for different periods, using HPLC and fluorometry. The stability of urinary creatinine was also investigated, because creatinine excretion is commonly used to estimate the exact timing of urine collection.

In the present experiments, urinary free catecholamines were assayed using a modification of a method described previously (7). Briefly, 0.5 or 1 mL of urine was adjusted to pH 8.4–8.6 with 1 mol/L NaOH after addition of 100 pmol of isoproterenol as an internal standard and 1 mL of 0.1 mol/L Na2EDTA (pH 8.6); the urine was then adsorbed to 150 mg of alumina packed in a glass column (6 mm i.d.). The alumina was washed twice with 5 mL of water and eluted with 1 mL of 0.25 mol/L acetic acid. The eluate was kept at –20 °C until HPLC analysis, unless otherwise stated. A small portion (100 μL) of the acidic extract was injected onto an HPLC column (Senshupak SCX-0201N 200 × 4 mm, Senshu Kagaku), using an autosampler, and developed with a mobile phase of 0.1 mol/L phosphate buffer (pH 3.5) containing 100 mL/L acetonitrile. Catecholamines in the eluate were derivatized by a postcolumn reaction, using the trihydroxyindole method, and detected by a spectrofluorometer with excitation and emission wavelengths of 420 and 520 nm, respectively. Absolute recovery from the alumina was 70–80%, but the amount of the amine was corrected using an internal standard. The CV of the calculated values was <6% (n = 10). The detection limit (signal-to-noise ratio = 5) was 0.05 pmol for adrenaline in the present HPLC analysis.

For analysis of free cortisol (8), 11-deoxycorticisol or tetrahydrocorticoestosterone was added to urine as an internal standard, and the urine was extracted with dichloromethane. The organic layer was washed with 0.1 mol/L sodium hydroxide and water and then evaporated to dryness. The residue was dissolved in 100 mL/L acetonitrile and injected onto an HPLC column (Capcell-pak C8, 250 × 4.6 mm, Shiseido). The column was eluted with 300 mL/L acetonitrile, and the effluent was mixed with sulfuric acid to produce a fluorescent derivative. The fluorescence intensity was recorded continuously using a spectrofluorometer with excitation and emission wavelengths of 465 and 530 nm, respectively. Urine creatinine concentrations were measured using the Jaffe reaction, by the AutoAnalyzer method (9).

Each sample was assayed once after initial thawing, and measurements were performed in duplicate. Urine catecholamine and cortisol concentrations are expressed as nmol/L, and creatinine as g/L of original urine, unless otherwise stated. Authentic noradrenaline, adrenaline, dopamine, and cortisol were obtained from Sigma Chemical Co., and the HPLC system used was purchased from Hitachi Co. Ltd. (L-6000 series). Because the CVs in cortisol and creatinine measurements were similar to those in catecholamine analysis, we used values within 6% of the initial baseline, in principle, as the criterion for stability in the present study.

In a preliminary experiment, the stability of catecholamines in unpreserved urine during storage at –80 °C was examined. Urine samples obtained from four healthy human volunteers were divided into two portions, one of which was acidified with 6 mol/L hydrochloric acid (1.5 mL/h of urine collection). The acidified (pH 1.7–2.3) and unpreserved (pH 5.4–6.8) urine samples were poured separately into small plastic tubes. After storage in a freezer (–80 °C) for periods of up to 4 months, each urine sample was thawed under tap water, and the concentrations of catecholamines, cortisol, and creatinine were determined as described above.

During storage at –80 °C for 4 months, catecholamine concentrations were within 6% of the baseline concentra-