Effect of Temperature on Plasma Renin Samples

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Plasma renin activity was measured in parallel in Na₂EDTA-containing plasma samples after storage at −20, 4, and 24 °C, and in the lyophilized state. In peripheral venous plasma from 22 hypertensive patients, the activity (range, 0.06–46.7 μg/liter per hour) remained stable during three days of storage at 4 °C, but decreased to a variable extent when plasma was kept at 24 °C: in one day by 9.2%, two days by 25.6%, and three days by 74.0%. Values were the same for samples handled at room temperature and chilled to 4 °C within 3 h and parallel samples immediately cooled in an ice bath and kept at 4 °C. Freezing (−20 °C) and thawing of plasma was associated with a 22% mean increase in activity (range, 0–83%). Lyophilization resulted in a smaller increase of plasma renin activity (mean 12%, range 0–46%). Blood for renin analysis need not be cooled immediately, but must be cooled to 4 °C within 2–3 h. It then is stable for at least three days. Freezing or lyophilization appears to be associated with some cold activation of "prorenin."

It is recommended that blood drawn for determination of plasma renin activity (PRA; EC 3.4.99.19) be promptly cooled, the cells separated in the cold, and the plasma stored below −20 °C until assayed (1–6). We have tested various temperatures of handling and storage of plasma samples. Our results show that PRA is not significantly altered if samples are kept at room temperature (24 °C) for 2–3 h, and that PRA remains unchanged for at least three days when samples are kept at 4 °C. Storage at −20 °C or lyophilization may result in cryoactivated "prorenin" (7, 8). Sample collection for PRA determination thus can be simplified, but temperature effects must be considered.

Materials and Methods

Reagents

Angiotensin I radioimmunoassay reagents. We used the reagents of the Phadebas angiotensin I test (Pharmacia Diagnostics, Piscataway, N. J. 08854).

Collection of blood. Samples of peripheral and renal venous blood were collected between 08:00 and 11:00 h from patients with hypertensive diseases, after 12 h of fasting. Their daily diet contained 140 mmol of sodium chloride on the average. Healthy subjects used as controls were laboratory personnel on an ad lib. diet, and blood samples were collected from them on an ambulatory basis. Blood was drawn into tubes containing disodium ethylenediaminetetraacetate, 6 g/liter of blood. Parallel tubes were kept in an ice bath and treated as reported earlier (6). Plasma was separated by centrifugation, either at room temperature or at 4 °C, and stored at 24, 4, or −20 °C until assayed. Frozen plasma was thawed by placing the tubes in cold tap water. In some experiments parallel samples were lyophilized.

Assay of renin activity. For this we used radioimmunoassay of angiotensin I, as recently described in detail (6, 9). The data were calculated by correction for the angiotensin I generated during the incubation (4 °C) in the ice bath.

Results

Instant cooling. There was no difference in PRA between samples of peripheral (n = 40) or renal venous (n = 18) blood, whether immediately cooled in an ice bath or handled at 24 °C until refrigerating to 4 °C within 3 h of blood collection.
Room temperature (24 °C). PRA decreased gradually and variably in 20 samples of peripheral venous plasma (Figure 1) kept at 24 °C for three days, 9.2% after one day, 25.6% after two, and 74.0% after three days.

Refrigerated samples (4 °C). PRA was essentially unchanged in 22 plasma samples of peripheral blood from hypertensive patients and from one patient with Bartter's syndrome when plasma was kept at 4 °C for three days (Figure 2). A minor increase of PRA was observed in plasma from a patient with primary aldosteronism, although PRA remained subnormal during the three-day storage period. In 18 of these samples, not shown in Figure 2, PRA was still the same after five days of storage at 4 °C. Similar results were obtained for 42 renal venous blood samples from 21 patients with suspected renovascular hypertension.

Frozen plasma samples (−20 °C). PRA increased (mean, 22%; range 0–82%) in 40 samples of peripheral venous plasma as compared to controls stored at 4 °C.

Lyophilized plasma. PRA was increased by 12.0% over initial values in 10 samples of peripheral venous plasma from healthy subjects (range, 0–46%) after storage in the lyophilized state. Parallel samples kept at −20 °C showed greater PRA (mean, 69%; range, 0–252%) as compared to 4 °C controls.

Discussion

Our data indicate that prompt cooling of blood samples drawn for determination of PRA is unnecessary if the plasma is refrigerated to 4 °C within 2–3 h. This accords with recent observations of Sealey et al. (8) that blood can validly be processed at room temperature for later assay of PRA, and simplifies collection of samples for PRA in clinical practice, PRA values reportedly increase, probably due to cryoactivation of prorenin, the greatest increase being at −5 °C (7, 8). This phenomenon is essentially avoided by using 4 °C as a storage temperature. Plasma samples containing Na₂EDTA maintain stable PRA for at least three days, but in many experiments we observed almost unchanged PRA values (change <20%) after keeping plasma samples at 4 °C for five days without EDTA. PRA in plasma stored at 4 °C was stable in samples of low, medium, or high PRA values (range 0.08–46.7 μg/liter per hour), although a proportionally higher increase in PRA was observed in samples with lower PRA. This may be due to the “prorenin,” activated at low temperatures, which comprises a relatively big part of the total PRA in low-PRA samples (8).

When kept at room temperature (24 °C), changes in PRA were unpredictable, precipitous decrements being seen in some plasma samples (Figure 1) after a day or more. This could be due to substrate consumption, deterioration of renin, or bacterial growth, or all of these. However, good correlation was observed between values obtained after one day at 24 °C and controls (n = 20, r = 0.962, P < 0.001). Blank values rapidly increase, however, when plasma is stored at 24 °C, and should be subtracted.

The increase in PRA observed particularly in samples stored at −20 °C, but also in lyophilized plasma, could be due to cryoactivation of “prorenin” (7, 8).

We conclude that in the handling of plasma renin samples, instant cooling is unnecessary, and that keeping samples at 4 °C minimizes cryoactivation of inactive “prorenin.” Samples should be stored below −20 °C (8) or lyophilized if not assayed within three to five days. Although freezing or lyophilization will result in some cryoactivation of “prorenin” when the sample passes through the optimal temperature of activation (−5 °C; 7), storage in the frozen state or lyophilized for a
pneval time does not appear per se to alter PRA (unpublished observations). Temperature effects may explain interlaboratory discrepancies in PRA data even when identical or very similar PRA assays have been used.

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References

An Aqueous Primary Standard for Use with the Technicon SMA 6/60

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An aqueous primary standard containing sodium, potassium, chloride, carbon dioxide, glucose, and creatinine was prepared for use with the Technicon SMA 6/60 continuous-flow analyzer. This standard, compared with a protein-base primary standard, showed no significant difference in slope, y-intercept, and linearity for these methods. Precision with use of the aqueous primary standard was acceptable, comparable to that for a commercial reference serum. The standard was stable for at least 30 days.

Additional Keyphrases: quality control • continuous-flow analysis

Primary standards are no less important in multichannel analysis than in single analysis. However, the calibration of the Technicon SMA 6/60 with “reference sera” is a widely accepted technique. The reported inaccuracies in the assigned values of reference sera (1-8) and the need for uniformity of standardization in the clinical chemistry laboratory prompted me to investigate an aqueous primary standard for use with the SMA 6/60.

Materials and Methods

Apparatus and Reagents

Two standard SMA 6/60's (Technicon Instruments Corp., Tarrytown, N. Y. 10591) were used, with no sample predilution. The chemical methods were those standard for the SMA 6/60 and include sodium and potassium by flame photometry, chloride by mercuric thiocyanate, carbon dioxide by cresol red, creatinine by alkaline picrate, and glucose by glucose oxidase-peroxidase, 3-methyl-2-benzothiazolinone hydrazine hydrochloride/N,N-dimethylaniline. All reagents used on the SMA 6/60 were obtained from Environmental Chemical Specialties Inc., Anaheim, Calif. 92807.

Standard

Analytical-grade sodium chloride, potassium chloride, sodium bicarbonate, and glucose were obtained from Mallinckrodt Inc., Saint Louis, Mo. 63147. Creatinine was obtained from Sigma Chemical Co., Saint Louis, Mo. 63178 (cat. no. C4255). Ion-free bovine serum (Chemvarion) was purchased from Clinton Laboratories, Santa Monica, Calif. 90404.

An aqueous stock standard, 1 liter of which contained 1500 mmol of sodium, 100 mmol of potassium, 1200 mmol of chloride, 400 mmol of carbon dioxide, 1.20 g of creatinine, and 45.0 g of glucose, was prepared by dissolving 64.4 g of NaCl, 7.46 g of KCl, 1.20 g of creatinine, 45.0 g of glucose, and 33.6 g of

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