Should Blood Samples for Assay of Plasma Renin Activity Be Chilled?

Rodica L. Emanuel and Gordon H. Williams

Collecting blood on ice for renin determination reportedly may produce falsely high results. To assess the probability of this occurring under actual collection conditions, we measured renin activity in duplicate aliquots of plasma from blood samples from 25 hypertensive patients, both supine and upright, and in 10 supine normotensive controls. One aliquot of the blood was collected on ice and processed at 4 °C, the other at room temperature. The two aliquots showed no significant differences in renin activity. If anything, values for samples collected at room temperature were higher. Repeat determination on the same specimens stored at −20 °C for nine and 12 months revealed no significant changes in results for any samples, although the amount of angiotensin I found in the sample before incubation at 37 °C significantly increased. We conclude that it makes little difference at what temperature one collects blood for renin determination, but because of the wide fluctuations in "room" temperature we recommend that blood samples be collected on ice.

Additional Keyphrases: variation, source of · specimen handling · enzyme activity

Sealey and colleagues (1, 3) reported the presence in plasma of a renin (EC 3.4.99.19) precursor, "prorenin," which is cold-activated. They suggest that blood for renin determinations should be collected at room temperature rather than on ice because the latter procedure may activate this precursor, thereby artifactually increasing renin activity. On the other hand, collecting and processing blood at room temperature introduces vagueness in the temperature to which the samples are exposed, and may be a source of inaccuracy in the method.

Thus, to assess the implications of these findings on our plasma renin activity (PRA) assay, we compared results for blood samples collected and centrifuged at 4 °C and identical samples handled at room temperature. PRA was measured in all specimens at the same time after storage at −20 °C for one week, and for nine and 12 months.

Methods

Two studies were performed. In the first, blood was collected from 25 patients with hypertension after balance had been achieved on a 10 mmol Na/100 mmol K intake, supine after an overnight fast, and again after ambulating for 3 h. In the second study there were 10 normotensive subjects, on an ad lib. diet, from whom blood was collected while they were supine. From both groups, blood was collected into two tubes each containing 1.5 mg of tripotassium ethylenediaminetetraacetate (K3-EDTA) per milliliter of blood. One tube was immediately chilled in ice water and the blood was centrifuged at 4 °C; the other tube was processed at room temperature. The plasma separated from each sample was quickly frozen in a mixture of acetone and solid CO₂ and stored at −20 °C. The interval from collection to freezing averaged 20 min. After about one week, the samples were defrosted and the pH was adjusted to 5.6 with HCl. Then PRA was measured by incubating an aliquot of each sample at 37 °C. For 11 subjects, the remainder of the plasma at pH 5.6 was refrozen, and PRA measured again after nine and 12 months.

PRA was measured by a modification of our previously reported radioimmunoassay (4). We now use borate buffer (pH 8.0, containing 5 g of bovine serum albumin and 10 μmol of ethylenediaminetetraacetate per liter) as assay diluent. The antiangiotsensin I, obtained by injecting sheep with a bovine serum albumin/angiotensin I conjugate, was used at a 90,000-fold dilution. I²⁵¹, labeled angiotensin I was used instead of I³¹ as published earlier.

The reaction mixture was incubated 24 h at 4 °C and then 0.5 ml of a mixture of 1 g charcoal and 0.1 g of Dextran T-70 in 170 ml of assay diluent was added to each tube. After vigorous vortex-mixing and a 20-min incubation in ice-water, the tubes were centrifuged and the free and bound peptide separated. Angiotensin I was measured after a 30-min incubation at both 4 and 37 °C. PRA was then calculated by the difference in angiotensin I found in the two samples, multiplied by two. The reliability of the assay is checked periodically by measuring the potency of our angiotensin I standard in a bioassay system and by measuring PRA in unknown samples from the American Association of Bioanalysts (205 West Levee, Brownsville, Tex. 78520).

For the statistical analysis we used the Fisher Exact Test or Wilcoxon rank order sum or Sign test (5). Values are reported as mean ± SEM and differences at P < 0.05 are considered significant unless otherwise indicated.

Results

Single Study

The PRA measured in samples obtained from hypertensive subjects in the upright position or normotensive subjects in the supine position were not significantly altered by collection on ice (Table 1). However, PRA values for specimens collected at ambient temperature from hypertensive subjects in the supine position significantly exceeded those for specimens collected on ice. Under all three circumstances (supine and upright hypertensive and supine normotensive) the "blank"
Table 1. Values for Plasma Renin Activity (μg/liter per hour) and “Endogenous” Angiotensin I (μg/liter) in Incubation Mixture at 4 °C

<table>
<thead>
<tr>
<th></th>
<th>Room temp.</th>
<th>Ice</th>
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<tbody>
<tr>
<td><strong>PRA</strong></td>
<td>3.81 ± 0.55</td>
<td>2.82 ± 0.55</td>
</tr>
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<td><strong>AI</strong></td>
<td>0.25 ± 0.02</td>
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</tr>
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<td><strong>PRA</strong></td>
<td>5.8 ± 1.1</td>
<td>4.8 ± 0.8</td>
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</tr>
<tr>
<td><strong>PRA</strong></td>
<td>15.8 ± 3.5</td>
<td>14.1 ± 3.1</td>
</tr>
<tr>
<td><strong>AI</strong></td>
<td>1.47 ± 0.25</td>
<td>0.57 ± 0.1</td>
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25 Hypertensive subjects, supine

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25 Hypertensive subjects, upright

Significance

NS

P < 0.05

P < 0.05

NS

P < 0.05

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References


We found no consistent changes in PRA during the short interval that the specimens were treated differently. Indeed, if anything, the PRA in specimens collected at room temperature exceeded that at 4 °C, suggesting that cryoactivation of prorenin does not occur very promptly or rapidly, or that other factors activated by the higher temperature counterbalance any effect from cryoactivation. Nevertheless, the ambient temperature in a laboratory may vary by several degrees and thereby introduce another intangible—collection temperature—in the determination of PRA; to eliminate concern on this point we recommend collecting and processing blood for PRA on ice and in a refrigerated centrifuge, thus assuring a relatively constant temperature for all samples. If blood specimens are processed promptly—say within 15–20 min—there evidently is no loss of precision or reliability; if anything, PRA values are reassuringly lower than if the blood is collected at room temperature.

Long-term storage of samples does not alter the calculated PRA, documenting that there is little if any increase in the total enzyme activity. However, the amount of angiotensin I measured in the 4 °C incubate is significantly increased, suggesting continued generation of angiotensin I in frozen samples. Although no error is produced in PRA determinations as long as the “blank” is subtracted, the potential exists at high renin, low substrate concentrations that PRA determinations after prolonged storage may be erroneously low.

Results of PRA determinations and, second, if PRA measurements are reliable on frozen plasma samples stored for prolonged periods at pH 5.6.

Time Study

PRA was determined a week and also nine and 12 months after sampling in specimens from 11 subjects. There were no significant differences in the calculated PRA during the 12-month period, whether the samples were processed at ambient temperature or at 4 °C (Figure 1). However, in all samples there was a clearly significant \((P < 0.01)\) increase with time in the angiotensin I concentration found in the 4 °C incubate, the samples collected at room temperature from upright subjects being the most affected (Figure 1).

Discussion

Our purpose in this study was to determine, first, if the temperature at which blood is collected and processed alters the apparent concentration of angiotensin I was significantly greater in the samples collected at room temperature.

\((4 °C \text{ incubation})\)