Increased Renin Concentration in Plasma and Amniotic Fluid During Storage

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Renin concentration was determined in plasma and amniotic fluid samples immediately after collection, after storage at −18 °C for up to 48 h, and after storage at 5 °C for as long as 48 h. Renin concentration increased, both in plasma ($P < 0.05$) and amniotic fluid ($P < 0.02$), after the storage at 5 °C, but no significant alteration occurred on storage at −18 °C. Partial activation of inactive renin could explain the observed increase in renin concentration at 5 °C.

Renin concentration is determined indirectly by measuring angiotensin I generated from exogenous substrate in the presence of angiotensinase inhibitors. There are variations among methods with regard to the pH and duration of incubation, selection of angiotensinase (EC 3.4.99.1) inhibitors, types of substrate used, and the possible inclusion of one or more periods of dialysis. Dialysis to low pH to remove endogenous substrate and angiotensinase activity before assay at pH 7.5 has been used (1) but was later shown to increase the rate of angiotensin I generation, possibly due to the irreversible activation of an inactive form of renin (2). Inactive and active renin have been determined both in plasma (3–5) and amniotic fluid (6). The physiological importance of the inactive form and the mechanism of conversion have still to be elucidated.

Increased rates of angiotensin I generation after storage of plasma at −20 and 4 °C have been reported (6, 7), respectively, although conflicting reports suggest that samples can be stored at −20 °C for short periods before assay (8, 9).

We have assessed the effect of time and temperature on the short-term storage of samples of both plasma and amniotic fluid before assay of renin concentration.

Methods

Blood samples were collected, with disodium ethylenediaminetetraacetate as anticoagulant, into iced tubes from 20
Fig. 1. Changes in amniotic fluid renin concentration after incubation at 5 °C compared with assaying immediately and freezing

normal individuals, immediately centrifuged at 4 °C, and the plasma was separated. Amniotic fluid samples were obtained from elective cesarean sections (n = 3) or by amniocentesis (n = 11). Samples contaminated with blood or meconium were discarded.

We assayed for renin in aliquots of amniotic fluid (n = 5) and plasma (n = 10) within 1 h of collection and again after 24 and 48 h at −18 °C. Other amniotic fluid and plasma samples were stored at −18 °C for a maximum of 24 h, assayed immediately on thawing and re-assayed after incubation at 5 °C for 24 and 48 h.

Renin concentration was measured by radioimmunoassay of angiotensin I using antiserum raised in rabbits against an (1-Asp,5-Ileu) angiotensin I/bovine serum albumin conjugate. We incubated samples with sheep substrate (4 × 0.1 ml) at 37 °C for 3 h in the presence of angiotensinase inhibitors: 15 mmol of disodium ethylenediaminetetraacetate, 5 mmol of dimercaptoopropanol, and 1 mmol of 8-hydroxyquinoline sulfate per liter (final concentration). Angiotensin I generation was stopped by adding 0.1 ml of cold acetonitrile after 0, 1, 2, and 3 h of incubation and, after centrifugation at 4 °C, an aliquot of each supernatant fluid was diluted fivefold with 50 mmol/liter 2-amino-2-(hydroxymethyl)-1,3-propanediol [tris(hydroxymethyl)methylamino] buffer, pH 7.5, containing 3 g of bovine serum albumin per liter, and assayed along with standards of angiotensin I. Antiserum and 125I-labeled angiotensin I were added and the samples were incubated at 4 °C for 18 h. Bound and free fractions were separated by treatment with dextran-coated charcoal and the radioactivity in both fractions was counted. Renin concentration was calculated in micrograms of angiotensin I produced per liter of sample per hour and expressed in terms of units per milliliter, with use of Medical Research Council renin 68/356 (1 unit being equivalent to 10^−8 international units) as standard.

Sheep plasma containing renin substrate was obtained six days after nephrectomy according to the method of Skinner (1) and used at a concentration of 1 mg/liter in the incubation mixture. Reproducibility (CV) of the method, as measured by replicate assays on a plasma pool, was 5.6% intra-assay and 10.7% interassay.

Results

Storage at −18 °C for 24 h had no significant effect on the apparent renin concentration in amniotic fluid (t = 0.31; P > 0.7) and the variation on re-assay was within the previously established inter assay variation. However, after incubation at 5 °C for 24 h, renin concentration was increased in all the amniotic fluid samples (Figure 1). The increases were significant by the paired t-test (t = 3.18; P < 0.002) and represented a mean increase of 77.9% over values for the aliquots held at −18 °C for the same period.

Results were similar for the plasma samples (Figure 2). Again, there was no significant change in renin concentration after storage at −18 °C for 48 h (t = 1.399; P > 0.2) but renin concentration increased significantly after incubation at 5 °C for 48 h (t = 2.209; P < 0.05). Overall, there was a mean increase in plasma renin concentration of 33.3%, although increases were not found in all plasma samples. No other similarities were observed among samples for which the renin concentration did not increase, when sex, renin activity, and blood pressure at the time of sampling were compared.

Discussion

Inability to measure renin directly, coupled with non-standardized methodology in the indirect assay of renin, has given rise to problems in the expression and interpretation of renin activity and concentration. Comparison of values among different laboratories may be further complicated by changes in renin concentration during storage of specimens before assay. Sealey and Laragh (6) reported substantial increases in plasma renin activity after prolonged storage at −20 °C accompanied by no change in renin substrate concentration, and Osmond et al. (7) found increased renin activity after plasma was stored at 4 °C. On the other hand Arakawa et al. (8) found no change in plasma renin activity after 2 months of storage deep frozen, and Fukuchi et al. (9) reported a decrease in plasma renin activity after more than four weeks at...
−20 °C. In this on-going study, we have initially looked at the effect of short-term storage on renin concentration, thus excluding unknown effects of storage on endogenous substrate that may interfere in the interpretation of renin activity measurements.

Although no other similar studies involving renin concentration have been performed, comparison with the findings of Sealey and Laragh (6) may be relevant because no change in renin substrate concentration accompanied the observed increases in renin activity. To account for the increases in plasma renin activity they suggested that inactive renin might be slowly activated in samples stored at −20 °C. Possibly this activation could occur more quickly at 5 °C and would explain our observed increases in the apparent renin concentration of both plasma and amniotic fluid. The greater increase in amniotic fluid renin concentration would support this hypothesis, because the proportion of inactive renin is reported to be greater than in plasma (2).

The increases we observed were significantly higher than could be accounted for by interassay variation. The pH optimum for the human renin–sheep substrate reaction does not peak sharply (1), thus a change in pH would have to be large to alter the angiotensin I generation rate considerably, but no differences in pH were detected after freezing or storage at 5 °C. The pH during incubation was stabilized by the addition of the buffered sheep substrate (pH 7.5).

If the increases in renin concentration is due to the activation of inactive renin, several considerations arise with regard to the investigation of the nature of inactive renin and its physiological importance. Inactive renin is normally converted to renin by low pH; conversion at 5 °C would not subject samples to harsh pH changes that denature some proteins and enzymes, while activating others. Methodologically it would appear inappropriate to dialyze or incubate samples at 5 °C before renin assay. Control samples, normally dialyzed at pH 7.5 in activation studies should be stored at −18 °C, so that renin concentration is not significantly altered.

References