Urinary Kallikrein and Plasma Renin Activity in Normal Human Pregnancy

GLORIA VALDÉS, M.D., PATRICIA ESPINOZA, B.SC, ROSARIO MOORE, M.D., AND HÉCTOR R. CROXATTO, M.D.

SUMMARY Urinary kallikrein excretion (UK), plasma renin activity (PRA), and 24-hour urine volume, sodium, and potassium excretion rates were determined sequentially in 16 normal pregnant women. Throughout gestation, UK was significantly elevated as compared to values obtained in 13 control women (1466 ± 152 vs 375 ± 90 U/g creatinine). The highest level was observed in Period 2 of gestation, corresponding to Weeks 17 to 24. PRA was also significantly elevated during pregnancy (11.97 ± 1.35 vs 1.06 ± 0.90 ng/ml/hr), with the highest level in Period 2. Mean 24-hour urine volume, sodium, and potassium excretion rates were significantly higher during pregnancy. No correlation was found between UK and: PRA, urine volume, and sodium and potassium excretions. These findings indicate a consistent activation of the renal-kallikrein-kinin system during pregnancy. We postulate that this vasodilator system might play a role in the maintenance of normotension in pregnancy, counteracting the effect of the renin-angiotensin-aldosterone system.

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KEY WORDS • sodium excretion • potassium excretion • urine volume

NORMAL pregnancy is associated with a marked increase in the renin-angiotensin-aldosterone system, plasma volume, and cardiac output. Maintenance of normotension, or even a decrease in blood pressure, has been attributed to the arteriovenous shunt created by the utero-placental junction. Nevertheless, a generalized decrease in peripheral resistance and a reduced vascular reactivity to angiotensin II suggest an endogenously increased vasodilator activity. Both urinary kallikrein and prostaglandin E in plasma and urine have been described as increased, possibly playing a role in cardiovascular homeostasis.

To our knowledge, this investigation designed as a prospective study represents the first sequential follow-up of urinary kallikrein in normal human pregnancy. Peripheral plasma renin activity (PRA), as well as water and electrolyte excretion, were determined serially.

Material and Methods

Subjects

Sixteen normal pregnant women aged 21 to 32 years (mean: 26 ± 0.78) were followed for periods ranging from 3 to 31 weeks (mean: 17.1 ± 2.1). Six were primigravida, seven para-1, and three para-2. Our criteria for normality were: a diastolic pressure persistently below 90 mm Hg in the sitting position, absence of proteinuria and edema, and monthly weight gain less than 2 kg. All patients were ambulatory, on free salt and fluid intake. We analyzed 101 urine collections from these women with urinary kallikrein, creatinine, sodium, and potassium.

The control group consisted of 13 healthy women aged 21 to 37 years (mean age, 28.3 ± 1.3), on free salt and fluid intake and no oral contraception. Thirty-one urine collections were analyzed as for the study group.

Urinary Kallikrein Determination

Urine was collected for 24 hours in a flask containing 5 ml toluene. After filtration, an aliquot was stored at -30°C, up to the moment of enzymatic determination. Before testing, the urine was dialyzed in the cold for 20 hours against a solution at pH 7.5 containing 5
g NaCl, 0.9 g Na HCO₃ and 1.1 g EDTA per liter. Kallikrein was determined according to its kininogenase activity. A urine sample (0.02-0.1) added to 0.2 ml tyrode solution was incubated for 2 minutes at 37°C with 0.2 ml saline containing low molecular weight (LMW) kininogen from human plasma. Every batch of this solution was previously assayed to assure an excess of substrate; it generated over 100 ng of kallidin when incubated for 2 minutes with an excess of kallikrein. Kallidin released was measured through its oxytocic effect upon the isolated rat uterus. This effect was compared to that induced by a highly purified, standardized human urinary kallikrein. Enzymatic activity was expressed in conventional units. One unit corresponds to the activity induced by 0.01 ml of standard kallikrein releasing 10 ng of kallidin from LMW kininogen, under the above mentioned conditions of incubation. Urinary kallikrein excretion was expressed as units per gram of creatinine (U/g cr).

**Peripheral Plasma Renin Activity (PRA)**

PRA was determined by radioimmunoassay (New England Nuclear kit) in 43 samples from 12 pregnant women and seven samples from seven control subjects; the blood samples were drawn with the patient in the sitting position, after 1 to 2 hours of normal activity.

**Urinary Sodium and Potassium**

They were measured in 24-hour urine samples by means of a flame photometer (IL 343).

**Urinary Creatinine**

Urinary creatinine was determined by the method of Jaffe.

**Statistical Analysis**

The samples were first averaged for the whole period of pregnancy and then grouped separately, according to the following periods: Period 1 = 0 to 16 weeks; Period 2 = 17 to 24 weeks; Period 3 = 25 to 32 weeks; and Period 4 = 33 to 40 weeks. Group means are presented with SEM as the index of dispersion. Statistical probability was evaluated with the Student's *t* test. Statistical significance was fixed at *p* < 0.05.

**Results**

**Urinary Kallikrein Excretion**

Throughout gestation, the average kallikrein excretion was significantly higher than that of controls (1466 ± 152 vs 375 ± 90 U/g cr, *p* < 0.0005). In each period of pregnancy the mean excretion was significantly higher than that of controls. The values for Period 3 were significantly lower than those in Period 2 (*p* < 0.05); the mean values for the latter period were the highest in pregnancy, although not significantly different as compared to Periods 1 and 4 (Table 1, fig. 1).

### Table 1. Peripheral Plasma Renin Activity (PRA), Urinary Kallikrein Excretion (UK), 24-Hour Urine Sodium (U_{NaV}), Potassium (U_{Kv}), and Volume (U_{vol}) in Controls and Normal Pregnant Women

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pregnancy</th>
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<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 43)</td>
<td>(n = 3)</td>
<td>(n = 9)</td>
<td>(n = 16)</td>
<td>(n = 15)</td>
<td>(n = 3)</td>
<td>(n = 101)</td>
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<tr>
<td><strong>PRA (ng/ml/hr)</strong></td>
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<tr>
<td>Mean</td>
<td>1.06</td>
<td>11.97†</td>
<td>6.73†</td>
<td>14.18†</td>
<td>12.24‡</td>
<td>11.23‡</td>
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<tr>
<td>SEM</td>
<td>0.90</td>
<td>1.35†</td>
<td>2.34</td>
<td>3.84</td>
<td>1.92</td>
<td>2.36</td>
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<td><strong>UK (U/g cr)</strong></td>
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<tr>
<td>Mean§</td>
<td>375</td>
<td>1466‡</td>
<td>1350†</td>
<td>1827†</td>
<td>1149*</td>
<td>1545†</td>
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<tr>
<td>SEM</td>
<td>90</td>
<td>152</td>
<td>331</td>
<td>370</td>
<td>231</td>
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<td><strong>U_{NaV} (mEq/day)</strong></td>
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<tr>
<td>Mean</td>
<td>156</td>
<td>188†</td>
<td>172 (NS)</td>
<td>188*</td>
<td>196†</td>
<td>185*</td>
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<td>SEM</td>
<td>11</td>
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<td><strong>U_{Kv} (mEq/day)</strong></td>
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<tr>
<td>Mean</td>
<td>59</td>
<td>67*</td>
<td>72†</td>
<td>63 (NS)</td>
<td>67†</td>
<td>66†</td>
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<td>SEM</td>
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<td><strong>U_{vol} (ml/day)</strong></td>
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<td>Mean</td>
<td>1095</td>
<td>1448‡</td>
<td>1336*</td>
<td>1308*</td>
<td>1511‡</td>
<td>1523‡</td>
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<td>SEM</td>
<td>66</td>
<td>39</td>
<td>60</td>
<td>72</td>
<td>80</td>
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*p* < 0.05; †*p* < 0.005; ‡*p* < 0.0005; vs control women.

§Similar values and trends were obtained when averaging 24-hour urinary kallikrein.
Peripheral Plasma Renin Activity

Throughout gestation and in all periods tested, PRA was significantly higher than that of controls (11.97 ± 1.35 vs 1.06 ± 0.90 ng/ml/hr, p < 0.001). Significant differences were also found between Periods 1 and 2 as well as between Periods 2 and 4 (p < 0.05 for both) (table 1, fig. 1).

Twenty-Four-Hour Urine Volume, Urinary Sodium, and Potassium Excretions

Throughout gestation, the mean 24-hour urine volume as well as sodium and potassium excretions were significantly higher than control values. Mean values for each of the four periods of gestation were also significantly higher with the exception of sodium excretion in Period 1, and potassium excretion in Period 2 (table 1, fig. 1). No correlation was found between urinary kallikrein excretion and: PRA, urine volume, urinary sodium and potassium when pairing individual samples.

Discussion

Results show a consistent and significant rise in urinary kallikrein excretion throughout normal pregnancy. This is in keeping with the observations of Elebute and Mills' during the first semester. These authors also found a significant negative correlation between kallikrein excretion and the stages of pregnancy. We did observe a fall in Period 3 although a rise was seen thereafter (fig. 2). This discordance might be ascribed to the influence of the high levels of urinary kallikrein preceding term, probably not detected in the Elebute and Mills series, which included fewer samples for the last weeks of pregnancy. On the other hand, a sequential study may avoid the distortion resulting from isolated sampling, given the wide range of kallikrein excretion levels among individuals (fig. 2).

The significant rise in urinary kallikrein throughout pregnancy reflects a consistent activation of this enzymatic vasodilator system. Similar rises in urinary kallikrein, concomitant with a reduction in total glandular renal kallikrein, have been recently observed in pregnant rats by Norambuena and Croxatto (personal communication). The most likely source of the high levels of urinary kallikrein is the maternal kidney, subjected to multiple humoral stimuli, especially of adrenal origin. Notwithstanding, we cannot rule out the possibility that the fetus, the placenta, or the uterus may contribute to raised kallikrein levels.

There is a growing body of evidence suggesting that renal kallikrein is involved in the regulation of renal
blood flow, water and electrolyte excretion, and prostaglandin synthesis\textsuperscript{11} and could thus be responsible for the changes observed in pregnancy. Important data support the idea that renal kallikrein can operate beyond the kidney.\textsuperscript{11} Roblero et al.,\textsuperscript{13} using isolated perfused kidneys, described the passage of kallikrein into the perfusion fluid. Although the lungs are an efficient barrier for kinins, pulmonary passage does not hamper the enzymatic activity of kallikrein "in vivo" (Rosas et al., personal communication). The occurrence of immunoreactive glandular kallikrein\textsuperscript{18} and kinins in plasma\textsuperscript{14} points to an extensive role of kallikrein in blood pressure regulation. The kallikrein-kinin system may counteract the effects of increased activity of the renin-angiotensin-aldosterone system in normal pregnancy. It is worth recalling that in this condition urinary kallikrein falls significantly below nonpregnant levels in patients that develop hypertension.\textsuperscript{7}

Evaluation of sequential changes in PRA throughout pregnancy shows an important increment similar to that described by Wilson et al.\textsuperscript{3} Such an increase is especially striking when considering that urinary sodium was persistently elevated, reflecting at this steady stage a higher salt ingestion. Relating this finding to data from Suzuki et al.\textsuperscript{16} and Sealey et al.\textsuperscript{14} that indicate a role for kallikrein in renin release and activation, we postulate that kallikrein can be one factor that promotes an increase of the renin-angiotensin system in pregnancy.

In pregnancy, the concomitant increase in the levels of the renin-angiotensin-aldosterone system, urinary kallikrein and prostaglandins is strikingly similar to that observed in Bartter's syndrome.\textsuperscript{17} The lack of hypokalemia, despite high mineralocorticoid activity, can be ascribed to the antagonistic effect of progesterone.\textsuperscript{19, 18} Prostaglandin synthetase inhibition by indomethacin normalizes all alterations except hypokalemia, pointing to a renal defect in potassium handling as the primary abnormality in Bartter's syndrome.\textsuperscript{17} In pregnancy, it is so far impossible to define the triggering mechanism(s), especially since there is such a close interrelationship among prostaglandins, corticoids, and the renin and kallikrein systems.\textsuperscript{9, 11} Very accurate studies of these systems — including steroids, prolactin, etc. — during the early stages of gestation will be needed to establish the sequence of hormonal changes.

\section*{Acknowledgments}

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