Inhibition of the Kallikrein-Kinin System and Vascular Reactivity in Bartter’s Syndrome

JOSE A. RODRIGUEZ-PORTALES, JOSE M. LOPEZ-MORENO, AND DANIEL MAHANA

SUMMARY To study the significance of the increased activity of the kallikrein-kinin system described in patients with Bartter’s syndrome, we investigated the pressor response to infused angiotensin II in four patients with the syndrome receiving no treatment and during the administration of aprotinin and of indomethacin. Five normal subjects served as controls. Aprotinin is a proteolytic enzyme that inhibits the formation of kinins by inhibiting plasma and glandular kallikrein. Indomethacin, a prostaglandin-synthesis inhibitor, can also inhibit the kallikrein-kinin system and normalizes vascular responsiveness to angiotensin II in Bartter’s syndrome. All patients had increased urinary kallikrein and prostaglandin E2 concentrations. Aprotinin significantly decreased the dose of infused angiotensin II required to induce a 20 mm Hg increase in diastolic blood pressure, from 11 ± 4 ng/kg/min to 7.0 ± 2.0 ng/kg/min (mean ± SD; p < 0.05) in normal subjects and from 135 ± 57 ng/kg/min to 70 ± 26 ng/kg/min (p < 0.05) in the patients with Bartter’s syndrome, without significantly changing plasma renin activity, mean control blood pressure, or urinary prostaglandin E2 concentration. Indomethacin normalized the pressor response to angiotensin II in three patients who had been pretreated for 4 days (pressor dose, 10 ng/kg/min) but not in one patient who received a single oral dose of indomethacin 5 hours before the test. Our results suggest that inhibition of the kallikrein-kinin system alone accounts for approximately a 50% decrease in vascular resistance to the pressor effect of angiotensin II in Bartter’s syndrome, while additional suppression of prostaglandins entirely normalizes the vascular response to angiotensin II. These observations underscore the importance of the kallikrein-kinin system as a vasodilator in Bartter’s syndrome and support the concept that this system may contribute to the regulation of blood pressure in human beings.

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KEY WORDS • prostaglandins • angiotensin II • aprotinin • indomethacin • blood pressure
Table 1. Clinical Data for Four Patients with Bartter's Syndrome

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25</td>
<td>4</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>37.9</td>
<td>11.5</td>
<td>23.0</td>
<td>19.2</td>
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</tr>
<tr>
<td>Height (cm)</td>
<td>154</td>
<td>87</td>
<td>124</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>102/80</td>
<td>100/80</td>
<td>100/60</td>
<td>100/80</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (mEq/L; n = 3)</td>
<td>143 ± 3</td>
<td>129 ± 5</td>
<td>138 ± 3</td>
<td>138 ± 4</td>
<td></td>
</tr>
<tr>
<td>Urinary (mEq/24 hr; n = 3)</td>
<td>105 ± 15</td>
<td>41 ± 8</td>
<td>84 ± 6</td>
<td>77 ± 10</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (mEq/L; n = 3)</td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Urinary (mEq/24 hr; n = 3)</td>
<td>54 ± 8</td>
<td>44 ± 10</td>
<td>61 ± 9</td>
<td>46 ± 8</td>
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<tr>
<td>Chloride</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Serum (mEq/L; n = 3)</td>
<td>101 ± 10</td>
<td>74 ± 3</td>
<td>87 ± 5</td>
<td>92 ± 8</td>
<td></td>
</tr>
<tr>
<td>Urinary (mEq/24 hr; n = 3)</td>
<td>138 ± 10</td>
<td>52 ± 6</td>
<td>122 ± 7</td>
<td>81 ± 7</td>
<td></td>
</tr>
<tr>
<td>Serum bicarbonate (mEq/L)</td>
<td>26.8</td>
<td>42.4</td>
<td>34.3</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m² BSA)</td>
<td>63</td>
<td>119</td>
<td>73.8</td>
<td>39.6</td>
<td></td>
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<tr>
<td>PRA, after overnight recumbency (ng/ml/hr)</td>
<td>103.7</td>
<td>370</td>
<td>44.6</td>
<td>54.9</td>
<td>1.0–2.5</td>
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<td>Aldosterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma 0900 hours (ng/dl)</td>
<td>48.4</td>
<td>52.4</td>
<td>42.6</td>
<td>50.2</td>
<td>1.0–26.0 ng/dl</td>
</tr>
<tr>
<td>Urinary (μg/24 hr)</td>
<td>(–)</td>
<td>8.6</td>
<td>9.3</td>
<td>15.9</td>
<td>6.0–25.0</td>
</tr>
<tr>
<td>Urinary kaliurein (μU kaliurein/g creatinine/24 hr)</td>
<td>329</td>
<td>13,261</td>
<td>956</td>
<td>21.0</td>
<td>5.0–15.0*</td>
</tr>
<tr>
<td>Urinary prostaglandin E₂ (ng/24 hr)</td>
<td>2.121</td>
<td>(–)</td>
<td>3.874</td>
<td>2.856</td>
<td>696.0±240.0</td>
</tr>
<tr>
<td>Fractional distal reabsorption of chloride</td>
<td>0.27</td>
<td>0.57</td>
<td>0.64</td>
<td>0.40</td>
<td>&gt;0.80</td>
</tr>
<tr>
<td>Juxtaglomerular hyperplasia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (± SD).

GFR = glomerular filtration rate; BSA = body surface area; PRA = plasma renin activity; + = moderate; ++ = marked; ? = no glomeruli obtained.

*Chromogenic assay, Patients 1, 3, and 4. †Bioassay, Patient 2.

Patient 1 was a 25-year-old woman who had been diagnosed in childhood but had abandoned treatment for 2 years. Patients 2, 3, and 4 were children who, in addition, showed failure to thrive. Patients 3 and 4 were siblings. None of the patients had a history of vomiting, diuretic intake, or laxative abuse. Their liver function, as judged from prothrombin time and serum bilirubin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and alkaline phosphatase levels, was normal. Results of urinalysis and intravenous pyelograms were also normal in all patients.

All patients had a decreased fractional distal reabsorption of solutes, measured as the ratio of the free water clearance (CH₂O) to the distal delivery of solutes (CH₂O + C כ or CH₂O + C Na + K) under conditions of maximal free water clearance with an intravenous infusion of 5% dextrose in water. The four patients had renal biopsies, which showed juxtaglomerular hyperplasia in three. In Patient 4 no glomeruli were obtained in the specimen. The control group was formed by five healthy adults (aged 17–42 yr; 4 men and 1 woman) who had a personal and family history of normal blood pressure and did not receive medications for at least 2 weeks. The patients or their parents signed consent forms for the protocol, which had previously been approved by the Ethics Committee of the Catholic University of Chile.

The patients were admitted to a metabolic ward where they received a diet constant in sodium (87 mEq/m² body surface area/day) and potassium (70 mEq/day). Blood pressure was monitored with an arm cuff mercury manometer every 4 hours throughout their hospital stay. A Foley catheter was inserted in the bladder for the clearance study and for the prostaglandin E₂ (PGE₂) and kaliurein urine collections.

Blood was collected on admission and on the study days for electrolytes, bicarbonate, and plasma renin activity (PRA). Urine was collected daily for electrolytes. Basal urinary aldosterone, PGE₂, and kaliurein were measured after equilibration on the diet was achieved. Sodium and potassium were measured by internal standard flame photometry in a spectrophotometer (Beckman Instruments, Inc., Fullerton, CA,
USA). Chloride was measured by titration in a chlorimeter (Beckman). Osmolarity was measured in a thermocouple osmometer (Wescor, Logan, UT, USA). Plasma renin activity was measured by radioimmunoassay of generated angiotensin I. 8 Aldosterone was measured in plasma and urine by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Urinary kallikrein was determined in three patients by absorbance at 405 nm of the derivative of the synthetic substance S-2266 (Kabi-Diagnostica, Stockholm, Sweden) generated by esterase activity. 9 In Patient 2, urinary kallikrein activity was determined as previously described, 10 by measuring on the isolated rat uterus the oxytocic effect of kinin liberated from dialysed urine incubated with human low molecular weight kininogen in excess for 2 minutes at 37 °C and pH 7.4. Results are expressed in units; 1 unit represents the oxytocic effect of purified standard human urinary kallikrein capable of generating 10 ng of kinin (2 min at pH 7.4 and at 37 °C) from purified human kininogen II. This substance was prepared according to the method of Jacobsen. 11 The kallikrein activity measured thus has a good correlation with that measured by radioimmunoassay. 12

The PGE₂ determinations in urine were performed by Dr. Meyer Lifschitz, Renal Division, Department of Medicine, University of Texas Health Science Center at San Antonio, Texas. Data were analyzed by Student’s paired t test. Differences were considered significant at a p less than 0.05. Results are given as means ± SD.

On the day of the study, an intravenous infusion of 5% glucose was started at a slow rate (0.2 ml/min). Blood pressure was monitored every 3 minutes for 30 minutes until a stable baseline was achieved. The mean diastolic blood pressure was obtained by averaging the last four determinations. Angiotensin II (Hypertensin, Ciba-Geigy) was infused at increasing rates, starting with 5 ng/kg/min, until an increase of 20 mm Hg in the mean diastolic blood pressure had been obtained (pressor dose). A basal period of 3 to 10 minutes of blood pressure monitoring without angiotensin II was included between each infusion period. The PRA was measured in blood drawn before starting the infusion and on reaching the pressor dose of angiotensin II. Urine was collected for PGE₂ measurements in three of the patients during the control period without angiotensin II.

When an increase of 20 mm Hg in the diastolic blood pressure had been obtained, the angiotensin II infusion was discontinued and new baseline measurements were made. After 30 minutes, aprotinin (Trasylol), 5 kallikrein-inhibiting units (KIU) per kilogram of body weight per minute, was infused, and blood pressure was monitored every 3 minutes for 30 minutes. After stable baseline values had been obtained, angiotensin II was added in the same way as previously described until an increase of 20 mm Hg had been obtained in the mean diastolic blood pressure. During the aprotinin infusion period, urine was collected for PGE₂ measurements in three of the patients. The PRA was measured basally, after infusion of aprotinin and after aprotinin plus the pressor dose of angiotensin II.

Indomethacin, 3 to 5 mg/kg/day, was given orally for 4 days to Patients 1, 2, and 3. On Day 4, the angiotensin II infusion was repeated in the manner just described. Patient 4 received the infusions 5 hours after a single oral dose of 100 mg of indomethacin (5.2 mg/kg). The PRA was measured before and at the end of the infusion.

To study whether repeated angiotensin II infusions per se can decrease pressor responsiveness, the pressor dose of angiotensin II was determined as previously described, on a separate day, in Patient 3. After a 20 mm Hg increase in the diastolic blood pressure had been obtained, the infusion was stopped for 30 minutes, which allowed the blood pressure to return to control values. Then the pressor dose of angiotensin II was again determined as before. A second period of 30 minutes without angiotensin II was observed, followed by a third infusion period to determine the pressor dose.

The effect of increasing doses of aprotinin on angiotensin II responsiveness was studied in Patients 3 and 4. The pressor response to angiotensin II was determined as described. Aprotinin then was added to the 5% glucose in water infusion, and the rate was adjusted to deliver 5, 10, 15, and 20 KIU/kg/min. Each dose was infused for 30 minutes before starting the angiotensin II infusion. Angiotensin II was given initially at a subpressor dose and increased stepwise until a 20 mm Hg increase of the control diastolic pressure had been obtained.

Results

Mean baseline diastolic blood pressures were 60, 70, 70, and 80 mm Hg, respectively, in Patients 1, 2, 3, and 4 (Figure 1). Infusion rates of 120, 60, 100, and 180 ng/kg/min of angiotensin II, respectively (mean ± SD, 135 ± 57 ng/kg/min), were required to increase their diastolic blood pressures by 20 mm Hg. Their systolic blood pressures were 90, 97, 105, and 103 mm Hg and increased to 114, 130, 108, and 120 mm Hg, respectively, with the pressor dose of angiotensin II. Levels of PRA were 67.2, 650, 54.9, and 115 ng/ml/hr before and 93.0, 631, 51, and 105 ng/ml/hr at the end of the infusion.

In normal subjects, the mean baseline blood pressures were 112 ± 11 mm Hg (systolic), and 66 ± 11 mm Hg (diastolic). The mean pressor dose of angiotensin II in this group was 11 ± 4 ng/kg/min. The PRA was 0.64 ± 0.74 ng/ml/hr before the infusion and 0.56 ± 0.38 ng/ml/hr at the end of it.

From control mean diastolic blood pressures of 60, 80, 70, and 80 mm Hg, doses of 60, 40, 80, and 100 ng/kg/min of angiotensin II were needed in Patients 1, 2, 3, and 4, respectively (mean ± SD, 70 ± 26 ng/kg/min; p < 0.05 with respect to control), to induce a similar 20 mm Hg rise in diastolic blood pressure during the simultaneous infusion of aprotinin (Figure 1). Therefore, a 50%, 33%, 55%, and 44% decrease in the vascular resistance to angiotensin II was obtained.
when angiotensin II was given together with aprotinin. Systolic blood pressures were 90, 100, 98, and 110 mm Hg in Patients 1, 2, 3, and 4 and increased to 100, 140, 108, and 120 mm Hg, respectively, with the pressor dose of angiotensin II. Levels of PRA were 104.9, 582, 61, and 112 ng/ml/hr before and 105.9, 570, 48, and 122 ng/ml/hr at the end of the aprotinin infusion in Patients 1, 2, 3, and 4 respectively. With the addition of angiotensin II, PRA changed to 106, 403, 50.7, and 115 ng/ml/hr on attainment of the pressor dose.

In normal subjects, the mean baseline blood pressures were 114 ± 11 mm Hg (systolic), and 68 ± 9 mm Hg (diastolic). The mean pressor dose of angiotensin II during aprotinin was 7 ± 2 ng/kg/min (p < 0.05 with respect to control). The PRA was 0.48 ± 0.2 ng/ml/hr before and 0.55 ± 0.3 ng/ml/hr at the end of the aprotinin infusion. With the addition of angiotensin II, PRA decreased slightly but not significantly to 0.32 ± 0.29 ng/ml/hr. Aprotinin did not change urinary PGE₂ significantly (control, 1.59 ± 0.7 ng/min, aprotinin, 5.15 ± 3.12 ng/min).

The angiotensin II infusion was repeated during treatment with indomethacin in all patients. In Patients 1, 2, and 3, who had received indomethacin for 4 days, the pressor dose was 10 ng/kg/min: blood pressure rose from 100/72, 102/74, and 88/61 mm Hg to 140/100, 120/94, and 96/81 mm Hg respectively.

Patient 4, who had received a single oral dose of 100 mg of indomethacin 5 hours earlier, had no response because his diastolic blood pressure increased by 20 mm Hg (from 101/78 mm Hg to 130/100 mm Hg) when 180 ng/kg/min of angiotensin II was infused. Levels of PRA were 7.2, 6.8, 15.0, and 96 ng/ml/hr in Patients 1, 2, 3 and 4, respectively, before and 5.5, 5.2, 11.0, and 81 ng/ml/hr at the end of the angiotensin II infusion. Figure 2 shows the increasing pressor responsiveness to angiotensin II in Patient 3 with aprotinin and with indomethacin.

The pressor responsiveness to repeated doses of angiotensin II was studied in Patient 3. A 20 mm Hg increase in diastolic blood pressure was found when 100 ng/kg/min of angiotensin II was infused. After 30 minutes without infusion, the pressor dose was found to be 192 ng/kg/min. After a second control period of 30 minutes, the pressor dose was 276 ng/kg/min.

The effect of increasing doses of aprotinin on angiotensin II responsiveness was studied in Patients 3 and 4. Figure 3 shows that Patient 3 required progressively less angiotensin II to induce a 20 mm Hg rise in diastolic blood pressure as the dose of aprotinin infused was increased: from a baseline pressor dose of 716
ng/kg/min, increasing doses of aprotinin decreased the angiotensin II pressor doses to 42 ng/kg/min.

In Patient 4 the pressor dose of angiotensin II without aprotinin was 655 ng/kg/min. With aprotinin, 5 KIU/kg/min, the pressor dose decreased to 308 ng/kg/min. With aprotinin, 10 KIU/kg/min, the pressor dose remained at 308 ng/kg/min. With 15 KIU/kg/min it decreased to 214 ng/kg/min of angiotensin II, but with aprotinin, 20 KIU/kg/min, the pressor dose increased to 308 ng/kg/min.

The initial pressor doses of angiotensin II before aprotinin infusion were considerably higher than in prior studies of Patients 3 or 4, possibly due to nausea and vomiting with an increased production of vasodilating prostaglandins since no change in technique occurred.

**Discussion**

Resistance to the pressor effects of angiotensin II in Bartter’s syndrome has been attributed to changes in receptor-hormone interaction as a result of chronically elevated concentrations of angiotensin II and to the vasodilating effect of prostaglandins and other substances. According to the pressor response to angiotensin II in Bartter’s syndrome can be enhanced by procedures whose main effect is to decrease endogenous angiotensin II, such as acute volume expansion, or to potentiate vascular responsiveness, such as by sodium loading, or to decrease the concentration of endogenous vasodilators, such as by the use of indomethacin.

Indomethacin not only inhibits prostaglandin production but also decreases the activity of the renin-angiotensin system, the excretion of urinary kallikrein, and the concentration of plasma bradykinin, effects that are also shared by volume expansion and by sodium loading. In fact, since all these mechanisms may account in part for the normalization of the pressor response to infused angiotensin II in Bartter’s syndrome, the use of either indomethacin, volume expansion, or sodium loading does not permit us to study separately the role of the vasodepressor substances or endogenous angiotensin II in the development of vascular resistance to pressor agents in Bartter’s syndrome.

Aprotinin is a proteolytic enzyme that inhibits the formation of vasodilating kinins by inhibiting plasma and glandular kallikrein. Direct evidence of kallikrein inhibition can only be obtained by measurements of plasma bradykinin. However, these measurements are still unreliable and technically cumbersome. In addition, plasma bradykinin levels may not correlate well with bradykinin generated locally at the blood vessel wall. In humans, aprotinin is a potent inhibitor of tissue kallikrein as well. Our results show that aprotinin can decrease vascular resistance to angiotensin II in Bartter’s syndrome. Additional indirect evidence that this effect may be mediated by kallikrein inhibition is provided by the dose-response relationship observed in Patient 3 (Figure 3). Patient 4, who had the lowest urinary kallikrein level and the lowest glomerular filtration rates, did not show such a clear dose-response relationship.

Aprotinin did not change PRA, blood pressure, or urinary PGE concentration, which confirms previous findings in Bartter’s syndrome. It is not surprising that aprotinin had no effect on the control blood pressure before the angiotensin infusion in the face of an unchanged PRA level because of the neural and humoral mechanisms that compensate for minor changes in blood pressure even if the kallikrein-kinin system is inhibited. It is unlikely that the effect of aprotinin on vascular reactivity could be mediated by prostaglandin inhibition, not only because urinary PGE did not change, but also because aprotinin had an immediate effect on vascular resistance while the effect of indomethacin was delayed, as observed in Patient 4.

That a decrease in vascular resistance is not a result of repeated angiotensin infusions per se is supported by the results obtained in Patient 3. In addition, evidence from the literature suggests that vascular resistance increases with increases in angiotensin II. Other authors have given aprotinin to human subjects in doses manyfold higher than those we used. It is possible that if we had used higher doses of aprotinin a greater degree of vascular reactivity to angiotensin II could have been achieved. That we observed a decrease in vascular resistance with small doses of aprotinin underscores the importance of the kallikrein-kinin system in the pathogenesis of vascular resistance to pressor agents in Bartter’s syndrome and emphasizes the need for further studies on the role of kinins in the pathophysiology of the syndrome.
Thus, it seems reasonable to conclude that in Bartter’s syndrome, the increased activity of the kallikrein-kinin system can contribute to the angiotensin II resistance. In normal human subjects, in whom the activity of the kallikrein-kinin system is much lower than in persons with Bartter’s syndrome, aprotinin also induced an increase in vascular sensitivity to angiotensin II. Therefore, the results of this study also support the concept that the kallikrein-kinin system is a modulator of blood pressure regulation in human beings.

In summary, in four patients with Bartter’s syndrome who had increased urinary kallikrein levels, the administration of aprotinin improved pressor responsiveness to exogenous angiotensin II, possibly as a result of the inhibition of the vasodilator effects of bradykinin on blood vessels. On the other hand, suppression of both the prostaglandin system and the kallikrein-kinin system with indomethacin entirely normalized the vascular response to angiotensin II.

References

Inhibition of the kallikrein-kinin system and vascular reactivity in Bartter's syndrome.
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