Renin Inhibition Improves Pressure Natriuresis in Essential Hypertension

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Abstract. Pressure natriuresis (PN), i.e., a rise in renal sodium excretion in response to a higher BP, is involved in long-term BP regulation. PN is blunted in essential hypertension, but the mechanism is unknown. This study assessed the role of the renin-angiotensin-aldosterone system (RAAS) in PN in eight essential hypertensive men from the individual correlations between spontaneous fluctuations in BP and time corresponding changes in sodium excretion (collected at 2- and 4-h intervals for 48 h), during strict sodium balance, without treatment, and during renin inhibition (remikiren, 600 mg oral compound). Without treatment, daily values for mean arterial pressure were 109.5 ± 1.9 and 107 ± 1.9 mmHg, for urinary sodium excretion were 37.2 ± 2.8 and 42.0 ± 2.8 mmol/24 h, and for plasma renin activity were 2.34 ± 0.48 and 2.23 ± 0.44 nmol/L per h, respectively, for two consecutive days. During remikiren treatment, mean arterial pressure was 101.9 ± 1.7 and 100.8 ± 1.7 mmHg (P < 0.05, versus baseline). Urinary sodium excretion was 39.3 ± 3.7 and 45.2 ± 5.3 mmol/24 h (not significant versus baseline), and plasma renin activity was 0.79 ± 0.11 and 0.82 ± 0.13 nmol/L per h (P < 0.05 versus baseline). During remikiren treatment, BP correlated positively with sodium excretion in all patients but in only three of eight patients without treatment. The slope of the regression equation was steeper during remikiren treatment in seven of eight patients. Thus, the relationship between BP and natriuresis was more readily apparent during RAAS blockade, suggesting that RAAS activity blunts PN in hypertensive patients. Improved PN may contribute to the hypotensive effect of RAAS blockade and to maintenance of sodium balance at a lower BP level without volume expansion.

Pressure natriuresis (PN) is the increase in renal sodium excretion in response to a rise in arterial pressure (1). This mechanism is part of a feedback loop that seems to play an important role in long-term BP control (2). The pressure-induced rise in renal sodium excretion with subsequent reduction in extracellular volume restores BP to its previous level. Animal studies demonstrated that short-term fluctuations in BP elicited corresponding changes in renal sodium excretion thought to constitute the key factor in the PN feedback loop (3).

PN is present in isolated kidneys and thus reflects an intrinsic renal property. However, in vivo, the PN curve is modified by neurohumoral factors such as the renin-angiotensin-aldosterone system (RAAS) (4), the sympathetic nervous system (5), and atrial natriuretic factor (6). In hypertension, the PN relationship is abnormal and shifted to the right (7,8). Several humoral and intrarenal hemodynamic mechanisms have been suggested to contribute to the abnormal PN curve in hypertension (9–11). Whereas PN has been the subject of much investigation, few data on the role of BP in promoting natriuresis are available in human hypertension. Nearly all studies on PN in humans were performed by testing the response of BP to a change in sodium status (12). No data, however, are available on the effect of a change in BP on sodium excretion in essential hypertensive individuals. We therefore investigated whether spontaneous fluctuations in BP are associated with corresponding changes in sodium excretion in patients in balance on a fixed sodium intake. To test the hypothesis that RAAS activity is involved in the impairment of PN in essential hypertension, we also investigated whether blockade of the RAAS by the specific renin-inhibitor remikiren could improve the pressure–natriuresis relationship.

Materials and Methods

Patients and Protocol

Eight essential hypertensive Caucasian men were studied. Their median age was 51 yr (range, 45 to 57 yr), and they weighed 84 kg (range, 74 to 102 kg). All subjects had mild to moderate essential hypertension without clinically relevant end-organ damage. Sitting diastolic BP at study entry was 100 mmHg (range, 95 to 104). Creatinine clearance was 111 ml/min (range, 98 to 125). Excluded were patients with secondary hypertension, patients who weighed more than 120% of their ideal body weight (Metropolitan Life Insurance Table), and patients with a history of alcohol or other drug abuse. All subjects gave their informed consent, and the study was approved by the local ethics committee. All antihypertensive medication had been withdrawn at least 3 wk before entry in the study.

The patients were hospitalized during the study. They adhered to an equicaloric diet containing 50 mmol of sodium, 100 mmol of potassium, 60 g of proteins, and 2500 ml of fluids per day, provided at fixed...
time points. Patients were allowed to walk around but were asked to adhere to comparable schedules of daily activities during all study days. The 24-h urine was collected daily to determine urinary creatinine, sodium, and potassium. BP was measured daily between 11:00 a.m. and 12:00 p.m. while patients were in supine position. After a run-in period of 7 d to ensure stabilization of BP and sodium balance, the patients were studied for a 3-d baseline period without treatment, followed by 7 d of treatment with the renin inhibitor remikiren, 600 mg orally administered at noon. Renal hemodynamics were assessed during baseline, at the first of the three baseline days, and on the fifth day of renin inhibition.

The pressure–natriuresis relationship was studied both during baseline, on the last two pretreatment days, and on the sixth and seventh days of remikiren treatment, after stabilization of urinary sodium excretion (UNaV) and body weight had been warranted. PN was assessed by performing 48-h continuous BP recordings, at 15-min intervals from 8:00 a.m. until 12:00 p.m. and every 30 min from 12:00 p.m. until 8:00 a.m.. Simultaneously, urine was collected every 2 h from 8:00 a.m. until 12:00 p.m. and every 4 h from 12:00 p.m. until 8:00 a.m. to determine the excretion of creatinine, sodium, and potassium in each portion. Blood was drawn to determine plasma renin activity (PRA), immunoreactive renin (irR), and immunoreactive angiotensin II (AngII) at noon when patients had been supine for 1 h.

**Methods**

BP was recorded by an automatic noninvasive device (Dinamap®; Criticon Inc., Tampa, FL). Mean arterial pressure (MAP) was calculated as diastolic BP plus one third of the difference between systolic and diastolic pressure. For each 2-h period, the mean value was calculated.

Urinary electrolytes and creatinine were measured by a standard autoanalyzer technique (SMA-C, Technicon®, Tarrytown, NY). Blood samples for measurement of PRA and irR were collected into Vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant, at room temperature. After immediate separation, plasma was stored at −30°C until analysis. PRA was assessed by the quantitation of generated AngI as measured by RIA (Rianen® Ang I RIA Kit; Cis Bio International, Gif-sur-Yvette, France). The irR was determined using the renin IRMA Pasteur kit, with a coefficient of variation of 15.9% (interassay) and 6.6% (intra-assay), both at 31 pg/ml. Blood samples for the measurement of AngII were collected into prechilled Vacutainer tubes containing an inhibitor cocktail consisting of ethylenediaminetetraacetic acid, the angiotensin-converting enzyme inhibitor cilazaprilat, and the renin-inhibitor Ro 42-5892 to prevent the formation of AngII during further analysis. After immediate separation, plasma was stored at −20°C until analysis. AngII was determined using the Nichols Institute Diagnostics AngII RIA with a coefficient of variation of 5.1% (interassay, mean value 31 pg/ml) and 4% (intra-assay, mean value 42 pg/ml). During analysis, plasma was first separated from plasma proteins by ethanol extraction. The extracted plasma AngII was measured in a sensitive and specific competitive protein-binding RIA with a detection limit of 3.6 pg/ml.

GFR and effective renal plasma flow (ERPF) were measured by constant infusion of $^{125}$I-iothalamate and $^{131}$I-hippuran, respectively. The day-to-day coefficient of variation of this method is 2.2% and 5.0%, respectively (13). GFR and ERPF are corrected for standard body surface area (1.73m$^2$). Two consecutive 2-h clearances were measured. Filtration fraction is calculated as GFR/ERPF and expressed as a percentage. Renal vascular resistance (RVR) is calculated as the ratio of mean arterial BP and ERPF.

**Statistical Analyses**

Results are expressed as mean ± SEM. Ranges are given when appropriate. During the assessment of the pressure–natriuresis relationship, the 15-min BP values were averaged according to corresponding real-time periods of urine collection. The impact of possible collection errors by incomplete bladder emptying was avoided by correcting urinary excretion of sodium or potassium for urinary creatinine excretion for each collection period.

The pressure–natriuresis relationship was assessed by performing a regression analysis between corresponding data on BP and natriuresis for each individual patient separately. Statistical analysis was performed by using a paired, nonparametric ANOVA (Friedman) for repeated measurements followed by Dunn’s correction for multiple comparisons and by using a paired Wilcoxon’s signed rank test. P values of less than 0.05 (two-sided) were considered to indicate statistical significance.

**Results**

All patients completed the protocol. During the run-in period all patients reached steady state with respect to BP, urinary excretion of sodium, potassium and creatinine, hormonal parameters, and body weight. The mean values for these parameters during the two baseline study days and the two remikiren study days are given in Table 1. These data show that dietary compliance was excellent. The presence of steady state during the baseline study days as well as the remikiren study days is demonstrated by the similarity of data on the two consecutive study days in both conditions.

As is apparent from Table 1, remikiren induced a significant fall in BP. Remikiren led to effective 24-h blockade of the renin angiotensin system, with a fall in PRA, a fall in AngII from 17 ± 4 to 8 ± 2 ng/L at trough, and a corresponding feedback increase in irR. A variable natriuresis was observed at onset of remikiren treatment with an overall cumulative sodium loss of 73 ± 30 mmol (not significant). Important is that natriuresis stabilized in all patients before repeating the PN analysis during remikiren treatment, as is apparent from the virtually identical UNaV on the two PN study days. The same applied for kaliuresis, which displayed stable values on the consecutive study days, after a cumulative potassium retention of 42 ± 11 mmol had been observed during the preceding treatment days. Body weight stabilized at 83.1 ± 2.9 kg.

The effect of remikiren on renal hemodynamics was assessed as changes in RVR could play a role in shifts of PN. GFR did not change (90 ± 4 to 88 ± 3 ml/min), whereas ERPF increased from 395 ± 25 to 426 ± 23 ml/min. As a result, filtration fraction fell from 23.1 ± 0.6 to 20.7 ± 0.6% and RVR fell from 24.0 ± 1.7 to 20.3 ± 1.3 mmHg/ml.min$^{-1}$. The group means of BP and UNaV at the different time points during the 48-h assessment of PN are depicted in Figure 1. It shows a normal diurnal rhythm for BP with a clear fall during the night. During remikiren treatment, BP is lower and the diurnal pattern is preserved. UNaV displays the normal diurnal pattern with higher values during the day and lower values during the night. This pattern was unchanged during remikiren treatment.

The relationship between BP at a given period and the corresponding real-time UNaV is depicted for the individual patients in
Figure 2, during baseline and remikiren treatment. The corresponding values of the linear regression parameters are given in Table 2. During the baseline assessment, a significant correlation between UNaV and BP values was observed in only three of eight patients, all three with a relatively low baseline PRA. During remikiren treatment, higher BP values were more closely associated with higher natriuresis values, as is apparent from a highly significant correlation in all studied patients and a steeper slope of the regression line in all patients but one. The slopes of the individual patients during remikiren treatment ranged from 0.63 to 2.4, indicating that a rise in natriuresis ranging from 0.63 to 2.4 umol/min was observed per mmHg rise in BP. No correlation was present between the excretions of creatinine or potassium and BP during either baseline or

Table 1. Mean values for BP, UNaV, hormonal parameters, and body weight during the two consecutive observation days during baseline and during remikiren treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Remikiren</th>
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<tbody>
<tr>
<td></td>
<td>Day 1 (0–24 h)</td>
<td>Day 2 (24–48 h)</td>
</tr>
<tr>
<td>MAP (mmHg)b</td>
<td>109.5 ± 1.9</td>
<td>107 ± 1.9</td>
</tr>
<tr>
<td>UNaV (mmol/24 h)</td>
<td>37.2 ± 2.8</td>
<td>42.0 ± 2.8</td>
</tr>
<tr>
<td>U_kV (mmol/24 h)</td>
<td>89.1 ± 2.8</td>
<td>91.6 ± 2.8</td>
</tr>
<tr>
<td>UCrV (mmol/24 h)</td>
<td>17.2 ± 0.7</td>
<td>17.4 ± 0.9</td>
</tr>
<tr>
<td>UVol (ml/24 h)</td>
<td>1657 ± 83</td>
<td>1780 ± 80</td>
</tr>
<tr>
<td>PRA (nmol/h)c</td>
<td>2.34 ± 0.48</td>
<td>2.23 ± 0.44</td>
</tr>
<tr>
<td>irR (ng/L)c</td>
<td>46.6 ± 8.8</td>
<td>44.8 ± 8.0</td>
</tr>
<tr>
<td>PAC (pmol/L)c</td>
<td>768 ± 98</td>
<td>689 ± 66</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>84.0 ± 3.2</td>
<td>84.0 ± 3.2</td>
</tr>
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</table>

a UNaV, urinary excretion of sodium, potassium, and creatinine; MAP, mean arterial pressure; U_kV, urinary excretion of potassium; UCrV, urinary excretion of creatinine; UVol, diuresis; PRA, plasma renin activity; irR, immunoreactive renin; PAC, plasma aldosterone concentration.

b Mean of all measured values during 24 h.

c Measured at noon.

d \( P < 0.05 \) study day versus baseline (two-way ANOVA).

Figure 1. The graphs in both panels indicate mean arterial pressure (MAP, mmHg, mean ± SEM). The bars in both panels denote urinary sodium excretion (UNaV, umol/min, mean ± SEM). For both parameters, measurements were performed during 2 × 24 h, both during baseline (left) and during remikiren (right) treatment.
remikiren, indicating that the observed change in the relationship between BP and sodium excretion is specific for sodium excretion.

**Discussion**

Blockade of the RAAS by the renin-inhibitor remikiren reinforced the relationship between spontaneous fluctuations in BP and UNaV in ambulant essential hypertensive individuals in balance on a fixed sodium diet.

The relationship between BP and renal sodium excretion has been hypothesized to be crucial to the homeostasis of BP and circulating volume. In humans, this relationship has been studied mainly as the response of BP to changes in sodium status, i.e., sodium sensitivity of BP. Sodium sensitivity of BP was shown to be modified by many factors, such as race (14), renal function impairment (15), and diurnal patterns of posture and activity (16). It is interesting that RAAS blockade increases sodium sensitivity of BP in essential hypertension, providing evidence for involvement of the RAAS in the relationship between BP and sodium status in humans (17). Evidence for a role of the RAAS in the PN curve in secondary forms of hypertension was provided by Kimura et al. (18), who demonstrated normalization of the PN curve after causal treatment of primary hyperaldosteronism (removal of the adenoma) and renovascular hypertension (angioplasty), respectively.

However, the other component of the PN feedback loop, the pressure dependency of sodium excretion in humans, has gained little attention. In isolated kidneys and in whole-animal preparations, the responsiveness of sodium excretion to short-term fluctuations in BP is well established. It was pointed out that the cumulated effects of short-term coupling of sodium excretion to fluctuations in BP would be an essential part of the PN feedback loop (3).

Our study is the first to analyze the pressure dependency of sodium excretion in humans from the spontaneous short-term fluctuations in BP in individual essential hypertensive patients. The value of such an individual assessment has been emphasized, referring to the heterogeneous nature of essential hypertension (4). We opted for the analysis of spontaneous fluctuations for two main reasons. First, we wanted to analyze the role of the PN response in the normal daily maintenance of BP and sodium homeostasis in hypertensive men. Second, this approach circumvents the need for deliberate induction of changes in BP as measures that can reproducibly and safely elicit changes in BP in men, such as infusion of vasopressor or -depressor agents, usually exert direct, pharmacologic effects on sodium excretion as well. For our approach, it is crucial that sodium balance be strictly controlled, to preclude that sodium excretion is confounded by fluctuations in overall sodium intake (19). Our patients, therefore, were hospitalized, and not only overall sodium intake but also the timing of food intake over the 48-h observation period was fixed and similar during the baseline study period and the remikiren study period. Patients were ambulant during those days, with fixed times for activity and rest. Accordingly, the pattern of sodium excretion over the 48-h period can be expected to be affected not only by BP but also by the modifying effects of activity, posture, and food intake. This approach was chosen because it mimics the conditions in normal life as closely as possible.

Under these conditions, a normal diurnal pattern of sodium excretion and BP was observed, consistent with the condition of uncomplicated hypertension without significant renal target organ damage in our patients (16-20). During baseline, no significant relationship was found between sodium excretion and BP in five of eight patients. Thus, under normal conditions, an effect of spontaneous fluctuations in BP on fluctuations in sodium excretion cannot be demonstrated in the majority of the patients. It should be noted that our study did not include normal controls; therefore, it cannot be ascertained from our data whether the absence of such a relationship is specific for the condition of essential hypertension, as such a conclusion would require a comparison with healthy control subjects.

Remarkably, during specific RAAS blockade, the relationship between spontaneous fluctuations in BP and sodium excretion was reinforced, as is apparent from a significant cor-

Figure 2. This graph shows the linear regression analysis between UNaV (Y-axis, umol/min) and MAP (mmHg) in all individual patients, both during baseline (○, dashed line) and during remikiren (●, solid line). From 8:00 a.m. to 12:00 p.m., each data point refers to a 2-h period, whereas from 12:00 p.m. to 8:00 a.m., each data point refers to a 4-h period. The patient numbers correspond with the parameters of regression analysis and plasma renin activity as denoted in Table 2.
relation between sodium excretion and BP in all patients, with a steeper slope of the regression equation in seven of eight patients. These findings strongly suggest that RAAS blockade improves the PN response and support the hypothesis that in hypertension, RAAS activity may play a role in blunting PN. Such a role for the RAAS is further supported by our finding that a correlation between BP and sodium excretion during baseline was more readily apparent in patients with a lower baseline PRA level. This is in line with the assumption by Staessen et al. (16) that the presence of a nocturnal correlation between BP and sodium excretion between different individuals, as opposed to the absence of such a relationship during daytime, is explained by the lower endogenous renin activity during the night.

It should be noted that the relationship between BP and sodium excretion during remikiren treatment was found at an overall BP level that was lower than that during the untreated condition. Thus, during RAAS blockade, the kidney is able to excrete sodium at a lower overall BP level and displays a more clearcut natriuresis in response to short-term elevations in BP.

Several mechanisms could account for the more clear-cut association between natriuresis and BP during renin inhibition. The renal vasodilation observed during RAAS blockade might result in a greater transmission of changes in renal perfusion pressure to the postglomerular renal microcirculation, i.e., peritubular and the medullary circulation, with concomitant transmission of BP changes to renal interstitial pressure. The latter has been shown to play an important role in mediating the tubular responses to elevated renal perfusion pressure (21).

It should be noted that our study did not include a control group with another renal vasodilator or a time control group. Thus, we cannot conclusively dissociate between the possible roles of renal vasodilation and the role of reduced AngII activity as such. Moreover, we cannot exclude that the pharmacologic intervention synchronized BP and the diurnal excretion pattern of sodium by an unidentified mechanism other than enhancement of the BP dependency of natriuresis. However, combining our data with those from the literature suggests that the alterations in the PN curve in our study likely are due to blockade of RAAS activity and consequently reduced AngII effects. The latter is likely to be relevant not only by allowing renal vasodilation but also by allowing a decrease in tubular sodium reabsorption in response to higher renal perfusion pressure (22). Experimental data support a specific effect of the RAAS in the relationship between renal sodium excretion and fluctuations in BP (4). It is interesting and in line with our data that blockade of the AngII (subtype 2) receptor was found to improve the PN response in rats even independent from renal hemodynamics (23). Recent strong supporting data for such a differential mechanism of the AngII blockade-induced shift in PN was obtained in hypertensive double-transgenic rats that contain both the human renin and angiotensinogen genes. In these rats, the ACE inhibitor cilazapril and the AT1 blocker losartan, both in submaximal doses, exerted additive effects on the PN curve. Whereas cilazapril improved sodium and water excretion mainly by increasing renal blood flow and glomerular filtration, losartan decreased tubular sodium reabsorption without marked effects on renal hemodynamics (24). Whether either of these mechanisms predominates during renin inhibition cannot be learned from our data and demands further study.

We conclude that in essential hypertensive patients, the relationship between natriuresis and BP is improved during renin inhibition, supporting a role for the RAAS in the PN mechanism. In addition, our data suggest that resetting of the PN mechanism by remikiren may contribute to its BP-lowering action. The stronger association between BP fluctuations and natriuresis during remikiren might well account for the kidneys’ ability to excrete sodium and maintain sodium balance without expansion of extracellular volume at a lower overall BP level.

Acknowledgments

We acknowledge the support of Prof. P. van Brummelen, Dr. W. Fischli, and G. Verweij (Hoffman-La Roche AG). Remikiren was kindly supplied by Hoffmann-La Roche AG, Basel, Switzerland. Furthermore, we acknowledge A. van Zanten for the skillful assessment of the hormonal parameters.

Table 2. Linear regression parameters (slope of the regression line, regression coefficient r, and P value) for BP and urinary sodium excretion for individual patients during baseline and remikiren, and individual values for PRA (PRA trough values measured at noon are given). Patients are arranged according to PRA during baseline

<table>
<thead>
<tr>
<th>PRA Slope</th>
<th>r</th>
<th>P</th>
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<tr>
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<td>Remikiren</td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
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<td>6</td>
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</tr>
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</tr>
<tr>
<td>8</td>
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<td>0.42</td>
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* PRA, plasma renin activity.
References


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