Abnormal Renal Responses to Calcium Entry Blockade in Normotensive Offspring of Hypertensive Parents

ALBERTO MONTANARI, DANIELE VALLISA, GIORGIO RAGNI, ANGELA GUERRA, ROSSANA COLLA, ALMERICO NOVARINI, AND PAOLO CORUZZI

SUMMARY In nine young normotensive subjects with no family history of hypertension and nine age-matched normotensive subjects with one parent with essential hypertension, effective renal plasma flow (p-aminohippuric acid clearance), glomerular filtration rate (inulin clearance), and excretion of sodium and exogenously administered lithium were measured for 90 minutes before and after administration of a single 20-mg oral dose of the calcium entry blocker nifedipine. Segmental tubular handling of fluid and sodium was estimated using lithium clearance as a marker of proximal tubular reabsorption. Nifedipine did not cause any change in subjects with no family history of hypertension, but in those with one hypertensive parent there was a marked increase in effective renal plasma flow (from 644 ± 39 to 847 ± 42 [SEM] ml/min x 1.73 m²; p < 0.001) and a decrease in filtration fraction (from 17.6 ± 1.0 to 12.6 ± 0.4%; p < 0.001), while the glomerular filtration rate was unchanged, thus suggesting a prevailing efferent vasodilation. Sodium excretion rate (p < 0.02) and fractional sodium excretion (p < 0.025) increased slightly but significantly in subjects with one hypertensive parent, but not in normotensive subjects with no family history of hypertension. Lithium clearance also rose (from 29.0 ± 2.0 to 32.8 ± 1.9 ml/min, p < 0.001), and the derived value of fractional proximal reabsorption diminished (from 75.8 ± 1.0 to 71.3 ± 1.2%, p < 0.001). Estimated distal delivery of sodium and absolute distal sodium reabsorption both increased significantly (p < 0.005), while fractional distal sodium reabsorption was unchanged. Our data show an exaggerated renal vasodilator response to calcium entry blockade in young normotensive subjects with one hypertensive parent that may be related to an abnormality in the efferent arteriolar tone sensitive to calcium entry blockade. The inhibitory effect of nifedipine on tubular reabsorption, conceivably located in the proximal tubule, may be either hemodynamically mediated or an expression of some alteration of tubular reabsorption present in prehypertensive young normotensive subjects. (Hypertension 12: 498-505, 1988)

KEY WORDS • prehypertensive state • nifedipine • renal plasma flow • lithium • sodium • tubular reabsorption

It is well known that there is an important genetic contribution to the development of essential hypertension.1 A number of studies in prehypertensive subjects, such as young normotensive members of hypertensive families, have been conducted to investigate functional disturbances preceding the onset of high blood pressure (BP) that may be involved in the pathophysiology of essential hypertension. Indeed, several abnormalities have been described in normotensive offspring of hypertensive persons, including alterations in membrane cation transport mechanisms2 and exaggerated cardiovascular responses to both physiological sympathetic stimuli3 and infusion of norepinephrine4. At the kidney level, although studies in early hypertension have shown an exaggerated vasoconstrictor response to psychological stimuli,4 elevated baseline values of renal blood flow have been observed in subjects with early hypertension5 and in young prehypertensive subjects.7 In addition, a higher glomerular filtration rate (GFR) and indirect evidence of increased tubular reabsorption of ions and water have been found in a large population of young normotensive subjects with two hypertensive parents.7
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Calcium entry blocking agents are known to produce exaggerated vasodilator and depressor responses in essential hypertension. In addition, diuresis and natriuresis are typically associated with the renal vasodilation induced by these drugs when administered to hypertensive subjects. Furthermore, recent studies have shown that the renal vasculature of normotensive relatives of hypertensive patients is abnormally sensitive to the dilating effect of intravenously infused diltiazem.

Taking these findings into account, we studied the renal responses to an acute oral administration of the calcium entry blocking agent nifedipine in young normotensive subjects with or without one hypertensive parent. The renal function study was extended to include not only the measurement of effective renal plasma flow (ERPF) and GFR but also lithium clearance as a marker of fluid reabsorption in the proximal tubule.

Our data show that marked renal vasodilation and reduction in tubular reabsorption of both lithium and sodium occurred as the result of calcium entry blockade in young normotensive offspring of one hypertensive parent but not in age-matched normotensive subjects who did not have a family history of hypertension.

Subjects and Methods

Eighteen subjects, recruited from among physicians, medical students, and nurses in our institution, participated in the study. All were found to be healthy by clinical and laboratory examination. They did not have hypertension before the study and their BPs were normal on at least three separate measurements at the clinic (Table 1).

To assess the prevalence of hypertension in the families, all first-degree relatives of the subjects (36 parents and 19 sisters and brothers) were clinically examined. Nine subjects, F(+), had one parent who was under long-term treatment with antihypertensive drugs for essential hypertension. The latter diagnosis had been made 3 to 12 years before the study on the basis of the usual clinical and laboratory criteria. The remaining nine subjects, F(-), had no parent or sibling with a personal or family history of hypertension. Their BPs did not exceed 140 mm Hg for systolic and 90 mm Hg for diastolic on at least three separate measurements during clinical examinations. Age distribution was the same in the two groups, but the sex distribution differed (eight out of nine subjects in the F(+) group were male). As a consequence, body weight and body surface area were greater in the F(+) group.

The study protocol was approved by the ethical committee of our institution, and informed consent was obtained from each subject.

Experimental Procedure

The study was performed on an outpatient basis. Each subject was placed on a diet providing 240 mM sodium, 80 mM potassium, and 1800 to 2100 kcal/day for 1 week. A relatively high sodium intake was adopted to rule out the possible confounding effect of sodium restriction on the validity of lithium clearance as a marker of proximal tubular reabsorption. After 1 week of the controlled diet, recumbent plasma renin activity (PRA) and plasma aldosterone were measured, a 24-hour urine collection was made to measure daily sodium excretion (see Table 1), and at 2200 on the day before the study, 24 mM lithium (in the form of a carbonate salt) was given. Then each subject was given 2 L of tap water to drink gradually through the night until 0800 of the following morning, when the experiment was initiated. In female subjects (four in the F(-) group and one in the F(+) group), experiments were performed in the early postmenstrual phase (between the 7th and 11th days) to minimize any confounding effect of both ovulatory and premenstrual periods.

After a plasma sample was drawn as the control for p-aminohippuric acid (PAH) and inulin measurement, a plastic indwelling catheter was placed in a cubital vein and a priming dose of inulin, 3000 mg/1.73 cm² body surface area (10% solution, J. Monico, Venice, Italy), and PAH, 600 mg/1.73 m² body surface area (20% solution, same source), was injected. Then, an infusion of PAH and inulin in saline solution was initiated using a double 50-ml syringe precision pump (Perfusor Secura, Braun, Melsungen, West Germany) at a rate adjusted to obtain plasma levels around 1.5 mg/dl for PAH and 20 mg/dl for inulin. The infusion was continued throughout the study, which was conducted with the subjects in a sitting position.

A second indwelling catheter for control sampling was immediately placed in the contralateral arm and kept patent by constant infusion of 0.9%
saline solution, 10 ml/hr. After a 50-minute equilibration (Time 0) subjects emptied their bladder, and a 90-minute baseline clearance period was initiated. After 90 minutes, subjects voided and then were given 20 mg of nifedipine in the following manner: Two 10-mg capsules of a commercially available product (Adalat, Bayer) were cut with a knife, and their liquid content was carefully placed in a teaspoon and ingested. This technique of administration ensures a very rapid absorption of nifedipine, according to previous pharmacokinetic reports. A second 90-minute clearance period then was initiated, and the experiment was stopped at 180 minutes, when subjects voided again. A 200-ml tap water load was administered hourly throughout the study to ensure appropriate urine flow. BP and heart rate were measured every 5 minutes using an automatic monitoring device (Arteriosonde, Model 1225, Roche, Basel, Switzerland). BP was also measured every 15 minutes with a standard mercury manometer. Sodium, potassium, and lithium were measured in the urine samples obtained during the two clearance periods. Blood for plasma lithium, sodium, and potassium concentrations was drawn at 0, 90, and 180 minutes; samples for plasma PAH and inulin were drawn at 0, 30, 60, 90, 120, 150, and 180 minutes.

A satisfactory steady state plasma concentration of PAH and inulin was obtained with our infusion technique. The variability in plasma PAH and inulin measured throughout infusion was of the same order of magnitude as the coefficient of variability found in duplicate analysis of single plasma samples (2.4% for PAH and 3.6% for inulin). Thus, ERPF and GFR were calculated without measuring PAH and inulin in urine, according to the method of Schnurr et al. To this purpose, PAH and inulin were measured in the infusate and infusion rates were obtained by multiplying their concentrations by the volume of infused solution per minute. By dividing the infusion rate (mg/min) for each measured plasma concentration (mg/ml), we obtained four values in the baseline period and three in the period of nifedipine administration for both ERPF and GFR.

The mean values for these measurements were used in the expression of data. Each value of GFR and ERPF was normalized for a 1.73 m² body surface area. This correction must be taken into account because of the substantial differences in body size between F(+) and F(−) groups, due mainly to the different sex distribution (see Table 1).

**Segmental Analysis of Tubular Fluid and Sodium Handling**

On the basis of the assumptions that lithium is reabsorbed in the proximal tubules in parallel with sodium and water and that lithium is neither reabsorbed nor secreted beyond the proximal tubules, the following calculations were made:

### Analytical Methods

Sodium and potassium were measured by flame photometry, creatinine with a Technicon autoanalyzer (Tarrytown, NY, USA), and lithium by atomic absorption spectrophotometry. Plasma and infusate PAH were determined with the method of Smith et al. and inulin with the anthrone method of Young and Raisz. Plasma aldosterone was measured by radioimmunoassay as previously described.

### Statistical Methods

Data are expressed as the means ± SE. The values obtained in the baseline and nifedipine periods were compared using Student’s t test or paired samples. The t test for unpaired samples was used to compare the baseline values between the two groups when necessary.

### Results

As shown in Table 2, BP and heart rate were not different in the two groups under baseline conditions. BP did not show any change after nifedipine administration, whereas heart rate increased slightly, but significantly. Table 2 shows also that mean plasma lithium and sodium concentrations were essentially the same in the two groups in both the basal and nifedipine periods.

**Table 3** summarizes the renal function data before and after nifedipine administration. Baseline values of urine flow and urinary sodium excretion were very close in the two groups. The measured baseline values of both ERPF (698 ± 48 vs 605 ± 35 ml/min) and GFR (121 ± 8 vs 107 ± 6 ml/min) were higher, although not significantly, in F(+) subjects than in F(−) subjects. However, both GFR and ERPF were identical when normalized for a 1.73 m² body surface area. The measured GFR was used to...
TABLE 1. Renal Function Before and After Acute Administration of 20 mg of Nifedipine in Normotensive Subjects With and Without One Hypertensive Parent

<table>
<thead>
<tr>
<th>Family history</th>
<th>Urine volume (ml/min)</th>
<th>GFR (ml/min x 1.73 m² BSA)</th>
<th>ERPF (ml/min x 1.73 m² BSA)</th>
<th>FF (%)</th>
<th>UnV (μmol/min)</th>
<th>UrV (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>F(−)</td>
<td>4.0±0.6</td>
<td>4.2±0.7</td>
<td>111±7</td>
<td>111±6</td>
<td>629±31</td>
<td>667±28</td>
</tr>
<tr>
<td>F(+)</td>
<td>4.3±0.8</td>
<td>4.8±0.6</td>
<td>111±5</td>
<td>107±6</td>
<td>644±39</td>
<td>847±42*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. GFR = glomerular filtration rate; BSA = body surface area; ERPF = effective renal plasma flow; FF = filtration fraction; UnV and UrV = urinary sodium and potassium excretion, respectively. F(−) and F(+) are defined in Table 1.

*p < 0.001, t p < 0.02, t p < 0.005, compared with respective values before nifedipine administration.

calculate the derived values for sodium and lithium tubular handling. Nifedipine did not elicit any significant change in renal function in F(−) subjects, whereas the F(+) group showed a marked elevation in ERPF. Individual values of ERPF at baseline and after nifedipine administration are shown in Figure 1. Since GFR did not change, the filtration fraction declined substantially. Urinary sodium and potassium excretion were also significantly increased.

Fractional sodium excretion was also slightly but significantly augmented during nifedipine administration in F(−) subjects: The fractional proximal reabsorption, which is derived from the ratio of C_u to GFR (fractional lithium excretion), was the same in the two groups. It was also not different between female (74.6 ± 0.7%; n = 4) and male (75.8 ± 0.7%; n = 5) subjects in the F(−) group. Nifedipine did not induce any change in F(−) subjects: The fractional proximal fluid reabsorption was 74.4 ± 0.7% in women and 75.5 ± 0.8% in men after nifedipine administration. In the F(+) group, a substantial increase in C_u was observed in the two groups. The lack of differences in baseline renal function between F(−) and F(+) subjects. Values for both GFR and ERPF, with indirect evidence of increased renal sodium reabsorption with impaired tubular response to a saline load has also been observed in normotensive offspring of hypertensive persons. Finally, elevated proximal tubular reabsorption has been suggested recently on the basis of a reduced fractional lithium excretion found in normotensive subjects with a family history of hypertension.

In the present study, we were unable to find any difference in baseline renal function between F(−) and F(+) subjects. Values for both GFR and ERPF, when normalized for body surface area were quite similar in the two groups. The lack of differences in female and male subjects in the F(−) group (1.44 ± 0.15 and 1.26 ± 0.21% respectively).

Discussion

The hypothesis that inherited abnormalities in kidney function may participate in the pathogenesis of essential hypertension has attracted interest in recent years. The finding that "hypertension follows the kidney" in studies of kidney transplantation in both human and experimental primary hypertension has given support to this hypothesis. In addition, alterations of renal function preceding the development of high BP have been detected in different strains of genetically hypertensive rats.

Studying young normotensive offspring of hypertensive parents is generally thought to provide an opportunity to detect primary alterations in human hypertension in a relatively pure state before the intervention of other mechanisms secondary to the hypertensive state. Previous investigations have found abnormalities of kidney function in young prehypertensive people, including elevated basal ERPF and GFR, with indirect evidence of enhanced tubular reabsorption. Abnormally increased renal sodium reabsorption with impaired tubular response to a saline load has also been observed in normotensive offspring of hypertensive persons. Finally, elevated proximal tubular reabsorption has been suggested recently on the basis of a reduced fractional lithium excretion found in normotensive subjects with a family history of hypertension.

Table 3 shows segmental analysis of fluid and sodium handling in proximal and distal tubules as estimated by C_u. Under baseline conditions, absolute C_u was slightly higher in the F(+) group. However, fractional proximal reabsorption, which is derived from the ratio of C_u to GFR (fractional lithium excretion), was the same in the two groups. It was also not different between female (74.6 ± 0.7%; n = 4) and male (75.8 ± 0.7%; n = 5) subjects in the F(−) group. Nifedipine did not induce any change in F(−) subjects: The fractional proximal fluid reabsorption was 74.4 ± 0.7% in women and 75.5 ± 0.8% in men after nifedipine administration. In the F(+) group, a substantial increase in C_u was observed in the two groups. The lack of differences in baseline renal function between F(−) and F(+) subjects. Values for both GFR and ERPF, with indirect evidence of increased renal sodium reabsorption with impaired tubular response to a saline load has also been observed in normotensive offspring of hypertensive persons. Finally, elevated proximal tubular reabsorption has been suggested recently on the basis of a reduced fractional lithium excretion found in normotensive subjects with a family history of hypertension.
Acute Administration of 20 mg of Nifedipine

ERPF and GFR may be due to the small number of subjects examined. In fact, elevated ERPF and GFR have been found by Bianchi et al.7 in a large number of young prehypertensive subjects, and Hollienbarg et al.6 were able to observe the same phenomenon in a subgroup of subjects with early hypertension.

Baseline fractional lithium excretion and the derived value of fractional proximal reabsorption did not differ between the two groups, unlike the results reported by Weder.15 However, Weder15 measured lithium excretion in subjects with a mean basal fractional sodium excretion of around 0.6%. Since some lithium reabsorption has been demonstrated to occur beyond the proximal tubule in sodium-restricted animals17-18 and in humans,19 lithium excretion may be of limited importance in estimating proximal tubular reabsorption under such experimental conditions. The high sodium intake used in the present study, which ensured a basal fractional sodium excretion of 1.2%, should prevent this confounding factor in estimating proximal tubular reabsorption.

Both glomerular hemodynamics and tubular reabsorption appeared to be clearly abnormal in F(+) subjects during acute calcium entry blockade with oral nifedipine administration. Nifedipine induced only small, nonsignificant changes in the ERPF of F(-) subjects, whereas the renal vasodilator response was quite exaggerated in the F(+) group, with a mean increase in ERPF of 31%.

An enhanced response of systemic BP to calcium entry blockade is well established in essential hypertensive patients.10 In addition, the regional circulation in different areas, such as limb8 and forearm,9 appears to be more sensitive in hypertensive than in normotensive subjects to the arteriolar dilation induced by verapamil. Since this phenomenon has not been observed with nonspecific vasodilators such as nitroprusside,9 it has been speculated that calcium entry blockade may disclose a primary defect in the calcium-dependent control of vascular smooth muscle.9,34

In the present study we showed that an abnormal sensitivity to calcium channel blockade is also detectable in renal vasculature and, more importantly, in normotensive offspring of hypertensive parents. This finding confirms the recent report of Blackshear et al.12 showing an enhanced renal vascular response to the dilating effect of diltiazem in normotensive subjects with a family history of hypertension. Blacksheer et al.12 have hypothesized that the exaggerated effect of diltiazem may reflect an abnormal contribution of the calcium blockade-sensitive, angiotensin II-mediated component of renal vascular tone.12 The present findings may be in keeping with such a view, since the increase noted in ERPF without changes in GFR is consistent with a prevailing efferent vasodilation resulting from calcium entry blockade. Since angiotensin II exerts its constrictor effect mainly in the efferent arterioles,35,36 one can speculate that calcium entry blockade can disclose an abnormality in angiotensin II-dependent efferent tone. A potentiated renovascular response to the converting enzyme inhibition

Table 4. Lithium Clearance and Derived Segmental Analysis of Tubular Fluid and Sodium Handling in Subjects Before and After Acute Administration of 20 mg of Nifedipine

<table>
<thead>
<tr>
<th>Family history</th>
<th>C_Li (ml/min)</th>
<th>APR (ml/min)</th>
<th>DDrn (μmol/min)</th>
<th>ADRn (μmol/min)</th>
<th>FPR (%)</th>
<th>FDRn (%)</th>
<th>FEN (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
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<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>F(-)</td>
<td>26.4±</td>
<td>26.±</td>
<td>80.±</td>
<td>80.±</td>
<td>3691±</td>
<td>3744±</td>
<td>3499±</td>
</tr>
<tr>
<td>F(+)</td>
<td>29.0±</td>
<td>32.±</td>
<td>91.±</td>
<td>91.±</td>
<td>4069±</td>
<td>4593±</td>
<td>3879±</td>
</tr>
</tbody>
</table>

Values are means ± SEM. C_Li = lithium clearance; APR = absolute proximal reabsorption; DDrn = distal delivery of sodium; ADRn = absolute distal sodium reabsorption; FPR = fractional proximal reabsorption; FDRn = fractional sodium distal reabsorption; FEN = fractional sodium excretion. F(-) and F(+) are defined in Table 1.

*p < 0.005, tp < 0.001, tp < 0.025, compared with respective values before nifedipine administration.
has been observed in normotensive subjects with a family history of hypertension,37 and at least some hypertensive patients show an enhanced sensitivity of ERPF to angiotensin II.38-39

Although in subjects with early hypertension an accentuated renovascular response to physiological sympathetic stimuli has been observed,2 our findings may be less easily explained by an abnormality in adrenergic regulation of the renal vasculature. In fact, noradrenergic stimulation is known to act preferentially in the afferent arterioles,40 and reversal of norepinephrine-induced renal vasoconstriction by calcium antagonist drugs produces an increase in GFR rather than in ERPF.41 Nifedipine was able to impair tubular reabsorption in the F(+) group. The fractional excretion of both lithium and sodium increased significantly and kaliuresis increased as well after nifedipine administration.

The difference in sex distribution between the F(−) group (four women, five men) and the F(+) group (one woman; eight men) may raise the question of whether the lack of increase in lithium and sodium excretion in the F(−) group reflects a salt-retaining condition related to the menstrual cycle. However, the prevalent belief of marked cyclical variations of salt balance in women has been questioned recently.20,42 In addition, our female subjects were studied in the early postmenstrual period, when there is no evidence of sodium retention.20 Finally, neither at baseline nor after calcium blockade were lithium and sodium excretion different in male and female subjects of the F(−) group. Thus, it is very unlikely that the observed abnormalities can be explained on the basis of sex differences between the two groups.

C₄, derived segmental analysis of tubular fluid and cation handling clearly shows that estimated proximal reabsorption was reduced under calcium entry blockade, with an increase in distal sodium handling. This finding suggests that the main site of tubular transport inhibition was the proximal tubule. The increased amount of sodium delivered through the proximal tubule seemed to be matched effectively in the postproximal sites of reabsorption, since the absolute distal sodium reabsorption increased significantly and the distally reabsorbed fraction of delivered sodium did not change. The amount of sodium that escaped from the distal tubule was statistically significant but very small, thus indicating that, even if there were some suppression of sodium reabsorption in postproximal sites as well, this had only a marginal role in the observed changes in tubular reabsorption. The quantitative estimate of segmental tubular reabsorption was based on the notion that lithium is a quantitative marker of proximal tubular reabsorption.13-15 Evidence supporting the belief that lithium is reabsorbed in the proximal tubule pari passu with sodium has been given by micropuncture and clearance studies in rats14,17,18 and clearance studies in humans.13,19,43 However, since some postproximal lithium reabsorption, such as in Henle's loop, cannot be completely excluded,44,45 our quantitative analysis must be interpreted with some caution. Nevertheless, since there is no doubt that the bulk of the filtered lithium is reabsorbed in the proximal tubule,14,17,44,45 the substantial rise in fractional lithium excretion would still indicate a suppression of proximal reabsorption. In addition, a major source of error in the lithium-derived estimate of proximal reabsorption (i.e., sodium restriction)17-19 was ruled out in our experiments.

In conclusion, acute calcium entry blockade with nifedipine produced abnormally accentuated hemodynamic and tubular responses in young normotensive offspring of hypertensive parents. Although any interpretation of these findings remains essentially speculative, our data give further support to the concept that abnormalities in kidney function are detectable in the prehypertensive state and may
contribute to the predisposition for and, perhaps, to the development of essential hypertension.

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