Normalization of Pressure–Natriuresis by Nisoldipine in Spontaneously Hypertensive Rats

Francisco J. Fenoy, Michael L. Kauker, Ivan Milicic, and Richard J. Roman

This study examined whether the calcium antagonist nisoldipine can shift the relations between sodium excretion, papillary blood flow, renal interstitial pressure, and renal perfusion pressure toward lower pressures in spontaneously hypertensive rats. Mean arterial pressure decreased similarly by 9% and 12% in Wistar-Kyoto and spontaneously hypertensive rats after nisoldipine (0.5 µg/kg bolus + 0.017 µg/kg/min). Urine flow and sodium excretion increased by 35% and 24% in Wistar-Kyoto rats after nisoldipine. In contrast, urine flow and sodium excretion rose by 121% and 132% in spontaneously hypertensive rats, and fractional sodium excretion rose from 1.9±0.3 to 4.2±0.4%. Control sodium excretion, papillary blood flow, and renal interstitial pressure were significantly lower in spontaneously hypertensive rats than in Wistar-Kyoto rats when compared at similar renal perfusion pressures. Sodium excretion, papillary blood flow, and renal interstitial pressure all increased in spontaneously hypertensive rats after nisoldipine, whereas it had no effect on papillary blood flow or renal interstitial pressure in Wistar-Kyoto rats. The relations among sodium excretion, papillary blood flow, renal interstitial pressure, and renal perfusion pressure were shifted toward lower pressures in spontaneously hypertensive rats given nisoldipine and became similar to those seen in Wistar-Kyoto rats. These results indicate that nisoldipine normalizes the relations among sodium excretion, renal interstitial pressure, papillary blood flow, and renal perfusion pressure in spontaneously hypertensive rats perhaps by correcting the defect in renal medullary perfusion associated with resetting of pressure natriuresis in this model of hypertension. (Hypertension 1992;19:49–55)

Previous studies have indicated that the pressure–natriuretic response is blunted in spontaneously hypertensive rats (SHR) and that this abnormality is associated with shifts in the relations among papillary blood flow, renal interstitial pressure, and renal perfusion pressure toward higher pressures.1,2 Papillary blood flow and the pressure–natriuretic response are also reduced in very young SHR, supporting the view that changes in renal function may play a role in the development of hypertension in this model.1,2 Recently, we reported that vascular tone is elevated in the preglomerular vasculature of juxtamedullary nephrons in SHR.3 This change appears to be functional rather than structural since it was abolished by removal of calcium from the bath and perfusate.3 We suggested that elevations in preglomerular vascular tone in deep nephrons may contribute to the alterations in renal medullary hemodynamics previously reported in SHR.2

Calcium antagonists are widely used in the treatment of hypertension.4–9 Unlike many vasodilators, they lower arterial pressure without causing sodium retention, indicating that these agents reset the pressure–natriuretic relation.4,7–9 In addition, calcium channel blockers increase sodium excretion when administered acutely to hypertensive patients8,9 or SHR,10–13 but they have little effect in normotensive animals. The mechanism by which calcium entry blockers alter renal function in hypertension remains obscure.4 The purpose of this study was to evaluate whether the natriuretic and diuretic effects of nisoldipine in SHR are associated with changes in papillary blood flow and renal interstitial pressure.

Methods

Experiments were performed on 38 SHR and 32 Wistar-Kyoto (WKY) rats purchased from Harlan...
Protocol 1: Laser-Doppler Blood Flow Measurements

One week before the experiments, the rats were anesthetized with ketamine (100 mg/kg) and acepromazine (2 mg/kg), and the left kidney was exposed through a flank incision. A small amount of renal cortical tissue overlaying the papilla on the dorsal surface of the kidney was surgically removed as previously described. The creation of this papillary window allowed for the later exposure of the renal papilla after removal of the ureter. On the day of the experiment, the animals were reanesthetized with ketamine and Inactin and were surgically prepared as described above. The left kidney was placed dorsal side up in a holder positioned above the abdominal aorta. The papilla was exposed by making a longitudinal incision in the ureter from the tip to the base of the papilla. Papillary red blood cell (RBC) flow was measured using a dual channel, PD6 laser-Doppler flowmeter (Perimed, Stockholm, Sweden) by placing a fiberoptic probe (Pf 316) 1 mm from the tip of the papilla. Cortical blood flow was measured by placing the probe at five random locations on the dorsal surface of the kidney, and the mean flow signal from these areas is reported.

After surgery and a 1-hour equilibration period, the relations between cortical and papillary RBC flow and renal perfusion pressure were determined during a control period. The laser-Doppler flow signals obtained from the renal cortex and the papilla were recorded as renal perfusion pressure was varied from 150 to 80 mm Hg in SHR and from 120 to 70 mm Hg in WKY rats by tightening the clamp on the aorta. Nisoldipine was then administered intravenously (0.5 µg/kg bolus plus 0.017 µg/kg/min sustaining infusion, n = 6 SHR and 6 WKY rats), and mean arterial pressure and papillary RBC flow were continuously recorded for 30 minutes. After this equilibration period, the relations between cortical and papillary RBC flow and renal perfusion pressure were determined.

Protocol 2: Renal Interstitial Pressure Measurements

These rats were prepared as described above. In addition, a capsule was implanted in the renal cortex for measurement of renal interstitial hydrostatic pressure (RIHP), as described previously. After surgery and a 1-hour equilibration period, the relation between RIHP and renal perfusion pressure was determined during a control period. The RIHP was recorded as renal perfusion pressure was varied from 150 to 50 mm Hg in SHR, and from 120 to 40 mm Hg in WKY rats by tightening the aortic clamp. Then, nisoldipine (0.5 µg/kg plus 0.017 µg/kg/min sustaining infusion, six SHR and six WKY rats) was administered intravenously, and mean arterial pressure and RIHP were continuously recorded for 30 minutes. After this period, the relation between RIHP and renal perfusion pressure was determined.

Protocol 3: Clearance Experiments

These rats were surgically prepared as described above, except cannulas were placed in both ureters for collection of urine and a flow probe was placed on the left renal artery for measurement of renal blood flow (RBF) using an electromagnetic flowmeter (model 501, Carolina Medical Instruments, King, N.C.). [3H]Inulin (1 µCi/ml) was included in the infusion solution to allow for measurement of glomerular filtration rate (GFR).

In these experiments, urine flow, sodium excretion, RBF, GFR, arterial pressure, and RIHP were measured during two 30-minute control periods. Then nisoldipine was administered intravenously (0.5 µg/kg plus 0.017 µg/kg/min sustaining infusion, seven SHR and six WKY rats), and after a 30-minute equilibration period, urine and plasma samples were collected again in two 30-minute experimental clearance periods.

Protocol 4: Pressure-Natriuresis Response

These rats were prepared as described in protocol 3. After a 1-hour equilibration period, either vehicle (eight SHR and eight WKY rats) or nisoldipine (0.5 µg/kg plus 0.017 µg/kg/min i.v.; five SHR) was administered. After a 30-minute equilibration period, renal perfusion pressure was lowered to 100 mm Hg in WKY rats and nisoldipine-treated SHR and to 125 mm Hg in untreated SHR by aortic occlusion; 10 minutes later, urine flow, sodium excretion, and GFR were measured during a 30-minute period. Renal perfusion pressure was then elevated by 25 mm Hg by releasing the clamp on the abdominal aorta, and after a 10-minute equilibration period, urine and plasma samples were collected during a 20-minute experimental period. Finally, renal perfusion pressure was increased 25 mm Hg above control by tying off the mesenteric and celiac arteries and the aorta below the kidneys, and urine and plasma samples were again collected during a second 15-minute experimental period.
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1.0

• WKT • SHH

80 80

100 140 180

1.5

1.0

60 80

100 120 140 160

Mean Arterial Pressure (mmHg)

1.0

2.0

3.0

4.0

Papillary Blood Flow (ml/min)

50 75 100 125 150

Control 5 10 15 20 25 30

Time (min)

FIGURE 1.  Plots show comparison of relation between cortical blood flow and renal perfusion pressure in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), before (top panel) and after (bottom panel) the administration of nisoldipine (0.5 μg/kg i.v. bolus plus 0.017 μg/kg/min maintenance infusion).

Analytical Methods

Urine volume was measured gravimetrically and factored by gram kidney weight. [3H]Inulin concentrations in urine and plasma samples were determined with the use of a liquid scintillation spectrophotometer (model 3320, Packard Instruments, Downers Grove, Ill.). GFR was calculated as the urine-to-plasma inulin concentration ratio times urine flow rate. The sodium concentration of urine and plasma samples was determined by flame photometry.

Statistical Methods

Data are presented as mean values±SEM. The significance of differences in the measured values within a group was evaluated using an analysis of variance for repeated measures followed by a Duncan multiple range test. Differences in measured values between groups were analyzed using a two-way analysis of variance followed by a Duncan multiple range test. Linear regression analysis was performed to determine the relations between cortical and papillary RBC flow, RIHP, and renal perfusion pressure. The slopes and y intercepts of these relations were compared using an unpaired t test. A value of \( p < 0.05 \) (two-tailed test) was considered statistically significant.

Results

Protocol 1: Laser-Doppler Blood Flow Measurements

The relation between cortical blood flow and renal perfusion pressure was similar in WKY rats and SHR during the control period (Figure 1, top). Treatment with nisoldipine had no effect on this relation in either group (Figure 1, bottom).

The time course effects of nisoldipine on papillary blood flow and arterial pressure are presented in Figure 2. Nisoldipine lowered mean arterial pressure in WKY rats (from 119±4 to 105±6 mm Hg) and had no effect on papillary blood flow over a 30-minute period. Arterial pressure also fell in SHR given nisoldipine (from 144±4 to 131±6 mm Hg), but papillary blood flow increased from a control value of 2.36±0.1 to a maximum of 3.14±0.2 V 30 minutes after infusion of nisoldipine was initiated.

The effects of nisoldipine on the relation between papillary RBC flow and renal perfusion pressure in WKY rats and SHR is depicted in Figure 3. The relation between papillary RBC flow and renal perfusion pressure was shifted toward higher pressures in SHR during the control period (Figure 3, top), and the slope of the regression line relating papillary RBC flow and renal perfusion pressure was significantly greater in WKY rats than in SHR (2.46±1.93 versus 0.89±0.45 mV/mm Hg). The relation between papillary RBC flow and renal perfusion pressure in SHR was reset toward lower pressures in SHR after
FIGURE 3. Plots show comparison of relation between papillary blood flow and renal perfusion pressure in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), before (top panel) and after (bottom panel) the administration of nisoldipine (0.5 μg/kg i.v. bolus plus 0.017 μg/kg/min maintenance infusion).

nisoldipine so that there was no significant difference in this relation in SHR and WKY rats during the experimental period (Figure 3, bottom).

Protocol 2: Renal Interstitial Pressure Measurements

The time course of the effects of nisoldipine on RIHP and arterial pressure in WKY rats and SHR are presented in Figure 4. Nisoldipine lowered mean arterial pressure (from 113±3 to 93±2 mm Hg 30 minutes after the drug) and had no effect on RIHP in WKY rats. Blood pressure also fell after nisoldipine in SHR (from 161±5 to 137±2 mm Hg). However, RIHP increased in SHR from a control value of 3.5±0.6 to a maximum of 5.3±0.6 mm Hg, 30 minutes after the administration of the nisoldipine.

The effects of nisoldipine on the relation between RIHP and renal perfusion pressure in SHR and WKY rats are presented in Figure 5. During the control period, the relation between RIHP and renal perfusion pressure was shifted toward higher pressures in SHR when compared with that seen in WKY rats (Figure 5, top). The slope of the regression line relating RIHP and renal perfusion pressure was significantly greater in WKY rats than in SHR (0.052±0.006 versus 0.019±0.008 mm Hg/mm Hg). After treatment with nisoldipine the relation between RIHP and renal perfusion pressure was reset toward lower pressures in SHR and was not significantly different from this relation in WKY rats given nisoldipine (Figure 5, bottom).

Protocol 3: Clearance Experiments

The results of these experiments are presented in Table 1. Mean arterial pressure fell by 9% and 12% in WKY rats and SHR, respectively, after nisoldipine. GFR and RBF were not significantly altered in either group. In WKY rats, nisoldipine increased sodium and water excretion by 35% and 24%, respectively. However, fractional excretion of sodium and urinary osmolality were not significantly altered. In SHR, urine flow increased 121%, and absolute and fractional excretion of sodium rose by 132% and 110%, respectively, after nisoldipine. The diuretic and natriuretic response observed in SHR was accompanied by a fall in urine osmolality.

Protocol 4: Pressure–Natriuresis Response

The effects of nisoldipine on the pressure–natriuretic relation in SHR are depicted in Figure 6. Control urine flow and sodium excretion measured at their spontaneous level of arterial pressure were not different in SHR and WKY rats and averaged approximately 25 μl/min/g kidney wt and 3.5 μeq/min/g kidney wt, respectively. The relations between urine flow, sodium excretion, and renal perfusion pressure were shifted toward higher pressures in SHR com-
pared with these relations in WKY rats. At a renal perfusion pressure of 150 mm Hg, urine flow and sodium excretion were significantly greater in WKY rats than in SHR (73.6±18.2 μl/min/g kidney wt and 11.95±3.0 μeq/min/g kidney wt versus 14.9±3.0 μl/min/g kidney wt and 2.6±0.6 μeq/min/g kidney wt, respectively). At a renal perfusion pressure of 150 mm Hg, fractional sodium excretion was also greater in WKY rats than in SHR, averaging 8.29±1.65% and 2.18±0.53% of the filtered load, respectively.

Nisoldipine shifted the relations between urine flow, absolute and fractional excretion of sodium, and renal perfusion pressure toward lower pressures in SHR, so that these relations were similar in SHR given nisoldipine and control WKY rats. At renal perfusion pressures of 150 and 175 mm Hg, fractional sodium excretion averaged 2.18±0.53% and 4.42±1.37% of the filtered load, respectively, in untreated SHR. Nisoldipine increased fractional excretion of sodium to 5.1±1.1 and 7.6±0.9% at renal perfusion pressures of 150 and 175 mm Hg, respectively. GFR was well autoregulated in all groups, and was similar in WKY rats and SHR at all renal perfusion pressures studied. However, GFR was about 30% higher in SHR given nisoldipine than in vehicle-treated rats.

**Discussion**

Calcium entry blockers are effective antihypertensive agents that usually lower arterial pressure in the absence of sodium retention. This observation suggests that this class of compounds in some way alters renal function and resets the pressure–natriuretic relation toward lower pressures in hypertension. In this regard, the natriuretic response to calcium antagonists is greater in SHR versus normotensive rats. Since this model of hypertension is characterized by a blunted pressure–natriuretic response, enhanced renal medullary vascular tone, and a shift in the relation between RBF, RIHP, and renal perfusion pressure, it is tempting to speculate that calcium antagonists may selectively reset the pressure–natriuretic response in SHR by reducing the elevated renal medullary vascular resistance and secondarily alter sodium reabsorption by raising renal interstitial pressure. However, very little is known about the effects of calcium channel blockers on the intrarenal distribution of blood flow especially in hypertensive animals.
In the present study, the relations between sodium excretion, papillary blood flow, RIHP, and renal perfusion pressure were shifted toward higher pressures in SHR, confirming previous reports.1,2,16,18 The administration of nisoldipine raised papillary blood flow and RIHP in SHR, even though renal perfusion pressure fell. Nisoldipine had little effect on papillary blood flow and RIHP in WKY rats, indicating that this compound selectively blocked the enhanced vascular tone that is present in the juxtamedullary nephrons of SHR.22 In addition, nisoldipine shifted the relations between sodium excretion, papillary blood flow, RIHP, and renal perfusion pressure in SHR so that they were not different from those seen in WKY rats. These observations suggest that this compound corrects the defect in vasa recta hemodynamics observed in SHR that we have suggested may be involved in resetting kidney function in this model of genetic hypertension.1,3,16 Overall, our findings are consistent with those of Abe et al.,19 who reported that calcium entry blockers caused a redistribution of RBF from the outer cortex to the juxtamedullary nephrons in anesthetized dogs. They are also consistent with a recent study showing that chronic administration of enalaprilat, an agent that also increases papillary blood flow,20 normalizes the pressure-natriuretic response in SHR.18 Therefore, it is reasonable to propose that changes in renal medullary hemodynamics and resetting of the pressure-natriuretic response may be important in the antihypertensive actions of calcium entry blockers.

Administration of nisoldipine produced only slight changes in sodium and water excretion in WKY rats. However, the calcium antagonist produced a significantly greater response in SHR. This observation is in agreement with previous reports that calcium entry blockers have a greater natriuretic effect in SHR versus normotensive animals.10-12 Tubular reabsorption was reduced by nisoldipine in SHR, as indicated by the large increase in fractional excretion of sodium. It has been reported that calcium antagonists inhibit tubular sodium reabsorption in the loop of Henle and distal tubule,13,21,22 and this effect likely contributes to the resetting of the pressure-natriuretic relation and the natriuretic effect of these agents. The present findings that nisoldipine reduces medullary vascular tone in SHR and increases renal interstitial pressure suggest that this compound may also raise sodium excretion by enhancing the back-leak of sodium in the proximal tubule and/or thin descending limb of Henle of deep nephrons.1,2,14 In this regard, Kauker et al.21 reported that a low dose of nisoldipine markedly reduced fluid reabsorption in the loop of Henle of superficial nephrons. Since the ascending limb of Henle is impermeable to water, these observations are compatible with a reduction in fluid reabsorption in the pars recta of the proximal tubule or thin descending limb of the loop of Henle. This effect was probably obscured in other studies13,22 in which a large drop in arterial pressure associated with the administration of calcium antagonists opposed the changes in medullary hemodynamics and RIHP produced by these agents. The fact that proximal tubular reabsorption is usually unchanged after the administration of calcium channel blockers despite a large fall in arterial pressure indicates that this class of compounds is probably maintaining RIHP and resetting the pressure-natriuretic relation in some way.13,22

### Table 1. Effect of Nisoldipine on Renal Function in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nisoldipine</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Nisoldipine</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>123 ± 2.8</td>
<td>111 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>11.1 ± 0.9</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>GFR (ml/min/g kidney wt)</td>
<td>9.5 ± 2.0</td>
<td>9.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>9.5 ± 2.0</td>
<td>9.5 ± 2.0</td>
</tr>
<tr>
<td>Renal blood flow (ml/min/g kidney wt)</td>
<td>18.3 ± 7.1</td>
<td>24.2 ± 7.8*</td>
</tr>
<tr>
<td></td>
<td>19.3 ± 7.5</td>
<td>22.6 ± 7.1*</td>
</tr>
<tr>
<td>Urine flow (µl/min/g kidney wt)</td>
<td>2.6 ± 1.0</td>
<td>3.7 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td>2.8 ± 1.0</td>
<td>3.6 ± 1.0*</td>
</tr>
<tr>
<td>Sodium excretion (µeq/min/g kidney wt)</td>
<td>1.6 ± 0.5</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>1.7 ± 0.6</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Fractional sodium excretion (%)</td>
<td>1,000 ± 198</td>
<td>868 ± 167</td>
</tr>
<tr>
<td></td>
<td>868 ± 167</td>
<td>887 ± 167</td>
</tr>
<tr>
<td>Urine osmolality (mosm/kg)</td>
<td>833 ± 117</td>
<td>843 ± 117</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; GFR, glomerular filtration rate.

*Significant difference from the control values of the same group.
†Significant difference from the corresponding value in WKY rats.
Previous studies have shown that calcium antagonists can attenuate the autoregulation of RBF and GFR.\(^4,22,24\) Impairment of autoregulation of RBF and GFR would be expected to shift the pressure-natriuretic relation to lower pressures and likely contributes to the natriuretic and diuretic actions of these compounds when used therapeutically.\(^4,23\) In the present study, the low dose of nisoldipine used did not change the autoregulation of GFR. However, GFR was greater in SHR given nisoldipine than in control SHR (Figure 6), and this contributed to the normalization of the pressure-natriuretic relation in SHR after nisoldipine.

In summary, the present study indicates that nisoldipine normalizes the relations between sodium excretion, renal interstitial pressure, papillary blood flow, and renal perfusion pressure in spontaneously hypertensive rats perhaps by correcting the defect in renal medullary perfusion associated with resetting of pressure-natriuresis in this model of hypertension.

**References**


**Key Words**: kidney medulla • kidney concentrating ability • kidney • glomerular function • hemodynamics • flowmeters
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