Effects of a Calcium Entry Blocker on Blood Pressure and Renal Function During Angiotensin-Induced Hypertension

JAN L. HUELSEMANN, R. BERND STERZEL, DOUGLASS E. MCKENZIE, AND CHRISTOPHER S. WILCOX

SUMMARY The effects of the calcium entry blocker nitrendipine on blood pressure (BP) and renal hemodynamics were studied in rats with angiotensin II (ANG II)-induced hypertension. The ANG II was infused subcutaneously by implanted osmotic minipumps for 14 to 16 days. There was a progressive rise in BP in ANG II-infused rats to levels 58 mm Hg above basal by Day 10, whereas control rats with sham pumps remained normotensive. Nitrendipine or vehicle was administered by gavage to groups of control and hypertensive rats for 5 days, and clearance experiments were performed with the rats under anesthesia on the last day. The prolonged infusion of ANG II increased the renal vascular resistance and reduced the glomerular filtration rate and renal Na⁺ excretion. At a dose of 3 mg/100 g body weight, nitrendipine had no consistent effects on BP or renal function of control rats. By contrast, in rats with ANG II-induced hypertension, nitrendipine normalized both the BP and the changes in renal vascular resistance and glomerular filtration rate. Despite the fall in BP, nitrendipine caused a marked diuresis and natriuresis. Moreover, nitrendipine increased Na⁺ excretion of conscious, ANG II-hypertensive rats but not of controls. Thus, nitrendipine appears to be highly effective in reversing ANG II-induced hypertension and Na⁺ retention. These findings also indicate that the hypertension, renal vasoconstriction, and Na⁺ retention accompanying prolonged ANG II infusions may be mediated by calcium-dependent mechanisms. (Hypertension 7: 374-379, 1985)

KEY WORDS • calcium entry blocker • nitrendipine • blood pressure • kidney • natriuresis • angiotensin II

ANGIOTENSIN II (ANG II) has an important homeostatic function in blood pressure (BP) and body fluid regulation and has been implicated in several categories of arterial hypertension. Although short-term infusion of ANG II causes systemic and renal vasoconstriction, prolonged administration of ANG II to human or experimental animals in doses that are initially subpressor leads to enhanced pressor sensitivity and to a progressive increase in BP to hypertensive levels. As systemic and renal vasoconstriction during short-term infusion of ANG II can be diminished by calcium entry blockers, these drugs might be effective in managing renin-dependent hypertension. Moreover, as calcium entry blockers can increase renal Na⁺ excretion, in addition to vasodilation, they might also reverse ANG II-induced salt retention. Indeed, the initial clinical experience with the calcium entry blockers verapamil and nifedipine has indicated that they differ from other vasodilators, such as hydralazine and minoxidil, in reversing hypertension without causing notable retention of NaCl or fluid.

We investigated the effects of a calcium entry blocker on BP, renal hemodynamics, and Na⁺ excretion in a rat model of hypertension caused by prolonged infusion of ANG II. We selected nitrendipine as the calcium entry blocker in our investigation because it is a structural analogue of nifedipine but nitrendipine may be more appropriate for treatment of hypertension because of its prolonged duration of action. There is presently little information about...
the effect of nitrendipine on renal hemodynamics and salt handling in normotensive or hypertensive animal models.

Materials and Methods
Experiments were performed on male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) with initial body weights (BW) of 170 to 220 g. The animals had free access to water and standard rat chow (Purina Laboratory, St. Louis, MO; Na⁺ content, 0.48%, K⁺ content, 1.1%). Two sets of experiments were conducted: prolonged ANG II infusion in conscious rats and short-term renal clearance studies in anesthetized rats.

Prolonged Angiotensin II Infusion
The protocol for the prolonged ANG II infusion experiments is shown in Figure 1A. Rats were anesthetized with ether, and an incision was made in the midline in the lumbar region for insertion of osmotic mini-pumps (Model 2002, ALZA Corporation, Palo Alto, CA; mean filling volume, 0.234 ml). The pumps generally were well tolerated, although a hematoma developed at the site of implantation in some rats and data from these animals were discarded. In pilot studies, we found that the growth of the rats was not impaired by pump implantation. Half the rats (Groups I, n = 10, and III, n = 6) were given empty pumps, while the other half (Groups II, n = 12, and IV, n = 7) were given pumps filled with ANG II (1-L-asparaginyl-5-L-valyl angiotensin octapeptide; Ciba-Geigy Corp., Summit, NJ) in a concentration of 10 μg/μL⁻¹. At the stated pump infusion rate of 10.32 ± 0.72 μL/day, this would deliver 76 ng/min of ANG II for approximately 21 days.

Rats were weighed daily, and systolic blood pressure (SBP) was determined by tail cuff measurements with a Doppler flowmeter (Model 802A, Parks Electronics Laboratory, Beaverton, OR). Rats infused with ANG II became hypertensive, and their SBP increased by more than 15 mm Hg within 3 to 7 days. From Day 11 onward, all rats received peroral gavages twice daily; those of Group III (sham pumps) and IV (ANG II-infused) received nitrendipine (Miles Pharmaceuticals, West Haven, CT) in a total daily dose of 3 mg/100 g BW divided between a morning dose of 1 mg/100 g and an afternoon dose of 2 mg/100 g. Because of its limited water solubility, nitrendipine was dissolved in 1 part of polyethylene glycol (PEG 400, Miles Pharmaceuticals) and 9 parts of saline. Nitrendipine was prepared daily from a stock solution containing 100 mg/ml of nitrendipine in PEG 400. Rats received 1 ml of saline daily with these gavages. Nitrendipine was kept in aluminum foil-covered tubes because of its instability in the light. Rats of Groups I (controls) and II (ANG II-infused) received the same amount of PEG 400 and saline but without nitrendipine. Pilot studies had shown that gavage with PEG 400 alone had no appreciable long-term effects on the rats' weight or BP.

Renal Na⁺ excretion was assessed in five rats of each group for 21 days while they were housed in metabolic study cages (Cage Model 4-640-000, Rack Model 5-000-350, Hazeltone Acme Div. of Hoeltge Inc., Aberdeen, MD). Rats had free access to tap water (Na⁺ content <1 mM) and ground rat chow. The animals were removed from the cages twice daily for measurements of SBP and for gavaging. The daily renal Na⁺ excretion was expressed as a percentage of daily intake of Na⁺, which was derived from the weight of chow consumed.

Renal Clearance Studies
The protocol for the renal clearance studies is shown in Figure 1B. Rats were studied 14 to 17 days after implantation of the pumps. Within 30 to 60 minutes after they had received their morning gavage with nitrendipine or vehicle, the rats were anesthetized with Inactin (BykGulden, Konstanz, West Germany; 0.01 g/100 g BW) and placed on a servocontrolled heating pad (body temperature, 38°C; Watlow, St. Louis, MO). After the rats had been tracheostomized, their right carotid artery, left external jugular vein, and bladder were cannulated. Fluid losses were replaced by 1 ml of 0.154 M NaCl solution, which contained the priming dose of 20 μCi [³H]-methoxyinulin (New England Nuclear, Boston, MA) and 40 μL/100 g BW of 20% p-amino-hippuric acid solution (PAH; Merck, Sharp & Dohme Research Laboratories, West Point, PA). The priming dose was followed by a maintenance infusion of 0.154 M NaCl (1% of BW/hr), containing 13 μCi/hour of [³H]-inulin and 30 μL/100 g BW/hour of 20% PAH solution. After allowing 1 hour for equil-
Hypertension developed within the first week of pump implantation in 19 of 21 rats receiving ANG II; the 2 rats that remained normotensive were excluded from further analysis. Hypertension was established in a mean of 5.47 ± 0.47 days and increased regularly thereafter (Figure 2). On Day 10, the SBP had increased by a mean value of 58 mm Hg above sham-operated control animals.

Administration of nitrendipine to rats receiving ANG II (Group IV) normalized their SBP in all instances. This drop of SBP occurred gradually over 30 to 90 minutes following the gavage with nitrendipine. A comparable reduction of SBP was recorded 90 minutes after nitrendipine gavage in seven awake, ANG II-infused rats (change in SBP measured by tail cuff method, -40.7 ± 7.5 mm Hg) and in five anesthetized rats (change in SBP measured by the carotid artery, -41.4 ± 8.5 mm Hg). After a further 1 to 3 hours, the SBP began to rise, and by 12 to 18 hours it had usually reverted to close to pretreatment values. When SBP was taken in the early morning (i.e., between gavages), the ANG II-infused rats had normalized values after 2 to 3 days of twice-a-day administration of nitrendipine (Figure 2). In contrast to the reproducible fall in BP in ANG II-treated rats, the BP of the controls (Group III) was unchanged after nitrendipine administration or showed only a slight and transient fall. Moreover, the BP of Group III rats was always unchanged compared with predrug values when it was taken before the subsequent gavage. The vehicle for nitrendipine (PEG 400) given alone did not change the SBP of Groups I or II.

The daily renal Na+ excretion, expressed as a percentage of Na+ intake, showed considerable day-to-day variations. During the observation time of 11 days, the mean values for rats with sham pumps (Group I) was 75.8 ± 3.6%, which was not significantly different from those with ANG II pumps (Group II; 77.6 ±
Renal Clearance Studies

As shown in Table 1, in comparison with the control rats of Group I, those receiving ANG II (Group II) had significantly higher values of SBP, MAP, and renal vascular resistance (RVR) but lower values for ERPF, GFR, and renal Na\(^{+}\) excretion (\(U_{Na}V\)). No significant differences were detected in urine flow rate (\(V\)) or fractional Na\(^{+}\) excretion (\(FE_{Na}\)). The decline in GFR in these ANG II-infused animals was proportionately more pronounced than that of ERPF, which resulted in a trend toward a drop in filtration fraction (\(FF\)), although the change did not achieve statistical significance (\(p = 0.059\)).

When given to control rats, nitrendipine had no significant effects on any parameter, except for a slight decline of MAP (from 107.0 ± 2.7 to 98.9 ± 3.8 mm Hg; \(p < 0.05\)). By contrast, nitrendipine given to rats receiving ANG II had dramatic effects: it reversed in full all the functional changes produced by ANG II. For example, there was a return of MAP to the normal range while RVR was actually reduced significantly below (\(p < 0.05\)) that of the control group (I). The GFR and ERPF of these rats were often higher than those of controls (Group I), although the group differences were not statistically significant. There was a two- to threefold increase in \(V\) to values that were significantly higher (\(p < 0.05\)) than those of the control group. The \(U_{Na}V\) of ANG II-treated rats also was markedly increased by nitrendipine administration; \(FE_{Na}\) rose but did not reach statistical significance.

### Table 1. Data from Clearance Experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>(V) ((\mu)L/min)</th>
<th>GFR (ml/min/100 g)</th>
<th>ERPF (ml/min/100 g)</th>
<th>FF</th>
<th>RVR (mm Hg, ml/min/100 g)</th>
<th>(U_{Na}V) ((\mu)mol/min)</th>
<th>(FE_{Na}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I* (n = 10)</td>
<td>116.4</td>
<td>107.1</td>
<td>13.39</td>
<td>1.066</td>
<td>2.542</td>
<td>0.443</td>
<td>25.29</td>
<td>1.124</td>
<td>0.302</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>2.7</td>
<td>1.52</td>
<td>0.045</td>
<td>0.128</td>
<td>0.020</td>
<td>1.22</td>
<td>0.106</td>
<td>0.061</td>
</tr>
<tr>
<td>II† (n = 12)</td>
<td>155.0</td>
<td>133.8</td>
<td>10.34</td>
<td>0.801</td>
<td>2.163</td>
<td>0.395</td>
<td>33.08</td>
<td>0.508</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>3.0</td>
<td>0.77</td>
<td>0.024</td>
<td>0.097</td>
<td>0.019</td>
<td>1.22</td>
<td>0.102</td>
<td>0.041</td>
</tr>
<tr>
<td>III‡ (n = 6)</td>
<td>117.5</td>
<td>98.8</td>
<td>15.21</td>
<td>1.051</td>
<td>2.250</td>
<td>0.483</td>
<td>23.54</td>
<td>1.369</td>
<td>0.457</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>3.8</td>
<td>1.47</td>
<td>0.027</td>
<td>0.089</td>
<td>0.021</td>
<td>1.04</td>
<td>0.308</td>
<td>0.124</td>
</tr>
<tr>
<td>IV§ (n = 7)</td>
<td>112.9</td>
<td>99.5</td>
<td>22.40</td>
<td>1.221</td>
<td>2.564</td>
<td>0.501</td>
<td>20.18</td>
<td>1.482</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>3.1</td>
<td>2.5</td>
<td>2.48</td>
<td>0.064</td>
<td>0.146</td>
<td>0.031</td>
<td>0.88</td>
<td>0.516</td>
<td>0.104</td>
</tr>
</tbody>
</table>

\(p\) Values

- Group I vs II <0.001 <0.001 NS <0.001 <0.05 NS <0.001 <0.05 NS
- Group I vs III NS <0.05 NS NS NS NS NS NS
- Group I vs IV NS NS <0.01 NS NS NS NS NS
- Group II vs IV <0.001 <0.001 <0.001 <0.001 <0.01 <0.001 <0.01 NS

* Sham-operated (control) rats.
† Rats receiving angiotensin II.
‡ Control rats receiving nitrendipine.
§ Rats receiving angiotensin II and nitrendipine.

\(p\) Values refer to results of unpaired \(t\) tests.

Values are means ± SEM.

ERPF = effective renal plasma flow rate; \(FE_{Na}\) = fractional excretion of sodium; \(FF\) = filtration fraction; GFR = glomerular filtration rate; MAP = mean arterial pressure; NS = not significant; RVR = renal vascular resistance; SBP = systolic blood pressure; \(U_{Na}V\) = urinary sodium excretion; \(V\) = urine flow rate.
Discussion

The main findings of this study are that prolonged infusion of ANG II, in a dose that is initially subpressor, led to a progressive rise in BP and RVR with a fall in GFR. Nitrendipine, given orally in a dose that had no consistent effects on BP or renal function of normal rats, reversed the ANG II-induced hypertension in conscious rats and caused a marked natriuresis despite a fall in BP. These effects of nitrendipine were also found in anesthetized rats. In addition, nitrendipine was found to reverse the renal vasoconstriction and fall of GFR observed with long-term infusion of ANG II.

The dose of ANG II infused was initially subpressor. After about 5 days, however, the BP rose progressively to more than 50 mm Hg above preinfusion values. A progressive increase in BP, despite a maintained increase in plasma levels of ANG II, has been shown previously and implies a progressive increase in sensitivity to ANG II that is not found with other pressor substances such as norepinephrine. The kidney may be critical for the development of hypertension in this setting, as the enhanced response to ANG II was accompanied in the dog and in humans by a marked retention of water and Na+ and an increase in extracellular fluid volume despite the increasing arterial pressure.

In the present study, we failed to detect a reduction in Na+ excretion in ANG II-infused, conscious animals (see Figure 3), which confirms previous reports in the rat. However, a subtle but important change in Na+ balance might not have been apparent in these prolonged metabolic studies. Moreover, at the time of the clearance experiment, we found that the $U_{\text{Na}}V$ of the anesthetized, ANG II-infused rats was reduced to approximately half that of the control group, which indicates that they were indeed in a Na+-retaining state. The reduction in $F_{\text{Na}}$ was less impressive, which suggests that the ANG II-induced fall in GFR contributed to the Na+ retention, at least in the anesthetized rats.

The prolonged infusion of ANG II led to parallel declines in GFR and ERPF with no significant change in FF; in fact, the FF tended to decrease. This finding contrasts with previous studies that have shown that the FF usually increases substantially with infusion of ANG II. The failure to rise with the FF in the present study is not clear but might relate to the duration of the ANG II infusion. This interpretation is supported by a comparison of the present findings on the effects of long-term ANG II administration with results obtained in anesthetized rats that received a short-term intravenous infusion of ANG II. Although BP elevation was comparable in both studies, short-term ANG II infusion led to a more pronounced fall of ERPF (to 67% of control values versus 85% found in the present study) and a striking rise of FF (to 151% of control values versus a fall to 89% in this study). As the same experimental preparations were used in both studies, we cannot attribute the present clearance results to the effects of anesthesia or operation. On the other hand, the prolonged infusion of ANG II might activate other, presently undefined neural or humoral mechanisms that also modulate renal function. For example, it has been reported that the plasma and renal levels of 6-ketoprostaglandin $F_1\alpha$, a metabolite of the vasodilator prostacyclin, rise markedly after 12 days of ANG II infusion in the rat. It is possible, yet presently unproved, that vasodilatory prostaglandins may have attenuated ANG II-induced increases in afferent, and especially efferent, arteriolar resistances and thereby countered the rise in FF. In view of the elevation of RVR in rats with long-term ANG II administration, however, other factors must be active in maintaining renal vasoconstriction and in reducing glomerular ultrafiltration.

The contractile action of ANG II at various smooth muscle sites is critically dependent on transmembrane calcium transport. In normal human subjects, nifedipine antagonizes the pressor and aldosterone-releasing actions of infused ANG II. In the kidney, calcium entry blockers have been shown to blunt the vasoconstrictor actions of several agents including ANG II, abolish renal autoregulation, and antagonize the rises in afferent and efferent arteriolar resistances produced by short-term infusion of ANG II. In the present study, although nitrendipine produced no detectable changes in GFR or $U_{\text{Na}}V$ of normal rats, when given at the same dose to ANG II-induced hypertensive rats, it caused a remarkable reversal of the systemic and renal vasoconstriction and led to a marked increase in V and $U_{\text{Na}}V$ with an accompanying increase in $F_{\text{Na}}$. The RVR actually fell below the values seen in control rats.

Previous studies have shown that nifedipine can prevent the development of hypertension in the spontaneously hypertensive rat and in the salt-sensitive Dahl rat. In contrast to the effects of other vasodilators such as minoxidil or hydralazine, the calcium entry blocker did not induce a hyperdynamic circulation nor did it activate the renin-angiotensin system. Moreover, there was a natriuretic action evident as an increase in $U_{\text{Na}}V$ despite a fall in BP. It has been proposed that a reduced volume load is an important mechanism of the long-term antihypertensive action of calcium entry blockers. Our results indicate that nitrendipine has a natriuretic effect on ANG II-induced hypertension in addition to its ability to overcome the pronounced renal vasoconstriction caused by ANG II.

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