Effects of Intrarenal Infusion of Calcium Entry Blockers In Anesthetized Dogs

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SUMMARY Renal function and renin release were studied in anesthetized, uninephrectomized dogs during intrarenal infusions of the calcium influx blockers, verapamil and nifedipine. Verapamil increased renal blood flow by 20% (p < 0.05) but did not alter glomerular filtration rate. Verapamil produced five-to-seven fold increases in urine flow and the rates of excretion of sodium and chloride (p < 0.01). Significant increases in the rates of excretion of potassium, calcium and magnesium were also observed. Despite its striking effects on renal function, verapamil, in nonhypotensive doses, failed to alter renin release. Intrarenal infusion of nifedipine had no consistent effect on renal blood flow or the rate of glomerular filtration but increased urine flow and the rates of excretion of sodium and chloride by more than three fold (p < 0.01). Nonhypotensive doses of nifedipine had no significant effect on renin release. In dogs with a denervated nonfiltering kidney, an intrarenal verapamil or nifedipine infusion did not produce a significant change in renin release. This study demonstrates a striking effect of calcium entry blockers on the reabsorption of sodium, chloride, and water by the renal tubules in intact dogs but renin release did not increase unless hypotension occurred.

(KEY WORDS • verapamil • nifedipine • renin secretion • natriuresis • diuresis • nonfiltering kidney)

ALTHOUGH renin release is controlled by several fairly well-defined mechanisms, the basic cellular processes involved in the secretion of renin from the juxtaglomerular cells are unknown. There have been attempts to relegate an important fundamental role to the calcium ion in the renin secretory process<sup>1,2</sup> and it has been suggested<sup>1</sup> that the calcium ion might be part of a final common pathway for renin release. An inverse relationship for calcium and renin release has been demonstrated by use of calcium free solutions to stimulate renin release in the isolated, perfused rat kidney<sup>3</sup> and by increasing plasma calcium concentration to inhibit renin secretion in the intact dog.<sup>4,5</sup>

Recently, calcium entry blockers such as verapamil and nifedipine have been studied extensively and found to be very useful in the treatment of coronary artery disease. Presumably, verapamil and nifedipine promote vascular smooth muscle relaxation by decreasing transmembrane calcium flux and intracellular calcium concentration. Little is known about their action on the kidney and on renin release. Nifedipine has been reported to increase plasma renin activity (PRA) in patients<sup>6</sup> but it is unclear whether this effect was produced by a direct action on the kidney or was an indirect result of arterial hypotension.

The purpose of the present study was to examine the effects of calcium entry blockers on renin release and renal function. The two structurally dissimilar calcium entry blockers, verapamil and nifedipine, were infused into the renal artery of anesthetized dogs at both nonhypotensive and hypotensive doses. Since these drugs produced striking increments in salt and water excretion, both calcium entry blockers were used in additional series in animals with a nonfiltering kidney in an attempt to eliminate some of the indirect influences of the drugs on renin secretion.

Methods

The experiments were conducted in female mongrel dogs weighing between 18 and 25 kg. The dogs were housed in individual metabolism cages for at least 4 days prior to the acute study and maintained on a prescription diet providing 60 mEq of sodium per day. For the acute study, the dogs were anesthetized with pentobarbital sodium (25 mg/kg) and supplemental
RENAL EFFECTS OF CALCIUM BLOCKERS/Dietz et al.

Kidneys (n = 6)

Doses were given as required. The animals were intubated and Fr. 8 polyethylene catheters were placed in a femoral artery and vein. Following a unilateral nephrectomy, the remaining kidney was isolated via a flank incision. In animals in which the left kidney was used for study, the ovarian vein was ligated. The ureter was catheterized with PE-90 tubing. A flow probe was placed on the renal artery and attached to a square wave electromagnetic flow meter for renal blood flow determinations (Carolina Medical Electronics, Inc., King, North Carolina). Needle tipped catheters were placed in the renal artery (22 gauge) and vein (18 gauge) for infusions and blood sampling. Sodium chloride (0.9%) was infused into the renal artery during the stabilization period and during the control and recovery period at a rate of 0.30 ml/min. Following the completion of the surgical procedures, a priming solution of creatinine (50 mg/kg) was injected intravenously and, subsequently, a constant infusion was given at 18 mg/min into the femoral venous catheter at a rate of 0.50 ml/min. An hour of stabilization was allowed before beginning one of the following series of experiments.

Group 1. Intrarenal Verapamil Infusion (n = 6)

Following two 15-minute control periods, verapamil was infused into the renal artery at doses of 50, 100, and 200 μg/min for two 15-minute periods at each dose. Verapamil was dissolved in 0.9% saline and the solution was delivered at rates of 0.15, 0.30 and 0.60 ml/min, respectively. After terminating the highest dose of the drug, a 1-hour waiting period was allowed to elapse and then two 15-minute recovery periods were obtained.

Group 2. Intrarenal Nifedipine Infusion (n = 7)

The protocol for this series was identical to that for Group 1. The doses of the drug used were 1, 5, and 20 μg/min and the rates of infusion were 0.15, 0.15, and 0.60 ml/min, respectively. Nifedipine was prepared by dissolving 3.5 mg of the drug in 15 ml of polyethylene glycol and 15 ml of ethanol. The final dilution was made with distilled water. Precautions were taken to prevent significant exposure to light.

Group 3. Intrarenal Verapamil in Denervated, Nonfiltering Kidneys (n = 6)

Four days prior to the acute study, the left kidney of the dogs was denervated and made nonfiltering by the method of Blaine et al. In brief, the renal artery was occluded for a period of 2 hours. Following restoration of blood flow, the ureter was ligated and cut and the kidney was denervated by surgical removal of all visible nerves and painting the renal vessels with 5% phenol. In several previous studies, this method of denervation was found to reduce renal norepinephrine content to near zero. This preparation abolishes the influence of the macula densa and renal nerves on renin release while leaving the renal vascular receptor intact. On the day of the acute experiment, the filtering kidney was removed and the animals were prepared for study as described above with the exceptions that the ureter was not cannulated and creatinine was not infused. In addition, a clamp was placed on the aorta just proximal to the origin of the renal arteries for constriction at the end of the experiment. The protocol was similar to that of Group 1, except that verapamil was infused at rates of 25, 50, and 200 μg/min. Following the recovery periods, renal perfusion pressure was reduced by approximately 50 mm Hg by means of the aortic clamp for an additional two 15-minute periods to stimulate renin release. This was done to determine if the renal vascular receptor mechanism for renin release remained functional.

At the end of each experiment, the kidney was examined for the presence or absence of glomerular filtration. The kidney was decapsulated and lissamine green dye was injected into the renal artery. Failure to observe the dye in surface tubules constituted a successful preparation. This was accomplished in all of the animals reported in this series.

Group 4. Intrarenal Nifedipine in Denervated, Nonfiltering Kidneys (n = 6)

The dogs in this group were prepared with a denervated nonfiltering kidney as described for Group 3. The experimental protocol was also similar to that of Group 3, and nifedipine was infused at doses of 1, 5, and 20 μg/min. Examination of the kidneys at the end of each experiment revealed that all were nonfiltering.

In all four groups, blood pressure was monitored throughout each experiment by a Statham pressure transducer connected to the femoral artery catheter. Arterial and renal venous blood samples were obtained during the final 4 minutes of each period and were placed in tubes containing 10% ethylenediaminetetra-acetate for measurement of PRA by the method of Sealey et al. Sodium and potassium were measured by flame photometry and creatinine was measured by standard techniques. Chloride was analyzed with a chloridometer and calcium and magnesium were determined on an atomic absorption-emission spectrophotometer. Hematocrit was determined by the microcapillary tube method. Renin secretion rate was calculated by multiplying the difference in renal venous and arterial PRA by renal plasma flow [renal blood flow × (1-hematocrit)].

All blood samples drawn from arterial and renal venous blood were immediately replaced with an equal volume of whole donor blood. No attempt was made to replace the electrolytes and water lost in urine; however, the two infusions of normal saline replaced approximately one-third of the sodium lost during verapamil infusion and two-thirds of the sodium lost during infusion of nifedipine. At the end of the drug infusion, water balance was negative to the extent of 120 cc with verapamil and only 52 cc with nifedipine.

Values obtained from each animal for each pair of 15-minute periods were averaged and statistical analysis was performed using the chi-square test. A p value of < 0.05 was considered significant.
Results

Group 1. Intrarenal Verapamil Infusion (n = 6)

Intrarenal verapamil infusion at a dose of 50 mg/min produced a significant increase in renal blood flow (fig. 1) from 217 ± 16 to 252 ± 21 ml/min (p < 0.05). Renal blood flow remained significantly elevated with the 100 and 200 μg/min doses of verapamil and decreased to a value not different from the control period following termination of the infusion. Mean arterial pressure (fig. 1) averaged 134 ± 6 mm Hg and was unchanged with the lowest dose of verapamil. Arterial pressure fell significantly by 7 mm Hg with the 100 μg/min dose (p < 0.05) and returned toward the control level during recovery period. Renin secretion rate (fig. 1) averaged 153 ± 47 ng AI/min during the control period and failed to show a significant change during the intrarenal verapamil infusion (p > 0.05). Renal plasma flow increased significantly with the first 2 doses of verapamil (table 1) but was not significantly different from the control period during the highest dose as hematocrit increased significantly from 41% ± 1% to 47% ± 2% (p < 0.05). Creatinine clearance and filtration fraction were not changed significantly by the verapamil infusion. At each of the doses of verapamil, urine flow and the rates of excretion of sodium and chloride (table 1) showed striking increases. Less marked, but significant increases in the rates of excretion of potassium, calcium and magnesium occurred. Plasma sodium and calcium concentrations were unchanged throughout the experiment; plasma potassium concentration showed a small but significant decrease during the highest dose of verapamil and remained depressed during the recovery period (p < 0.05). Heart rate decreased by 26 beats/min during the verapamil infusion (p < 0.05).

Group 2. Intrarenal Nifedipine Infusion (n = 7)

Renal blood flow (fig. 2) failed to show a consistent change with any dose of nifedipine used in the study (p > 0.05). Mean arterial pressure (fig. 2) was unchanged with the lowest dose of nifedipine but decreased by 9 and 21 mm Hg with the 5 and 20 μg/min doses, respectively (p < 0.05). Renin secretion (fig. 2) was not changed significantly with the 1 and 5 μg/min doses of nifedipine but increased to 267 ± 67 ng AI/min (p < 0.05) during the 20 μg/min dose. Renal plasma flow, creatinine clearance, and filtration fraction (table 2) were unchanged throughout the experiment. Hematocrit increased from 41% ± 2% to 50% ± 3% (p < 0.05) during the largest dose of nifedipine. Urine flow and the rates of excretion of sodium and chloride were markedly elevated during all three doses of nifedipine. Less marked but significant increases in the rates of excretion of potassium, calcium and magnesium also were observed (p < 0.05). While the plasma concentrations of sodium and calcium were not significantly changed throughout the study, plasma potassium and magnesium concentrations showed small but significant decrements (p < 0.05). Heart rate was unchanged during the nifedipine infusion.

Figure 1. Effects of an intrarenal verapamil infusion in six anesthetized dogs. MAP = mean arterial pressure; C = control; R = recovery. Values are means ± sem, and each category represents the average of two 15-minute periods.
TABLE 1. Effects of an Intrarenal Verapamil Infusion on Renal Function and Plasma Electrolytes in Seven Anesthetized Dogs

<table>
<thead>
<tr>
<th>Control</th>
<th>Verapamil (μg/min)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF (ml/min)</td>
<td>±9</td>
<td>141*</td>
</tr>
<tr>
<td>Ccr (mM/l)</td>
<td>±2</td>
<td>31.3</td>
</tr>
<tr>
<td>FF (%)</td>
<td>±3</td>
<td>22</td>
</tr>
<tr>
<td>UF (ml/min)</td>
<td>±0.09</td>
<td>2.03*</td>
</tr>
<tr>
<td>UNaV (μM/min)</td>
<td>±5</td>
<td>303*</td>
</tr>
<tr>
<td>UKV (μM/min)</td>
<td>±5</td>
<td>34</td>
</tr>
<tr>
<td>UCaV (μM/min)</td>
<td>±4</td>
<td>2.7*</td>
</tr>
<tr>
<td>PK (mM/l)</td>
<td>±0.6</td>
<td>4.09</td>
</tr>
<tr>
<td>PCa (mM/l)</td>
<td>±0.17</td>
<td>2.18</td>
</tr>
<tr>
<td>PMg (mM/l)</td>
<td>±0.60</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

RPF = renal plasma flow; Ccr = creatinine clearance; FF = filtration fraction; UF = urine flow; U = rate of electrolyte excretion; P = plasma electrolyte concentration.

*p < 0.05 from control.

TABLE 2. Effects of an Intrarenal Nifedipine Infusion on Renal Function and Plasma Electrolytes in Seven Anesthetized Dogs

<table>
<thead>
<tr>
<th>Control</th>
<th>Nifedipine (μg/min)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF (ml/min)</td>
<td>±9</td>
<td>103</td>
</tr>
<tr>
<td>Ccr (mM/l)</td>
<td>±4</td>
<td>35</td>
</tr>
<tr>
<td>FF (%)</td>
<td>±2</td>
<td>0.31</td>
</tr>
<tr>
<td>UF (ml/min)</td>
<td>±0.09</td>
<td>1.16*</td>
</tr>
<tr>
<td>UNaV (μM/min)</td>
<td>±13</td>
<td>45</td>
</tr>
<tr>
<td>UKV (μM/min)</td>
<td>±16</td>
<td>30</td>
</tr>
<tr>
<td>UCaV (μM/min)</td>
<td>±2</td>
<td>0.7</td>
</tr>
<tr>
<td>UMgV (μM/min)</td>
<td>±0.20</td>
<td>2.05*</td>
</tr>
<tr>
<td>PK (mM/l)</td>
<td>±0.13</td>
<td>4.14</td>
</tr>
<tr>
<td>PCa (mM/l)</td>
<td>±0.04</td>
<td>2.24</td>
</tr>
<tr>
<td>PMg (mM/l)</td>
<td>±0.06</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

RPF = renal plasma flow; Ccr = creatinine clearance; FF = filtration fraction; UF = urine flow; U = rate of electrolyte excretion; P = plasma electrolyte concentration.

*p < 0.05 from control.

FIGURE 2. Effects of an intrarenal infusion of nifedipine in seven anesthetized dogs. MAP = mean arterial pressure; C = control; R = recovery. Values are means ± SEM and each category represents the average of two 15-min periods.
Group 3. Intrarenal Verapamil Infusion in Nonfiltering Kidneys (n = 6)

Mean arterial pressure (fig. 3) was unchanged with the 25 μg/min dose of verapamil but fell by 5 mm Hg during the 50 μg/min dose (p < 0.05) and by 16 mm Hg during the 200 μg/min dose (p < 0.05). Mean arterial pressure was not significantly different from the control value during the recovery period. Renal blood flow was unchanged from control during the 25, 50, or 200 μg/min doses of verapamil. Renal blood flow fell to 64 ± 17 ml/min during the recovery period, and this value was significantly lower than the control value (p < 0.05). Renin secretion did not change significantly from control during the verapamil infusion in dogs with nonfiltering kidneys but fell during the recovery period. Following the recovery period aortic clamping resulted in a decrease in renal perfusion pressure (femoral artery pressure) from 134 ± 5 to 78 ± 5 mm Hg (p < 0.05) (fig. 3) and stimulated renin secretion from 68 ± 28 to 161 ± 37 ng/min, an increase of 137% (p < 0.05).

Group 4. Intrarenal Nifedipine Infusion in Nonfiltering Kidneys (n = 6)

Mean arterial pressure did not change significantly with the 1 and 5 μg/min doses of nifedipine (table 3) but decreased significantly during the 20 μg/min infusion (p < 0.05). Renal blood flow and renin secretion (table 3) failed to show a significant change during the nifedipine infusion but the recovery value for renin secretion was lower than the value observed during the 20 μg/min infusion (p < 0.05). Aortic constriction (table 3) resulted in a significant decrease in renal perfusion pressure (p < 0.05) and renal blood flow (p < 0.05) and produced a striking increase in renin secretion compared to the recovery value (p < 0.05).

Discussion

The present study demonstrates that intrarenal infusions of verapamil and nifedipine in nonhypotensive doses into intact dogs produced consistent and striking increases in sodium, chloride, and water excretion, but did not increase renin release. Renal blood flow increased even with the lowest dose of verapamil but was

Table 3. Effects of an Intrarenal Nifedipine Infusion in Anesthetized Dogs with a Single Denervated, Nonfiltering Kidney (n = 7)

<table>
<thead>
<tr>
<th>Nifedipine (μg/min)</th>
<th>Control</th>
<th>1</th>
<th>5</th>
<th>20</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin secretion (ng/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* p < 0.05 from control.
† p < 0.05 from recovery.
unchanged with the highest dose of nifedipine which decreased arterial pressure by 21 mm Hg and increased renin release. Under these circumstances, activation of the renal vascular receptor in the renal afferent arteriole by decreased renal perfusion pressure could account for the increased renin release. With this experimental design, these findings provide information which is relevant to the response in renin release to calcium entry blockers in patients receiving these drugs.

Two factors might explain the failure of verapamil to increase renin release. First, verapamil is an alpha blocking agent which decreases renal arteriolar smooth muscle tone and could decrease the response of the renal vascular receptor. Second, it should be mentioned that the failure of both verapamil and nifedipine in nonhypotensive doses to stimulate renin release might be attributable in part to their natriuretic properties. If calcium entry blockers inhibit sodium reabsorption at a site proximal to the macula densa then an increase in sodium chloride delivery to the macula densa would favor inhibition of renin release. One or the other of these actions could conceivably mask any direct stimulatory effect of verapamil or nifedipine on the juxtaglomerular cells.

To evaluate the influence of increased sodium chloride delivery to the macula densa on renin release, intrarenal infusions of verapamil and nifedipine were given to dogs with a denervated, nonfiltering kidney, a model in which the macula densa is nonfunctional. Both drugs failed to produce a significant change in renin secretion from nonfiltering kidneys even with the highest doses which produced a fall in mean arterial pressure. At the end of the study, renal perfusion pressure was reduced by use of an aortic clamp; this stimulated renin release and demonstrated the ability of the renin secretory mechanism to respond. It appears, therefore, that changes in tubular sodium chloride delivery cannot be the sole explanation for the failure of verapamil and nifedipine to stimulate renin release by a direct action on the juxtaglomerular cells.

Another factor that might contribute to the failure of verapamil to increase renin release is that verapamil has been reported to be effective only when there is augmented calcium influx through activated calcium channels in the cell membrane and the present experiments were done during basal calcium influx. It has been reported previously that verapamil had no effect on basal renin release in the isolated perfused cat kidney or in renal cortical slices but blocked the inhibitory action of high perfusion pressure, high potassium, angiotensin II, and ADH on renin release. However, the other calcium entry blocker studied here, nifedipine, had no significant effect on renin release in nonhypotensive doses and nifedipine has been demonstrated to inhibit basal calcium flux.

In a preliminary report, Roy et al. indicated that an intrarenal verapamil infusion in anesthetized dogs increased renal blood flow, glomerular filtration rate and sodium excretion but decreased renin release. They also reported that in dogs with a single denervated, papavarine treated, nonfiltering kidney verapamil increased renin secretion. No mention was made of the effects on arterial pressure which if reduced might account for the increased renin response in the nonfiltering kidney. In chronic studies in conscious rats on a low sodium diet by Roy, et al., plasma renin activity was not altered by verapamil. Thus, the results of Roy, et al. were quite variable with a decrease, no change and an increase in the renin responses to verapamil.

While renal blood flow increased with verapamil, no change occurred with nifedipine. The drop in arterial pressure with nifedipine probably reflects peripheral arteriolar dilatation which occurred in the absence of a change in renal blood flow. It seems, therefore, that this drop in arterial pressure was accompanied by renal autoregulation while blood flow increased in other arteriolar beds.

One of the most striking effects of verapamil and nifedipine was the increase in sodium, chloride and water excretion. This occurred in the absence of a significant change in creatinine clearance. Okahara et al. reported similar changes in electrolyte excretion during an intrarenal verapamil infusion at a dose of 100 μg/min in anesthetized dogs and also found no change in glomerular filtration rate. It has also been reported that verapamil inhibited the short-circuit current and reduced sodium transport in the toad urinary bladder. Studies with isolated tubules have suggested that sodium and calcium are cotransported in the proximal tubule but it is unclear if co-transport of sodium and calcium occurs in the distal portions of the nephron. Regardless of the specific sites of action on the renal tubules, it seems likely that these calcium entry blockers increased salt and water excretion by decreasing the renal tubular reabsorption of sodium.

In the present study verapamil and nifedipine also increased slightly the rates of excretion of potassium, calcium and magnesium which probably can be attributed to the mild osmotic diuresis produced by the drugs. In addition, in the verapamil series plasma potassium concentration fell slightly and with nifedipine both plasma potassium and magnesium concentrations showed small decreases. The urinary losses of electrolytes and water seem to account, at least in part, for the changes in plasma electrolytes but other factors may be involved.

It is concluded that verapamil and nifedipine given intrarenally in nonhypotensive doses failed to influence renin release. It should be emphasized that these intrarenal doses provided a much higher rate of delivery of these drugs to the kidney than occurs during systemic administration. On the other hand, the doses of both drugs were sufficient to produce marked increases in salt and water excretion. These observations on the renal effects of verapamil and nifedipine in intact dogs strongly suggest that in the doses used in patients there is no affect on renin release unless hypotension occurs.

Acknowledgment

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