EFFECTS OF EDTA AND VERAPAMIL ON RENIN RELEASE IN DOGS

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Abstract—EDTA or verapamil was infused into the renal artery of the anesthetized dog, and the effects on renin release and renal function were examined in an attempt to elucidate the role and action site of calcium ion in the renin secretory system. Both EDTA (25 mg/min) and verapamil (60 μg/min) increased RSR and RBF with a concomitant increase of urine flow, although there was no change in systemic arterial pressure. An infusion of verapamil in combination with ouabain produced a significant increase in RBF which was similar to that seen with verapamil alone. Urine flow and urinary excretion of electrolytes were also increased, but these changes did not differ from those seen with ouabain infusion alone. Ouabain alone did not affect RSR, but its infusion combined with verapamil resulted in a significant increase in RSR. Therefore, the effects of EDTA and verapamil on renin release may reflect the action of both drugs on the vascular component of the juxtaglomerular apparatus. If a change in calcium movement similar to the one in vascular smooth muscle occurs in the juxtaglomerular cells, the altered concentration of calcium may be considered to induce, in part, the stimulation of renin release by EDTA or verapamil.

Renin release from the juxtaglomerular cells is affected by multiple factors, i.e., renal perfusion pressure, renal sympathetic nerves and circulating catecholamines, and the sodium and chloride load to the macula densa in the distal tubules (1, 2). However, the cellular mechanism by which these factors trigger the juxtaglomerular cell to release renin is unknown.

The importance of calcium ion to secretory process in many cells has been stressed in review articles (3, 4). Renin release from the juxtaglomerular cell might be mediated by a calcium-dependent mechanism similar to those in other secretory cells. We have previously reported that an intrarenal arterial infusion of calcium chloride resulted in a transient increase in renin release (5). Haulica et al. (6) also showed that calcium chloride increased renin release from the perfused dog kidney. Conversely, Kotchen et al. (7) and Watkins et al. (8) have demonstrated that intrarenal calcium administration inhibits renin release in the dog with normal or nonfiltering kidneys. In in vitro experiments, Fray (9, 10) and Park and Malvin (11) also reported that low calcium medium stimulates renin release in isolated perfused kidney and in renal cortical slices.

In view of the conflicting information on the role of calcium in the control of renin release, the present in vivo experiments were performed. The experiments were designed to test whether the reduction of free calcium in the perfusate by EDTA or the inhibition of calcium influx by verapamil (12) affected renin release in the anesthetized dog. The modification of actions after the adminis-
tration of ouabain are also discussed.

**Materials and Methods**

Experiments were carried out using adult mongrel dogs weighing 14–16 kg which had been maintained on standard laboratory chow for one week. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and were given additional doses as necessary. Catheters were inserted into the right brachial vein and artery for infusion of saline or drug and for arterial blood sampling. A catheter was also placed in the abdominal aorta via the right femoral artery, and systemic blood pressure was continuously monitored with a pressure transducer. The left kidney was exposed with a retroperitoneal flank incision. The kidney was carefully denervated by dissecting all visible nerve fibers and the tissue connecting the renal hilum cephalic to the renal artery. Renal blood flow (RBF) was measured by an electromagnetic flowmeter (Nihon Kohden MF-25). A 23-gauge needle was introduced into the left renal artery proximal to the flow probe for intrarenal infusion of saline and drug solution at a rate of 0.5 ml/min. For measurement of the glomerular filtration rate (GFR), a priming dose of creatinine (100 mg/kg) was administered into the right brachial vein, followed by a continuous infusion at a rate of 50 mg/kg-hr to maintain a constant blood level of creatinine. A polyethylene catheter was inserted into the left ureter and urine was collected.

After the completion of surgery, the dog was left for 60–90 min to allow for stabilization of systemic blood pressure, RBF and urine flow. Urine was then collected for each of the three consecutive 10-min control clearance periods. At the midpoint of each period, systemic arterial and renal venous blood was collected from the right brachial artery and from the left renal vein via a catheter introduced through the left spermatic or ovarian vein. The blood and urine samples were tested for plasma renin activity (PRA), creatinine and electrolytes. PRA was determined by radioimmunoassay of angiotensin I (13) and expressed as nanogram of angiotensin I per ml-hr. Renin secretion rate (RSR) was calculated as the product of renal plasma flow and the difference between renal venous PRA and arterial PRA and expressed as nanogram of angiotensin I per g of kidney per min.

The experiments were carried out according to the following protocols:

1. **Effect of EDTA**: EDTA was infused into the renal artery of 8 dogs after the third control period at the rate of 25 mg/min for 40 min, and urine was collected for each of the four consecutive 10-min clearance periods. At the midpoint of each period, blood samples were withdrawn for plasma analysis. Following the 40 min infusion of EDTA, saline was infused into the renal artery at the rate of 0.5 ml/min for 60 min to assess the effectiveness of EDTA. Samples were then collected at 20-min intervals.

2. **Effect of verapamil**: After the three 10-min control periods, verapamil was infused into the renal artery of 9 dogs at the rate of 50 µg/min for 40 min. Urine and blood samples were taken in the same way as described for EDTA.

3. **Effects of ouabain infusion alone and of verapamil infusion in combination with ouabain**: Ouabain was infused into the renal artery of 6 dogs at the rate of 1.2 µg/kg-min for 60 min. Blood samples were obtained at 30 and 60 min. Urine samples were also collected during the time between 25 and 30 min as well as between 55 and 60 min after the infusion started. To study the combined effect of ouabain and verapamil, first, ouabain alone was infused into the artery of 8 dogs at the rate of 1.2 µg/kg-min for 30 min, and then verapamil (50 µg/min) plus ouabain were infused for 30 min. Blood
and urine samples were taken in the same way as described for ouabain alone.

The values presented in this paper are mean±S.E. Statistical analysis was performed with the Student’s t-test and paired t-test.

Chemicals: Verapamil was a gift from the Eizai Pharmaceutical Company (Tokyo, Japan).

Results

1. Effects of intrarenal infusion of EDTA on renal function and renin release: Intrarenal arterial infusion of EDTA at a rate of 25 mg/min resulted in an increase in RBF without any significant change in systemic arterial pressure. GFR did not change significantly throughout the experiment. However, urine flow and urinary excretion of sodium, potassium and calcium increased significantly (Table 1. Urinary excretion of potassium was not presented.). The urinary excretion of calcium increased from 0.05±0.01 to 1.42±0.09 µEq/g·min. 20 min after the start of infusion, and the magnitude of the change in calcium excretion was greater than those of sodium and potassium excretion. The above responses reached maximum about 20 min after the start of infusion and were maintained at high levels in the consecutive periods. Plasma concentrations of sodium and potassium were not affected by EDTA infusion, but plasma calcium concentration decreased slightly from 3.79±0.16 in the control period to 3.46±0.15 mEq/L in the fourth experimental period (P<0.05).

Renal venous PRA was significantly increased by EDTA infusion from 4.6±0.8 to 11.2±1.5 ng/ml·hr at 40 min. Arterial PRA was also increased from 4.2±0.7 to 7.2±0.7 ng/ml·hr. Thus, RSR was significantly increased from 1.0±0.3 in the control to 7.2±2.3 and 10.7±3.0 ng/g·min·hr at 30 and 40 min, respectively, after the start of EDTA infusion (Fig. 1). RSR returned to the control value 60 min after the cessation of infusion.

2. Effect of intrarenal infusion of verapamil: The intrarenal arterial infusion of verapamil (50 µg/min) produced an immediate increase in RBF which was maintained throughout the infusion (Table 2). Systemic blood pressure decreased gradually, and its fall became significant at the fourth period of verapamil infusion (P<0.05). Urine flow increased immediately after the start of infusion without any significant change in GFR. Urinary excretion of sodium, potassium and calcium also increased significantly (Table 2. Urinary excretion of potassium was not presented).

Intrarenal infusion of verapamil resulted in a progressive rise in arterial and renal venous PRA. The calculated RSR increased from 1.9±0.4 at control to 17.2±3.1 and 20.2±4.0 ng/g·min·hr at 30 and 40 min, respectively, after the start of infusion (Fig. 1). RSR returned to the control level in the recovery period, but arterial and renal venous PRA were still maintained at the higher levels.
Table 1. Effects of EDTA on renal hemodynamics and plasma renin activity

<table>
<thead>
<tr>
<th></th>
<th>BP (mmHg)</th>
<th>RBF (ml/g-min)</th>
<th>GFR (ml/g-min)</th>
<th>UF (μl/g min)</th>
<th>U NaV (μEq/g·min)</th>
<th>UCaV (μEq/g·min)</th>
<th>PRA A (ng/ml·hr)</th>
<th>PRA V (ng/ml·hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>128±3</td>
<td>3.35±0.25</td>
<td>0.88±0.04</td>
<td>18±3</td>
<td>2.4±0.3</td>
<td>0.05±0.01</td>
<td>4.2±0.7</td>
<td>4.6±0.8</td>
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<tr>
<td>EDTA infusion (25 mg/mg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>126±4</td>
<td>3.89±0.40*</td>
<td>0.67±0.05</td>
<td>46±6*</td>
<td>5.6±1.0*</td>
<td>1.25±0.10*</td>
<td>4.5±0.8</td>
<td>6.0±1.0</td>
</tr>
<tr>
<td>20 min</td>
<td>125±4</td>
<td>4.13±0.42*</td>
<td>0.70±0.60</td>
<td>50±6*</td>
<td>6.1±1.1*</td>
<td>1.42±0.09*</td>
<td>6.3±0.9</td>
<td>9.2±1.4*</td>
</tr>
<tr>
<td>30 min</td>
<td>125±4</td>
<td>4.30±0.41*</td>
<td>0.70±0.05</td>
<td>50±5*</td>
<td>6.4±0.9*</td>
<td>1.40±0.11*</td>
<td>7.0±1.0*</td>
<td>9.8±2.0*</td>
</tr>
<tr>
<td>40 min</td>
<td>124±3</td>
<td>4.22±0.45*</td>
<td>0.71±0.05</td>
<td>47±5*</td>
<td>6.0±1.2*</td>
<td>1.46±0.09*</td>
<td>7.2±0.7*</td>
<td>11.2±1.5*</td>
</tr>
<tr>
<td>Recovery</td>
<td>(60 min)</td>
<td>125±4</td>
<td>3.48±0.39</td>
<td>0.70±0.05</td>
<td>15±3</td>
<td>2.1±0.3</td>
<td>0.17±0.03</td>
<td>3.4±0.5</td>
</tr>
</tbody>
</table>

Each of the values is a mean±S.E. BP=Systemic blood pressure, RBF=Renal blood flow, GFR=Glomerular filtration rate, UF=Urine flow, U NaV and UCaV=Urinary excretion of sodium and calcium, PRA=Plasma renin activity, A=Arterial blood, V=Renal venous blood. *P<0.05 as compared to the control.

Table 2. Effects of verapamil on renal hemodynamics and plasma renin activity

<table>
<thead>
<tr>
<th></th>
<th>BP (mmHg)</th>
<th>RBF (ml/g-min)</th>
<th>GFR (ml/g-min)</th>
<th>UF (μl/g min)</th>
<th>U NaV (μEq/g·min)</th>
<th>UCaV (μEq/g·min)</th>
<th>PRA A (ng/ml·hr)</th>
<th>PRA V (ng/ml·hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>131±4</td>
<td>3.24±0.21</td>
<td>0.63±0.04</td>
<td>12±2</td>
<td>1.9±0.3</td>
<td>0.03±0.01</td>
<td>3.0±0.6</td>
<td>3.5±0.7</td>
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<tr>
<td>Verapamil infusion (50 μg/min)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 min</td>
<td>129±4</td>
<td>4.51±0.42*</td>
<td>0.66±0.05</td>
<td>67±7*</td>
<td>8.4±1.0*</td>
<td>0.21±0.03*</td>
<td>3.8±0.9</td>
<td>5.5±1.1</td>
</tr>
<tr>
<td>20 min</td>
<td>126±4</td>
<td>4.48±0.45*</td>
<td>0.64±0.05</td>
<td>75±7*</td>
<td>9.1±1.1*</td>
<td>0.24±0.03*</td>
<td>5.4±1.1</td>
<td>8.3±1.5*</td>
</tr>
<tr>
<td>30 min</td>
<td>125±3</td>
<td>4.50±0.40*</td>
<td>0.63±0.05</td>
<td>79±8*</td>
<td>10.7±1.2*</td>
<td>0.26±0.03*</td>
<td>10.1±1.2*</td>
<td>14.7±2.0*</td>
</tr>
<tr>
<td>40 min</td>
<td>118±3*</td>
<td>4.40±0.40*</td>
<td>0.64±0.04</td>
<td>86±8*</td>
<td>10.2±1.2*</td>
<td>0.25±0.03*</td>
<td>10.6±1.2*</td>
<td>19.4±3.0*</td>
</tr>
<tr>
<td>Recovery</td>
<td>(60 min)</td>
<td>126±4</td>
<td>3.12±0.32</td>
<td>0.58±0.04</td>
<td>21±3</td>
<td>3.5±0.5</td>
<td>0.08±0.02</td>
<td>7.2±0.9*</td>
</tr>
</tbody>
</table>

Each of the values is a mean±S.E. See legend to Table 1 for abbreviations. *P<0.05 as compared to the control.
3. Effect of intrarenal infusion of ouabain:
The data obtained with the dogs infused with ouabain at the rate of 1.2 \( \mu g/\text{kg-min} \) are shown in Fig. 2. After the infusion of ouabain, the systemic arterial pressure rose significantly by about 10 mmHg, 60 min after the start of infusion, and RBF decreased significantly, showing significant renal vasoconstriction. Urine flow and urinary excretion of sodium increased, despite a marked reduction of GFR.

Arterial and renal venous PRA and RSR slightly decreased, although this was statistically insignificant (Fig. 3). There was no significant difference between RSR at 30 and 60 min after the start of infusion.

4. Effects of verapamil infusion in combination with ouabain: Following the control period, ouabain (1.2 \( \mu g/\text{kg-min} \)) was infused for 30 min, and a verapamil infusion (50 \( \mu g/\text{min} \)) was then superimposed on the ouabain infusion for a further 30 min. The results are shown in Fig. 2. The combined infusion produced a significant increase in RBF, similar to that seen with verapamil alone. Urine flow and urinary excretion of sodium, potassium and calcium were also increased, but these changes did not differ from those seen with ouabain infusion alone (Fig. 2).

Ouabain infusion alone did not affect RSR, but the combined infusion of verapamil resulted in a significant increase in RSR from 0.9±0.5 to 16.7±4.2 ng/g·min (Fig. 3). Thus, there was no significant difference between the magnitudes of renal responses to verapamil with and without ouabain except the urine flow.

Fig. 2. Effects of ouabain alone and of verapamil infusion in combination with ouabain on renal function and renal venous plasma renin activity (PRA). Intrarenal infusion of ouabain at a rate of 1.2 \( \mu g/\text{kg-min} \) resulted in a significant rise in blood pressure and in decreases in RBF, GFR and urine flow. Renal venous PRA decreased slightly. The combined infusion produced significant increase in RBF, urine flow and renal venous PRA. C=Control, O-I=Ouabain (30 min after the start of infusion), O-II=Ouabain (60 min after the start of infusion), O-II+V=Combined infusion of verapamil and ouabain (30 min after the start of combined infusion). *P<0.05 as compared to the control.

Fig. 3. Effects of ouabain infusion alone and of verapamil infusion in combination with ouabain. Intrarenal infusion of ouabain alone (1.2 \( \mu g/\text{kg-min} \)) did not affect RSR, but the infusion in combination with verapamil resulted in a significant increase in RSR. See legend to Figure 2 for abbreviations. *P<0.05 as compared to the control.
Discussion

The present experiments, in which an intrarenal infusion of EDTA or verapamil showed a marked increase in renin release, clearly indicate that calcium plays a role in the control of renin release. The present findings obtained from this in vivo study extend the results of the in vitro experiments that EDTA or low calcium in the perfusate stimulated renin release in the isolated and perfused rat kidney (9, 14).

Although the direction of calcium movement across the cell membrane during the infusion of EDTA or verapamil is completely opposite to that during the infusion of calcium chloride, the present results are consistent with those of Kotchen et al. (7) and Watkins et al. (8) who reported that an intrarenal infusion of calcium chloride decreased renin release. These experimental data indicated that renin release was inversely related to the perfusate calcium concentration. Although the intracellular concentration of calcium could not be measured in the present study, it may be reduced during EDTA or verapamil infusion for the following reason: Since the change in vascular tone is probably associated with that in the concentration of free calcium within the smooth muscle cells (15), the vasodilation observed during the infusions may result from the reduction of free calcium concentration in plasma by EDTA and from the inhibition of calcium flux to the smooth muscle cells by verapamil. If the changes in calcium movement similar to those in smooth muscle cells occur in the juxtaglomerular cells, it is likely that the reduction of calcium in the cytoplasm of the juxtaglomerular cell triggers the release of renin. However, the mechanisms by which hemodynamic and chemical signals are translated into a reduction of calcium are unknown.

Both EDTA and verapamil affected several renal parameters that are related to the renin secretory mechanism, i.e., the renal vascular receptor, the macula densa and the adrenergic nervous system. In the present experiments, EDTA and verapamil did not affect the renal perfusion pressure until the third experimental period, although renin release was by then seen to be on the increase. We have previously reported that a small change in renal perfusion pressure above 100 mmHg and the renal vasodilation induced by acetylcholine did not affect renin release (16). Therefore, it seems unlikely that the fall in renal perfusion pressure and the renal vasodilation induced by EDTA and verapamil stimulate renin release. Both EDTA and verapamil infusions resulted in the increase in urine flow and urinary excretion of sodium. The increased sodium load to the macula densa may be responsible for the increase in renin release since Thurau and associates (17) have presented evidence that increased sodium concentration at the macula densa increases renin release. However, a number of papers indicate an inverse relationship between sodium load to the macula densa and renin release (18). In the present experiment, verapamil in combination with ouabain infusion caused a significant increase in renin release without any additional changes in sodium excretion. Watkins et al. (8) reported that an intrarenal infusion of calcium chloride inhibited renin release in dogs with a nonfiltering kidney. Thus, these results may suggest that the natriuresis during infusions of both drugs is not responsible for the increased renin release. Another possibility which must be considered is that EDTA and verapamil increased renin release via the adrenergic nervous system. However, it seems unlikely that the adrenergic nervous system is involved since hypocalcemia could be expected to inhibit rather than stimulate adrenal medullary secretion (19). Furthermore, Peart et al. (14) reported that pro-
pranolol and phenoxybenzamine had no effect on the increase in renin release produced by EDTA in an in vitro experiment. Therefore, the effects of EDTA and verapamil on renin release may reflect the action of both drugs on the vascular component of the juxtaglomerular apparatus, most likely on the juxtaglomerular cells. If the change in calcium movement similar to the one in vascular smooth muscle occurs in the juxtaglomerular cells which contain myofilaments (20, 21), the altered concentration of calcium in the juxtaglomerular cells may be considered to induce, in part, the stimulation of renin release by EDTA or verapamil.

References