Chronic Hypotensive Effects of Verapamil in Angiotensin Hypertension Are Steroid Independent

Thomas E. Lohmeier, Jean-Pierre Montani, Manis J. Smith Jr., and Eric Lane Rushing

This study was designed to examine the mechanisms that contribute to the chronic hypotensive effects of verapamil during angiotensin II–induced hypertension. Hypertension was induced in five dogs by continuous intravenous infusion of angiotensin II (5 ng/kg/min) for 17 days. On the sixth day of angiotensin II infusion when daily sodium balance was achieved, mean arterial pressure (control, 92±4 mm Hg), plasma aldosterone concentration (control, 5.2±0.9 ng/dl), and renal resistance (control, 0.28±0.01 mm Hg/ml/min) were increased 37±8 mm Hg, 13.6±5.0 ng/dl, and 0.20±0.05 mm Hg/ml/min, respectively. At this time there were no significant changes in glomerular filtration rate, effective renal plasma flow, net sodium and water balance, or extracellular fluid volume. Subsequently, when verapamil was infused (at 2 μg/kg/min) simultaneously with angiotensin II (days 7–13), there was a net loss of 55 ±10 meq sodium, a 7.0±0.7% fall in extracellular fluid volume, and approximately a 70% reduction in the chronic effects of angiotensin II on mean arterial pressure and renal resistance; in contrast, verapamil failed to attenuate the long-term aldosterone response to angiotensin II. Further, although glomerular filtration rate and effective renal plasma flow tended to increase during verapamil administration, there were no consistent chronic long-term changes in these renal indexes. In comparison with these responses in hypertensive dogs, when verapamil was infused for 7 days before the induction of angiotensin II hypertension, there were no significant changes in any measurements except mean arterial pressure, which fell 11±1 mm Hg. Thus, these data fail to support the hypothesis that the chronic stimulatory actions of angiotensin II on aldosterone secretion are dependent on a sustained increase in transmembranal calcium influx. Moreover, these data indicate that the pronounced long-term hypotensive effects of verapamil in angiotensin II hypertension are due to impairment of the direct renal actions of angiotensin II rather than the indirect sodium-retaining effects that are mediated via aldosterone secretion. (Hypertension 1989;13:273-282)

In vitro studies have established firmly the primary role of cytosolic calcium in mediating vascular smooth muscle contraction and hormonal secretion. One of the important events that mediates increases in intracellular calcium concentration is activation of membrane-associated calcium channels, which increases the rate of calcium entry into the cytosol. The recent development of calcium channel blockers, which interact with calcium channels and inhibit calcium entry into cells, has made it possible to assess the importance of transmembranal calcium influx to vascular tone and to hormonal secretion in vivo.

Numerous acute studies now have shown that calcium channel blockers attenuate the vasoconstrictor and steroidogenic effects of angiotensin II (Ang II). In addition, calcium channel blockers greatly decrease the renal vasoconstrictor effects of Ang II and promote natriuresis. Thus, in light of these acute renal actions of calcium channel blockers and in view of the pivotal role of the kidneys in long-term blood pressure control, it perhaps is not surprising that calcium antagonists have proven to have considerable efficacy in the treatment of hypertension. However, in spite of a large number of acute studies, there is relatively
little information about the chronic effects of calcium channel blockers on the long-term effects of Ang II on blood pressure, renal function, and aldosterone secretion.

Therefore, because calcium channel blockers have been shown to act acutely as antagonists to the actions of Ang II and because of the paramount importance of the renin-angiotensin system in affecting sustained changes in renal excretory capability, the primary objective of this study was to evaluate the chronic effects of verapamil on mean arterial pressure (MAP), renal function, aldosterone secretion, and body fluid balance in dogs made hypertensive by long-term infusion of Ang II. An important feature of this study was that plasma levels of Ang II were fixed at an elevated level by infusion, and, therefore, responses to verapamil infusion in hypertensive dogs could not be influenced by compensatory changes in renin secretion. Also, to circumvent the complicating effects of anesthesia on renal function, the dogs were studied in the conscious state. Finally, to achieve comparative data in the normotensive state, the dogs were studied before as well as after the induction of Ang II–induced hypertension.

Materials and Methods

Six male dogs weighing 24.8±3.1 kg were used in this study. The dogs were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and chronic indwelling Tygon catheters (0.05 in. i.d., 0.06 in. o.d., Norton Plastics, Akron, Ohio) were placed in the femoral arteries and veins. The tips of the femoral arterial catheters were advanced into the aorta distal to the origin of the renal arteries, and the ends of the femoral vein catheters were positioned in the lower inferior vena cava. A silastic elbow prevented kinking of the catheters in the femoral area. The catheters were tunneled subcutaneously and exteriorized at the intrascapular region of the back. Patency of vascular catheters was maintained by flushing with sterile isotonic saline every 2–3 days and by filling the catheters with heparin (1,000 units/ml). The catheters were protected within a canvas jacket.

Two weeks after surgery, the dogs were placed in metabolic pens and fitted with an aluminum and canvas backpack housing a Statham arterial blood pressure transducer (model P23 ID, Statham Laboratories, Inc., Hato Rey, Puerto Rico) at heart level. Restraining lines from the sides of the pen were connected to the four corners of the backpack; this permitted the dogs to move freely in the cage but not to turn completely around. The electrical connections to the arterial blood pressure transducer and an intravenous infusion line were brought to the top of the cage through a flexible tube attached to the top of the backpack. Continuous intravenous infusions were made through one of the femoral vein catheters by means of a Sage tubing pump (model 375A, Sage Instruments, Cambridge, Massachusetts). MAP was recorded continuously from the femoral arterial catheter on a Grass polygraph (model 7D, Grass Instrument Company, Quincy, Massachusetts) and simultaneously on a PDP 11/70 Digital Equipment Corporation computer (Maynard, Massachusetts) through an analog-to-digital converter. The analog signal from the Grass polygraph was sampled every 60 seconds and digitized to provide 60 sample points per hour. Daily values presented for MAP were calculated from the 960 data points generated during the 16-hour period extending from 4 PM to 8 AM.

At least 1 week before collection of the control data and throughout the experimental period, the dogs were given free access to water and maintained on a fixed daily diet of two 15.5 oz cans of H/D prescription diet (Hills Pet Products, Inc., Topeka, Kansas) supplemented with 5 ml vitamin syrup (V.A.L. Syrup, Fort Dodge Laboratories, Fort Dodge, Iowa). Two cans of H/D provide about 5 meq sodium and 45–50 meq potassium. Isotonic saline was infused at a rate of 400 ml/day so that total sodium intake was approximately 67 meq/day. When appropriate, [Asp¹,Val⁵]angiotensin II (Ciba Pharmaceutical Company, Summit, New Jersey) and verapamil hydrochloride (Calan, Searle Pharmaceuticals, Inc., Chicago, Illinois) were added to the intravenous saline infusion. A Millipore filter (Cathivex Millipore, Bedford, Massachusetts) was connected in series with the infusion line to prevent passage of bacteria and other contaminants. To ensure the dogs were afebrile, body temperature was measured daily, and ampicillin (Princen, E.R. Squibb and Sons, Princeton, New Jersey) and a trimethoprim-sulfamethoxazole combination (Bactrim, Roche Laboratories, Nutley, New Jersey) were given prophylactically.

At noon each day, just before feeding, samples were taken from the 24-hour urine collections for determination of the daily excretion rates of sodium, potassium, and urine volume. In addition, water intake was measured at this time. Daily water balance was calculated from the difference between water intake (water drunk + saline infused) and urinary volume excretion. The water content of the feces or the canned food (approximately 300 ml/can) was not included in this calculation; however, food intake, and therefore dietary intake, of water was constant throughout the experiment.

Before the control period, the dogs were trained to lie quietly while blood samples were drawn, and measurements of heart rate and renal function were performed. In all experiments, collection of arterial blood samples and measurements of heart rate and renal function were begun at approximately 8 AM each day, about 20 hours after feeding.

Experimental Protocol

After 7–10 days of saline infusion, three of the dogs were infused intravenously with Ang II at 5 ng/kg/min for a total of 17 days; additionally, on
days 7–13, verapamil was infused simultaneously with the Ang II at 2 μg/kg/min. After the 17 days of Ang II infusion, a 9-day recovery period was observed. Subsequently, two of the dogs (the third dog was not used because catheter problems developed) were infused with verapamil alone for an additional 7 days, and this was followed by another 9-day recovery period. In two additional dogs, the order of the experiments was reversed; that is, verapamil was infused first while the dogs were normotensive and then after hypertension was induced by Ang II. A sixth dog was infused only while normotensive. Thus, four of the six dogs were infused with verapamil both before and after the induction of Ang II hypertension. The other two dogs were infused with verapamil only while either normotensive or hypertensive.

In all dogs, 5-ml arterial blood samples were taken intermittently for determination of hematocrit, plasma renin activity (PRA), and the plasma concentrations of aldosterone, cortisol, sodium, potassium, and protein. Additionally, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined during the control period, on the sixth day of Ang II infusion (before verapamil infusion), on days 7, 9, and 13 of Ang II (days 1, 3, and 7 of verapamil infusion), on day 17 of Ang II (4 days after verapamil infusion), and on the last day of the recovery period. Renal function in normotensive dogs was determined during the control and recovery periods, and on days 1, 3, and 7 of verapamil infusion.

## Analytical Methods

PRA was measured by radioimmunoassay according to the method of Haber et al., and PRA is expressed as nanograms of angiotensin I (Ang I) generated per milliliter of plasma per hour incubation (ng Ang I/ml/hr). Commercially available radioimmunoassay kits were used to measure plasma aldosterone concentration (Diagnostic Products, Los Angeles, California) and plasma cortisol concentration (Diagnostic Products). Plasma and urine concentrations of sodium and potassium were determined by flame photometry (IL 343, Instrumentation Laboratories, Lexington, Massachusetts), plasma protein concentration by refractometry (American Optical, Buffalo, New York), and hematocrit by a micromethod (Autocrit II, Clay Adams, Franklin, New Jersey). GFR and ERPF were determined from clearances of [125I]iothalamate (Glofil, Iso-Tex Diagnostics, Inc., Friendswood, Texas) and [131I]iodohippurate (Hippuran, Mallinckrodt Nuclear, St. Louis, Missouri), respectively. Renal blood flow was calculated from the ERPF and hematocrit, and renal resistance from the quotient of MAP and renal blood flow. The volume of sodium iothalamate distribution, an index of extracellular fluid volume, was estimated by using the technique of Sapirstein et al.,

## Statistics

All values presented are mean±SEM. Comparisons were made by one-way analysis of variance for repeated measurements followed by a paired t test using the Bonferroni adjustment for making multiple comparisons. Statistical significance was considered to be p<0.05.

## Results

### Effects of Chronic Angiotensin II Infusion in Normotensive Dogs

Figure 1 illustrates the effects of chronic Ang II infusion on MAP, urinary water and electrolyte excretion, and water balance. MAP increased from a control level of 92±4 to 123±3 mm Hg after 3 days and stabilized near that level during chronic Ang II infusion. After 6 days of Ang II infusion, MAP was 129±7 mm Hg or 37 mm Hg above control levels. There were no significant changes in heart rate (control, 55±2 beats/min) during chronic Ang II infusion. Urinary sodium excretion decreased from a control value of 45±1 to 11±1 meq/day on day 2 of Ang II infusion, but by day 2 it returned to control levels and remained near control levels for the next 4 days of infusion, with no significant accumulation of sodium for the 6-day period. Water balance paralleled changes in urinary sodium excretion. On day 1 of Ang II infusion there was net retention of approximately 450 ml of water.
TABLE 1. Effects of Chronic Verapamil Infusion on Renal Hemodynamics in Dogs With Angiotensin II Hypertension

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min)</th>
<th>ERPF (ml/min)</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63±3</td>
<td>176±7</td>
<td>0.36±0.01</td>
</tr>
<tr>
<td>Ang II (day 6)</td>
<td>59±5</td>
<td>148±15</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>Ang II+verapamil</td>
<td>62±5</td>
<td>166±20</td>
<td>0.38±0.02</td>
</tr>
<tr>
<td>Ang II+verapamil</td>
<td>62±4</td>
<td>164±15*</td>
<td>0.38±0.02</td>
</tr>
<tr>
<td>Ang II+verapamil</td>
<td>61±4</td>
<td>157±15</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>Ang II recovery</td>
<td>55±3</td>
<td>144±14</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>Recovery</td>
<td>59±3</td>
<td>168±11</td>
<td>0.36±0.02</td>
</tr>
</tbody>
</table>

* p<0.05, verapamil versus Ang II (day 6).

Values are mean±SEM. GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; Ang II, angiotensin II.

Effects of Chronic Verapamil Infusion in Dogs With Angiotensin II Hypertension

As shown in Figure 1, chronic verapamil infusion greatly attenuated the hypertensive effects of Ang II. During the first 24 hours of verapamil infusion, MAP decreased dramatically from 129±7 to 115±5 mm Hg; subsequently, MAP gradually fell even further to 104±5 mm Hg over the next 6 days of verapamil infusion. Thus, by day 7 of verapamil infusion there was a 25 mm Hg decrease in MAP or a 68% reduction in the severity of the hypertension. In contrast, renal resistance increased markedly from a control value of 0.28±0.7 to 1.15±0.5 mm Hg/ml/min by day 6 of Ang II infusion, these changes were not statistically significant. In contrast, renal resistance increased markedly from a control value of 0.28±0.01 to 0.48±0.06 mm Hg/ml/min by day 6 of Ang II infusion.

The natriuretic and hypotensive effects of verapamil in dogs with Ang II hypertension occurred without associated changes in plasma aldosterone.
concentration (Figure 2). In addition, not only did verapamil fail to attenuate the aldosteronism, but also the hypokalemia and the renin suppression associated with Ang II infusion (Figure 2). While both hematocrit and plasma protein concentration increased during verapamil infusion, only the 11% elevation in hematocrit was statistically significant (Figure 2). Finally, there were no significant changes in either plasma sodium concentration or plasma cortisol concentration during chronic administration of verapamil.

The renal hemodynamic response to verapamil infusion was opposite to that produced by Ang II (Table 1 and Figure 3). Both GFR and ERPF tended to increase during verapamil infusion, but the changes (with the exception of ERPF on day 3 of verapamil) were not statistically significant. In addition, there were no significant changes in filtration fraction during verapamil infusion. Renal blood flow also tended to increase, and on the final day of verapamil infusion there was a 13% increase in renal blood flow; however, the increments in renal blood flow during verapamil infusion (other than on day 3) were not statistically significant. The only significant renal hemodynamic change that consistently occurred during chronic verapamil administration, as with chronic Ang II infusion, was renal resistance. Verapamil greatly attenuated the increase in renal resistance produced by Ang II. After 7 days of verapamil infusion, renal resistance was reduced from 0.48±0.06 mm Hg/ml/min on the sixth day of Ang II to 0.34±0.04 mm Hg/ml/min. This represents approximately a 70% reduction in the increase in renal resistance produced by Ang II.

Effects of Chronic Verapamil Infusion in Normotensive Dogs

As illustrated in Figure 4, verapamil also decreased MAP when infused chronically in the dogs without preexisting hypertension. When the dogs were normotensive, MAP decreased gradually from a control level of 94±2 to 83±2 mm Hg after 7 days of verapamil infusion (p<0.05). Thus, there was a smaller hypotensive response when verapamil was infused into the dogs when they were normotensive (11 mm Hg) than when they were hypertensive (24 mm Hg). Further, in contrast to dogs with Ang II hypertension, verapamil infusion in normotensive dogs failed to produce measurable increases in urinary sodium and water excretion, or a fall in sodium iothalamate space (Table 2). As before, there were no significant changes in either heart rate or urinary potassium excretion during verapamil infusion. Control values for heart rate, urinary sodium, potassium, and water excretion were 56±2 beats/min, 65±3 meq/day, 52±1 meq/day, and 922±64 ml/day, respectively.
TABLE 2. Effects of Chronic Verapamil Infusion in Normotensive Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GFR (ml/min)</strong></td>
<td>66±4</td>
<td>65±3</td>
</tr>
<tr>
<td><strong>ERPF (ml/min)</strong></td>
<td>180±16</td>
<td>178±9</td>
</tr>
<tr>
<td><strong>RBF (ml/min)</strong></td>
<td>309±34</td>
<td>299±23</td>
</tr>
<tr>
<td><strong>RR (mm Hg/ml/min)</strong></td>
<td>0.31±0.03</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td><strong>Na loth space (ml)</strong></td>
<td>6323±266</td>
<td>6275±387</td>
</tr>
<tr>
<td><strong>Pm (meq/l)</strong></td>
<td>146±1</td>
<td>146±1</td>
</tr>
<tr>
<td><strong>Pc (meq/l)</strong></td>
<td>4.3±0.2</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td><strong>HCT (%)</strong></td>
<td>42±2</td>
<td>41±1</td>
</tr>
<tr>
<td><strong>Aldo (ng/dl)</strong></td>
<td>5.6±0.8</td>
<td>5.3±0.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; RBF, renal blood flow; RR, renal resistance; Na loth space, sodium iothalamate space; Pm, plasma sodium concentration; Pc, plasma potassium concentration; HCT, hematocrit; Aldo, plasma aldosterone concentration.

In the normotensive state, as when Ang II hypertension was established, the chronic hypotensive response to verapamil infusion occurred without concurrent changes in plasma aldosterone concentration (Table 2). Also, as in the hypertensive dogs, there were no concomitant changes in plasma sodium concentration, plasma potassium concentration, hematocrit, plasma protein concentration (control, 7.0±0.1 g/dl), or plasma cortisol concentration (control, 1.3±0.2 µg/dl) during verapamil infusion. Finally, in normotensive dogs, the hypotensive response to verapamil infusion was associated with an increase in PRA from a control level of 0.56±0.08 to 0.87±0.12 ng Ang I/ml/hr on day 7 of verapamil administration (Figure 5); however, changes in PRA during verapamil infusion were not statistically significant.

In large part, the renal hemodynamic response to verapamil infusion was qualitatively similar in the presence and absence of Ang II hypertension (Tables 1 and 2). In normotensive dogs, as when Ang II hypertension was established, there were no significant long-term changes in GFR, ERPF, or filtration fraction during verapamil infusion. However, although verapamil produced a large fall in renal resistance in dogs with Ang II hypertension, when infused in normotensive dogs the changes in renal resistance were much smaller and the average change was not statistically significant.

Discussion

There is now considerable evidence that indicates the kidneys play a dominant role in the chronic regulation of arterial pressure.21 Central to this role is the capability of the kidneys to regulate body fluid volume through changes in the renal excretion of salt and water. Long-term changes in arterial pressure occur when the "set point" of the kidney-body fluid pressure control mechanism is altered.21 This occurred in the present study during verapamil infusion. Verapamil altered the kidney's excretory response to pressure, allowing salt and water balance to be achieved at a lower arterial pressure level. If verapamil lowered arterial pressure without altering the excretory function of the kidneys, the initial hypotensive response would have caused the kidneys to retain salt and water and increased body fluid volume until arterial pressure returned to the renal set point. Consequently, in the steady state, arterial pressure before and during verapamil infusion would have been the same. Clearly, this was not the case. The fall in arterial pressure during verapamil infusion was sustained and was associated with loss of salt and water and a reduction in extracellular fluid volume. Conversely, when verapamil infusion was terminated, salt and water retention occurred, extracellular fluid volume increased, and arterial pressure returned to hypertensive levels observed before administration of verapamil. Therefore, while reduced peripheral resistance undoubtedly contributed to the initial fall in arterial pressure during verapamil infusion, a shift in the set point of the kidney-body fluid pressure control mechanism to a lower arterial pressure level must have been of paramount importance in determining the chronic hypotensive response to verapamil.

In the present study, increased sodium excretion occurred during the first 24 hours of verapamil infusion in all dogs with Ang II hypertension except one. Presumably, in this dog, the very pronounced acute fall in arterial pressure, due to decrements in cardiac output or total peripheral resistance,
exceeded the reduction in the set point of the kidney–body fluid control mechanism. Consequently, even though verapamil increased renal excretory capability in this dog (and in all others), an initial natriuresis and diuresis did not occur. However, negative sodium and water balance did ensure on subsequent days and in all dogs with Ang II hypertension, sodium and water balance was negative and extracellular fluid volume was reduced during chronic verapamil infusion. Verapamil also decreased the renal set point in normotensive dogs but in this setting the hypotensive response was not associated with significant changes in sodium balance or extracellular fluid volume. In patients with essential hypertension, sodium excretion is calcium dependent and can be depressed by calcium channel blockers suppress the acute vasoconstrictor or pressor responses to Ang II. Additionally, numerous acute studies have shown that calcium channel blockers promote natriuresis and attenuate the renal vasoconstrictor effects of Ang II and the short-term stimulatory effects of Ang II on aldosterone secretion. Thus, if these renal actions of calcium channel blockers were maintained chronically, one would expect enhanced renal excretory capability and a persistent reduction in arterial pressure. Indeed, Huelemann et al recently reported that chronic oral administration of nifedipine markedly attenuated the hypertension produced by long-term subcutaneous infusion of Ang II in rats. However, it is not clear from their study what mechanisms accounted for the hypotensive response to the calcium channel blocker. Although there is considerable evidence from in vitro studies that the aldosterone response to Ang II is calcium dependent and can be depressed by calcium channel blockers including verapamil, results from in vivo experiments are more variable. In humans, most studies, but not all, have shown that acute administration of calcium channel blockers attenuates the short-term aldosterone response to Ang II. In support of these findings, McDougall et al found that acute infusion of verapamil directly into the arterial supply of the transplanted adrenal gland of sheep blocked the acute stimulatory effects of Ang II on aldosterone secretion. In contrast, all but one study in humans indicate that chronic administration of calcium channel blockers does not impair the short-term aldosterone response to Ang II. Under conditions of chronic aldosteronism, aldosterone responses to calcium channel blockers have been variable also. Roy et al found that chronic verapamil administration decreased plasma aldosterone concentration (but not PRA) in sodium-depleted rats, whereas Johnson and associates observed that acute infusion of verapamil directly into the arterial supply of the transplanted adrenal gland failed to alter aldosterone secretion in sodium-depleted sheep. In patients with primary aldosteronism, Nadler et al reported that chronic administration of nifedipine controlled blood pressure while reducing plasma aldosterone concentration; in contrast, Bravo et al found no significant effect of nifedipine on either blood pressure or plasma aldosterone concentration in patients with primary aldosteronism. Finally, most studies indicate that chronic administration of therapeutic levels of calcium channel blockers in patients with essential hypertension has little long-term effect on either PRA or plasma aldosterone concentration. To our knowledge there have been no previous studies that have evaluated the chronic effects of calcium channel blockers on the long-term aldosterone response to Ang II. Our results demonstrate very clearly that the hypotensive response to verapamil infusion, both before and after induction of Ang II hypertension, was not associated with a fall in plasma aldosterone concentration. Therefore, these data, while inconclusive, fail to support the hypothesis that the chronic stimulatory effects of Ang II on aldosterone secretion are dependent on a sustained increase in transmembrane calcium influx into zona glomerulosa cells. Thus, it is possible that the stimulatory effects of Ang II on aldosterone secretion are more calcium dependent under acute than chronic conditions. Alternately, it is conceivable that increased calcium influx is maintained chronically through a membrane calcium channel that is not inhibited by verapamil. A very important finding in our study was that the hypotensive effects of verapamil were independent of a fall in plasma aldosterone concentration.
tion. In another study, a very low infusion rate of Ang II directly into the renal artery of unilaterally nephrectomized dogs produced marked sodium retention acutely; moreover, within 48 hours the direct sodium-retaining effects of Ang II were manifested in hypertension that persisted for the entire 10-day infusion period. Since the effects of Ang II were confined largely to the kidneys, this study demonstrates that the direct renal effects of Ang II can reduce renal excretory capability and produce chronic hypertension. We have also observed during chronic intravenous infusion of Ang II, in both intact dogs and adrenalectomized dogs given maintenance levels of steroids, that increases in plasma levels of aldosterone, achieved by infusion, have virtually no effect on the severity of Ang II-induced hypertension. Finally, in addition to these findings during Ang II hypertension, other studies during chronic sodium depletion also indicate that the major effects of the renin-angiotensin system on arterial pressure are independent of its indirect sodium-retaining effects that are mediated via aldosterone secretion.

Although several acute studies have shown that verapamil and other calcium channel blockers markedly attenuate the renovascular effects of Ang II, there is relatively little information on the long-term effects of calcium channel blockers on renal function, particularly under conditions associated with high circulating levels of Ang II. As in previous studies, there was a marked increase in renal resistance during chronic Ang II infusion but no significant changes in GFR, ERPF, or filtration fraction. Therefore, although Ang II transiently decreases GFR, ERPF, and sodium excretion and increases filtration fraction during the chronic hypertensive phase of Ang II infusion, these renal indexes return to control levels. Apparently, establishment of sodium balance and normalization of ERPF (in part) are dependent on the rise in arterial pressure. Conversely, although verapamil acutely increases GFR, ERPF, and sodium excretion in the presence of elevated plasma levels of Ang II, our results indicate that the only consistent long-term effect of verapamil on renal function in Ang II hypertension is a decrease in renal resistance. Similar renal findings have been reported in patients with essential hypertension subjected to chronic verapamil therapy. We cannot ascertain from our experiments to what degree, if any, renal autoregulation contributed to the fall in renal resistance during chronic verapamil infusion. However, if verapamil impairs renal autoregulatory capability chronically, as it does acutely, there would be little additional decrease in renal resistance as arterial pressure fell. Further, because the renal vasodilatory response to verapamil would be highly pressure dependent if renal autoregulation were impaired, one would expect acute increases in GFR and ERPF induced by verapamil to wane chronically because of the attendant hypotension. Finally, our calculations of renal resistance would be expected to be in error to the extent that changes in the extraction ratio of iodohippurate occurred during either Ang II or verapamil infusion.

Our renal function findings differ from those of Huelsemann et al., who reported that chronic nitrendipine administration actually increased GFR, ERPF, and filtration fraction in rats with chronic Ang II hypertension. However, besides the fact that different calcium channel blockers were used, other important differences exist between our two studies. First, in Huelsemann's study Ang II was infused subcutaneously and nitrendipine was given by gavage twice daily. In our study, constant blood levels of Ang II and verapamil were achieved by continuous intravenous infusion. Second, in Huelsemann's study renal function was not measured in conscious animals but in rats subjected to the stresses of surgery and anesthesia. Under these conditions, one would expect an enhanced renal response to calcium channel blockers. Finally, although GFR and ERPF in hypertensive rats given nitrendipine were greater than in rats infused with Ang II, these latter animals were in positive sodium balance and had depressed values for GFR and ERPF. It is likely that if this group of rats with Ang II hypertension were studied under steady-state conditions when sodium balance was achieved, the values for GFR and ERPF would have been comparable with those of both the control group (as discussed above) and the hypertensive rats given nitrendipine.

In the absence of statistically significant changes in either GFR or ERPF, it is not clear what intrarenal mechanisms contributed to the chronic hypotensive effects of verapamil. Increased ERPF and decreased filtration fraction would tend to increase peritubular capillary hydrostatic pressure and decrease peritubular capillary osmotic pressure, which would decrease tubular reabsorption of salt and water. However, although ERPF (and especially renal blood flow) increased during verapamil infusion, only on the third day of verapamil administration was the increment statistically significant. Nonetheless, if verapamil does chronically impair renal autoregulatory efficiency (as discussed above), it would be difficult to demonstrate a statistically significant long-term change in either GFR or ERPF because only very small increments in these renal indexes would be expected at reduced levels of arterial pressure. Therefore, it is not clear from these data whether a small increase in GFR or changes in peritubular physical forces contribute to the chronic antihypertensive effects of verapamil. In addition, there is also evidence, mostly indirect, from other acute studies that suggests verapamil may have direct effects on tubular transport to decrease sodium reabsorption. However, at the present time, we can only speculate as to the importance of direct tubular actions of calcium channel blockers in mediating their long-term effects
on sodium excretion. Whatever the exact intrarenal mechanisms responsible for chronically enhancing renal excretory capability, the data in the present study clearly indicate that the hypotensive effects of verapamil are not due to a decrease in plasma aldosterone concentration.

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