Continuous Angiotensin II Blockade throughout the Acute Phase of One-Kidney Hypertension in the Dog

BARRY E. WATKINS, JAMES O. DAVIS, RONALD H. FREEMAN, JACK M. DEFORREST, AND GREGORY A. STEPHENS

SUMMARY The importance of the renin-angiotensin system in the development and maintenance of one-kidney renal hypertension in the dog was assessed by chronically inhibiting angiotensin by continuous infusion of [Sar\(^{\alpha-1}\),Ala\(^{\alpha-8}\)]angiotensin II or a converting enzyme inhibitor (SQ 20881). Angiotensin blockade was begun 1–2 days before renal artery constriction and continued for 6–7 days throughout the high renin phase of hypertension. Chronic hypertension was produced despite continuous angiotensin inhibition with [Sar\(^{\alpha-1}\),Ala\(^{\alpha-8}\)]angiotensin II or SQ 20881. Mean arterial pressure rose to hypertensive levels during the [Sar\(^{\alpha-1}\),Ala\(^{\alpha-8}\)]angiotensin II infusion, but the increase occurred more slowly than when the renin-angiotensin system was intact. Although chronic infusion of the angiotensin II analogue itself produced no measurable vasopressor agonism, the analogue appeared to be mildly agonistic for the kidney and adrenal cortex. Chronic SQ 20881 infusion consistently lowered mean arterial pressure by about 10 mm Hg in unilaterally nephrectomized normotensive dogs and potentiated the vasodepressor action of bradykinin. Renal artery constriction during the SQ 20881 infusion for 6 days produced a gradual increase in mean arterial pressure (18 mm Hg above the control level); discontinuation of SQ 20881 was followed by a further elevation in arterial pressure. The present observations demonstrate that chronic hypertension developed and was sustained in spite of minimal or no activation of the renin-angiotensin system during the acute high renin phase of one-kidney hypertension.

THERE IS considerable evidence that activation of the renin-angiotensin system is closely associated with the onset of renovascular hypertension. The acute responses to angiotensin blockade have been studied during both the acute and chronic phase of one- and two-kidney renovascular hypertension. Short-term inhibition of angiotensin II during the first few days after renal artery constriction, when plasma renin activity (PRA) is elevated, reduced arterial pressure toward normal levels in the dog, rabbit, and rats with two-kidney hypertension. It also has been reported that partial blockade of the renin-angiotensin system produced a depressor response during acute one-kidney hypertension in dogs and rats. Indeed, evidence from constant infusion of the converting enzyme inhibitor (SQ 20881) for 4 days in two dogs suggested that renal artery stenosis failed to induce one-kidney hypertension the time of angiotensin blockade, but the dogs were not observed to determine if chronic hypertension developed. It should be emphasized, however, that the renin-angiotensin system appears to be less important for maintenance of chronic renal hypertension. Chronic one- and two-kidney renal hypertension in dogs is characterized by a normal PRA and failure of angiotensin inhibition to lower mean arterial pressure. However, various reports indicate that acute angiotensin blockade may or may not reduce arterial pressure during chronic one- or two-kidney renal hypertension in rabbits and rats. The depressor effectiveness of angiotensin inhibition in these instances appears to be related to the prevailing level of PRA in the presence of a normal sodium balance.

There are, however, no data for experiments in which the renin-angiotensin system has been rendered nonfunctional throughout the entire acute phase of one-kidney experimental renal hypertension (3–6 days in the dog) to evaluate the necessity of this system for the subsequent development of chronic hypertension. Our experiments were designed to determine whether activation of the renin-angiotensin system is essential for production of sustained renovascular hypertension. Angiotensin blockade was produced by continuous infusion of either [Sar\(^{\alpha-1}\),Ala\(^{\alpha-8}\)]angiotensin II or the converting enzyme inhibitor (SQ 20881) throughout the acute 6-day period of elevation of PRA in dogs with one-kidney hypertension.

Methods

Female hounds (14–22 kg) were laparotomized during sodium pentobarbital anesthesia (30 mg/kg), and a unilateral nephrectomy was performed after it had been determined that there was a single renal artery to the other kidney. Chronic femoral arterial and venous catheters (Tygon microbore tubing) were inserted and exteriorized between the shoulder blades. The dogs were allowed to recover for 7 days before beginning control observations prior to renal artery constriction. Each morning, arterial pressure and heart rate were measured from the femoral
Constriction of the Renal Artery

During sodium pentobarbital anesthesia, the renal artery of the remaining kidney was exposed retroperitoneally through a flank incision. After bathing the area with a 2% lidocaine solution, an adjustable constricting device and an electromagnetic flow probe were placed around the renal artery. The preparation was left undisturbed for 10-20 minutes while renal blood flow stabilized. The renal artery then was constricted to reduce renal blood flow by 55-60%. This degree of constriction produces a 2% lidocaine solution, an adjustable constricting device and an electromagnetic flow probe were placed around the renal artery. The preparation was left undisturbed for 10-20 minutes while renal blood flow stabilized. The renal artery then was constricted to reduce renal blood flow by 55-60%. This degree of constriction produces benign one-kidney renovascular hypertension in dogs within 24 hours, this study showed that PRA was elevated for the first 6 days only after induction of hypertension.

Studies with \([\text{Sar}^1, \text{Ala}^8]\) Angiotensin II

\([\text{Sar}^1, \text{Ala}^8]\) angiotensin II was infused continuously during the acute high renin phase of renal hypertension. Before infusion of the angiotensin II analogue, several daily measurements were made of the pressor response to intraarterial bolus injections of 0.5 and 1 \(\mu\)g of synthetic angiotensin II (Hypertension; CIBA). The dogs then received a continuous intravenous infusion of \([\text{Sar}^1, \text{Ala}^8]\) angiotensin II for as long as 9 days. The angiotensin II analogue was dissolved in heparinized bacteriostatic saline and delivered from portable hydrolytic infusion pumps (graciously supplied by Dr. David Young, Department of Physiology, University of Mississippi Medical Center). These pumps, which were carried within canvas dog jackets, delivered 20-30 ml/24 hours. The effectiveness of angiotensin blockade was verified daily by evaluation of the pressor effect of 1 \(\mu\)g of exogenous angiotensin II. In a group of six dogs, the angiotensin II analogue was infused at a rate of 1 \(\mu\)g/kg per min for 2 days before renal artery constriction, and infusion was continued for as long as 7 additional days. Data were collected daily throughout the period of analogue infusion and for at least 1 week afterwards. In two dogs, chronic measurements were made for 28 days after renal artery constriction.

Two additional dogs were studied in a similar manner with continuous infusion of \([\text{Sar}^1, \text{Ala}^8]\) angiotensin II at 2 \(\mu\)g/kg per min. Infusion of the angiotensin II analogue was started 1 day before renal artery constriction and continued for 6 days after constriction in both of these animals. Observations of recovery were made for 25 and 28 days after renal artery constriction. Effectiveness of angiotensin blockade was assessed twice daily with doses of 0.5, 1, and 2 \(\mu\)g of exogenous angiotensin II.

A control series of four normal dogs was infused with the angiotensin II analog at 1 \(\mu\)g/kg per min for 9 days but did not undergo renal artery constriction. As in the other groups, data were collected daily and the effectiveness of angiotensin II blockade was evaluated using 1-\(\mu\)g injections of angiotensin II.

Studies with the Converting Enzyme Inhibitor (SQ 20881)

Additional continuous infusion experiments were conducted with the converting enzyme inhibitor (CEI), SQ 20881. As in the studies with the angiotensin II analogue, the CEI was infused throughout the acute high renin phase of renal hypertension. In six dogs, the pressor response to 1, 2, 4 \(\mu\)g of synthetic angiotensin I (Squibb) was determined before the initiation of CEI infusion. The CEI then was infused continuously, iv, at 10 \(\mu\)g/kg per min for 7 days. After 1 day of infusion, surgery was performed to reduce renal blood flow to the remaining kidney by 55-60%. Measurements were made in the same manner as in the series with angiotensin II analogue infusion; two dogs were studied for 20 and 28 days after renal artery constriction. The effectiveness of the CEI in producing angiotensin blockade was verified daily by evaluating the pressor response to 4 \(\mu\)g of angiotensin I. Continuous SQ 20881 infusions also were conducted in two unilaterally nephrectomized dogs for 3 days and in one chronic renal hypertensive dog for 2 days.

Studies during Bradykinin Administration

Because SQ 20881 also inactivates bradykinin, it was decided to investigate a possible role for bradykinin in these experiments. Depressor dose-response curves were determined in the two unilaterally nephrectomized dogs and in one chronic hypertensive dog by bolus injections of bradykinin triacetate (Sigma) ranging from 0.1 to 30 \(\mu\)g/kg. Similar dose-response curves were obtained in the same dogs during the last day of the 2 to 3 day infusion of SQ 20881.

Statistical Analyses

Data within all experiments were analyzed by use of Student's paired t-test, critical to a 5% level of significance. Analysis of data between experimental groups was accomplished by use of Student's t-test for group comparisons.

Results

Studies with \([\text{Sar}^1, \text{Ala}^8]\) Angiotensin II Infusion

\([\text{Sar}^1, \text{Ala}^8]\) angiotensin II (1 \(\mu\)g/kg per min) was continuously infused for 9 days in four normal dogs. Before infusion of the analogue, bolus injections of 1 \(\mu\)g of synthetic angiotensin II increased mean arterial pressure by 26 ± 2 mm Hg. Daily verification of angiotensin blockade during infusion of the analogue showed that 1 \(\mu\)g of exogenous angiotensin II was without any pressor effect in over 80% of the daily trials. When there was a response to the bolus of angiotensin II, mean arterial pressure rose by only 6 ± 1 mm Hg. Continuous infusion of \([\text{Sar}^1, \text{Ala}^8]\) angiotensin II did not change mean arterial pressure in these four dogs (Table 1; Fig. 1). Heart rate and cumulative sodium balances likewise were not significantly affected by the infusion; however, the values of...
TABLE 1  Effects of Continuous [Sar',Ala+] Angiotensin II Infusion (1 μg/kg per min) for 9 Days in Normal Dogs

<table>
<thead>
<tr>
<th>Days</th>
<th>MAP (mm Hg)</th>
<th>P Na (mEq/liter)</th>
<th>P K (mEq/liter)</th>
<th>PA (ng %)</th>
<th>PRA (ng ang/ml per 3 hr)</th>
<th>U Na V (mEq/day)</th>
<th>U vo (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>108 ± 5</td>
<td>139.5 ± 2.9</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.9</td>
<td>5.2 ± 1.0</td>
<td>56.5 ± 5.2</td>
<td>510 ± 45</td>
</tr>
<tr>
<td>Control 2</td>
<td>109 ± 5</td>
<td>139.8 ± 1.9</td>
<td>4.4 ± 0.3</td>
<td>4.8 ± 0.5</td>
<td>4.5 ± 1.1</td>
<td>48.6 ± 6.2</td>
<td>450 ± 35</td>
</tr>
<tr>
<td>Infusion 1</td>
<td>107 ± 5</td>
<td>139.6 ± 1.0</td>
<td>4.4 ± 0.4</td>
<td>12.9 ± 3.6</td>
<td>4.1 ± 1.9</td>
<td>36.2 ± 11.0</td>
<td>600 ± 160</td>
</tr>
<tr>
<td>2</td>
<td>110 ± 2</td>
<td>142.8 ± 1.9</td>
<td>4.2 ± 0.4</td>
<td>8.3 ± 1.2</td>
<td>4.7 ± 2.1</td>
<td>34.3 ± 14.9</td>
<td>485 ± 80</td>
</tr>
<tr>
<td>3</td>
<td>112 ± 3</td>
<td>139.2 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>8.3 ± 1.2*</td>
<td>4.7 ± 2.1</td>
<td>34.3 ± 14.9</td>
<td>485 ± 80</td>
</tr>
<tr>
<td>4</td>
<td>112 ± 2</td>
<td>140.0 ± 1.3</td>
<td>4.2 ± 0.1</td>
<td>3.2 ± 1.4</td>
<td>4.7 ± 2.1</td>
<td>34.3 ± 14.9</td>
<td>485 ± 80</td>
</tr>
<tr>
<td>5</td>
<td>108 ± 8</td>
<td>139.1 ± 1.5</td>
<td>3.9 ± 0.2</td>
<td>3.2 ± 1.4</td>
<td>4.7 ± 2.1</td>
<td>34.3 ± 14.9</td>
<td>485 ± 80</td>
</tr>
<tr>
<td>6</td>
<td>109 ± 6</td>
<td>141.1 ± 2.9</td>
<td>3.6 ± 0.4</td>
<td>8.5 ± 6.7</td>
<td>44.6 ± 8.3</td>
<td>590 ± 95</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>108 ± 7</td>
<td>145.8 ± 0.7*</td>
<td>4.2 ± 0.6</td>
<td>8.8 ± 3.1</td>
<td>44.6 ± 8.3</td>
<td>590 ± 95</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>113 ± 5</td>
<td>145.2 ± 2.5</td>
<td>3.7 ± 0.5</td>
<td>8.8 ± 3.1</td>
<td>44.6 ± 8.3</td>
<td>590 ± 95</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>112 ± 4</td>
<td>144.6 ± 3.2</td>
<td>3.8 ± 0.4</td>
<td>9.3 ± 0.6*</td>
<td>1.9 ± 0.3*</td>
<td>55.6 ± 7.6</td>
<td>435 ± 86</td>
</tr>
</tbody>
</table>

Recovery 1 108 ± 4 141.6 ± 1.8 4.4 ± 0.3 4.8 ± 1.1 1.3 ± 0.4* 90.2 ± 21.9 655 ± 130
Recovery 2 115 ± 8 141.5 ± 1.6 4.3 ± 0.3 2.3 ± 0.6* 62.0 ± 26.8 520 ± 155
Recovery 3 110 ± 8 144.9 ± 1.6 4.4 ± 0.1 6.6 ± 2.6 70.3 ± 19.2 735 ± 265
Recovery 4 104 ± 9 145.6 ± 1.2 3.9 ± 0.2 1.6 ± 0.6 50.5 ± 10.4 640 ± 95
Recovery 5 107 ± 7 142.6 ± 2.5 4.2 ± 0.2 6.2 ± 0.7 4.0 ± 2.1 49.0 ± 11.7 652 ± 160

Values are means ± se; n = 4. MAP = mean arterial pressure, P Na = plasma sodium, P K = plasma potassium, PA = plasma aldosterone, PRA = plasma renin activity, U Na V = urinary sodium excretion, and U vo = urine volume. * Different from average pre-infusion control value (P < 0.05).

In the experimental group of six dogs, continuous infusion of the analogue at 1 μg/kg per min was begun 2 days before renal artery constriction and sustained as long as 7 days thereafter. The effectiveness of angiotensin II blockade in these animals was essentially the same as in the control dogs with two exceptions; infusion of the analogue was discontinued in two of these animals at 4 and 6 days after renal artery constriction when it was found that inhibition of angiotensin II had first become ineffective. Infusion of the angiotensin II analogue alone for 2 days before renal artery constriction produced a slight increase in mean arterial pressure from preconstriction control levels on the first day only (Table 2). In contrast to dogs not receiving infusion in which mean arterial pressure increased within 24 hours after renal artery constriction (Fig. 1), arterial pressure increased more slowly in the dogs infused with the angiotensin II analogue. A significant elevation from preconstriction control levels did not occur until 3 days after renal artery constriction (Table 2). Also, mean arterial pressure did not increase significantly until 5 days after renal artery constriction in these animals, compared to normal dogs.
Consequently, two additional dogs were infused with angiotensin II given 2 times each day. The time course of totally unresponsive to 2 μg bolus injections of exogenous responses to renal artery constriction were almost identical with the two different doses of the angiotensin analogue.

Studies with the Converting Enzyme Inhibitor (SQ 20881)

Continuous infusion of SQ 20881 in six dogs before and after renal artery constriction produced excellent angiotensin blockade, as evidenced by complete failure of 4-μg bolus injections of synthetic angiotensin I to elevate mean arterial pressure. However, the results obtained from one of these animals were markedly different from those observed in the remaining dogs of this group and, conse-

![Figure 2 Effects of [Sar\(^1\), Ala\(^8\)]angiotensin II infusion during the development of one-kidney hypertension in dogs. Open symbols show the days of angiotensin blockade. The arterial pressure responses to renal artery constriction were almost identical with the two different doses of the angiotensin analogue.](image-url)
should be emphasized that the level of arterial pressure of value during the first 2 days after renal artery constriction. Finding is questionable since control plasma potassium values were low. Renal artery constriction during the days of CEI infusion, a change which was sustained for several days after CEI was discontinued. Although infusion of the CEI was accompanied by a slight increase in plasma potassium concentration, the importance of this finding is questionable since control plasma potassium values were low. Renal artery constriction during the continuous infusion of the CEI produced only minimal transient changes in sodium balance or in water turnover. Infusion of the CEI for 24 hours increased PRA from an average value of 6.0 to 15.3 ng angiotensin/ml per 3 hr (P < 0.05), and renal artery constriction combined with CEI infusion further increased PRA to 25-30 ng angiotensin/ml per 3 hr throughout the infusion period (Table 3). PRA was not significantly elevated above the normal level during the recovery period. Heart rate and plasma aldosterone (Table 3) did not change appreciably in these dogs with renal artery constriction during the CEI infusion; the

<table>
<thead>
<tr>
<th>Days after RAC</th>
<th>MAP (mm Hg)</th>
<th>$P_{ml}$ (mEq/liter)</th>
<th>$P_{x}$ (mEq/liter)</th>
<th>PA (ng %)</th>
<th>PA (ng ang/ml per 3 hr)</th>
<th>PRA (ng ang/ml per 3 hr)</th>
<th>$U_{\text{NaV}}$ (mEq/day)</th>
<th>$U_{\text{K}}$ (mEq/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c = 3</td>
<td>101 ± 3</td>
<td>141.4 ± 1.4</td>
<td>3.9 ± 0.2</td>
<td>8.0 ± 3.0</td>
<td>6.6 ± 0.9</td>
<td>50.8 ± 6.3</td>
<td>455 ± 80</td>
<td>460 ± 80</td>
</tr>
<tr>
<td>c - 2</td>
<td>105 ± 3</td>
<td>143.8 ± 1.5</td>
<td>3.7 ± 0.1</td>
<td>6.7 ± 2.2</td>
<td>5.5 ± 1.6</td>
<td>48.8 ± 11.3</td>
<td>455 ± 80</td>
<td>460 ± 80</td>
</tr>
<tr>
<td>Infusion $c'$ = 1</td>
<td>94 ± 2*</td>
<td>145.7 ± 1.0</td>
<td>4.3 ± 0.2*</td>
<td>3.6 ± 0.7</td>
<td>15.3 ± 4.6*</td>
<td>67.6 ± 14.0</td>
<td>755 ± 185</td>
<td>755 ± 185</td>
</tr>
</tbody>
</table>

Values are means ± se; c and $c'$ represent control values before and during CEI infusion. * Different from mean pre-infusion control value (P < 0.05). † Different from mean pre-constriction blocker infusion value (P < 0.05).
Table 4  Effects of Infusion of SQ 20881 (10 µg/kg per min) in Two Unilaterally Nephrectomized Dogs

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Control</th>
<th>Control</th>
<th>CE1</th>
<th>CE1</th>
<th>Recovery</th>
<th>Recovery</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 57</td>
<td>No. 58</td>
<td>No. 57</td>
<td>No. 57</td>
<td>No. 57</td>
<td>No. 57</td>
<td>No. 57</td>
<td>No. 57</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>102</td>
<td>110</td>
<td>108</td>
<td>110</td>
<td>98</td>
<td>112</td>
<td>112</td>
<td>112</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ang per ml per 3 hr)</td>
<td>1.6</td>
<td>2.5</td>
<td>2.4</td>
<td>2.8</td>
<td>18.3</td>
<td>3.1</td>
<td>1.4</td>
<td>4.3</td>
</tr>
</tbody>
</table>

first recovery value of 27.8 for the plasma aldosterone level appears elevated but the change was not statistically significant.

The remaining dog of the group infused with the CEI showed a prompt elevation in mean arterial pressure from a control level of 109 to 125, 132, 138, and 135 mm Hg on the first four days after renal artery constriction. Since plasma aldosterone increased 10-fold after renal artery constriction during the CEI infusion, it seems likely that angiotensin blockade was incomplete. Therefore, this dog was excluded from the analysis of the data for the group.

Continuous infusion of the CEI in the two control unilaterally nephrectomized dogs consistently decreased mean arterial pressure by about 10-15 mm Hg in one animal (dog no. 58), and some decrease in pressure in the other dog is suggested by the recovery data (Table 4). This depressor response was completely reversed within 24 hours after the end of SQ 20881 infusion. Infusion of the CEI markedly increased PRA in both dogs (Table 4). A similar depressor response of about 15 mm Hg was observed in one chronic hypertensive dog 24 and 25 days after renal artery constriction (Fig. 3); PRA increased markedly in association with the 2-day period of angiotensin blockade.

The data for the bradykinin dose-response curves obtained before and during SQ 20881 infusion for these two control unilaterally nephrectomized dogs and the one chronic hypertensive dog are presented in Table 5. The observations show that the depressor responses were much greater in magnitude and lasted considerably longer during SQ 20881 infusion than during the control preinfusion period.

Discussion

There are still major gaps in our knowledge of the role of the renin-angiotensin system in the pathogenesis of renal hypertension. It has been uncertain whether the transient activation of the renin-angiotensin system is essential for induction of chronic hypertension or is just a coincidental occurrence. In an attempt to resolve these uncertainties, long-term continuous infusions of [Sar1,-Ala8]angiotensin II or SQ 20881 were given in doses which produced almost complete blockade of the renin-angiotensin system in the one-kidney renal hypertensive

Table 5  Effects of Intravenous Bradykinin Administration before and during Infusion of SQ 20881 in Three Dogs

<table>
<thead>
<tr>
<th>Bradykinin (µg/kg) as a bolus injection</th>
<th>0.1</th>
<th>1.0</th>
<th>3.0</th>
<th>10.0</th>
<th>30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Prior to SQ 20881 infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 47 (hypertensive)</td>
<td>ΔMAP*</td>
<td>Duration†</td>
<td>-8</td>
<td>-26</td>
<td>-50</td>
</tr>
<tr>
<td>No. 57 (unilaterally nephrectomized control)</td>
<td>ΔMAP</td>
<td>Duration</td>
<td>-12</td>
<td>-15</td>
<td>-26</td>
</tr>
<tr>
<td>No. 58 (unilaterally nephrectomized control)</td>
<td>ΔMAP</td>
<td>Duration</td>
<td>-15</td>
<td>-22</td>
<td>-32</td>
</tr>
<tr>
<td>II. During SQ 20881 infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 47 (hypertensive)</td>
<td>ΔMAP</td>
<td>Duration</td>
<td>-35</td>
<td>-49</td>
<td>-70</td>
</tr>
<tr>
<td>No. 57 (unilaterally nephrectomized control)</td>
<td>ΔMAP</td>
<td>Duration</td>
<td>-24</td>
<td>-38</td>
<td>-42</td>
</tr>
<tr>
<td>No. 58 (unilaterally nephrectomized control)</td>
<td>ΔMAP</td>
<td>Duration</td>
<td>-40</td>
<td>-45</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mean arterial pressure (mm Hg).
† Duration of depressor response (sec).
model. It should be emphasized that both compounds are competitive antagonists so that blockade was never complete; also, it is not known how blockade of the response to a bolus injection relates to blockade of the circulating level of the endogenous peptide. For example, the degree of blockade needed to prevent a pressor response to 1 μg of a peptide might be more than adequate to block 95% of the action of the endogenous peptide.

In the present study, chronic infusion of [Sar¹, Ala¹]angiotensin II in the sodium replete normal dogs produced no change in mean arterial pressure. This indicates that the administration of the angiotensin II analogue produced no measurable pressor agonism at vascular receptors. These data agree with and extend the findings of several studies in normal sodium replete dogs which show that this angiotensin II analogue produced only a transient (less than 5-minute) elevation in mean arterial pressure which was followed promptly by a return to the control level.15-20 The absence of a long-term vascular agonism is an important prerequisite for interpreting the results of the present study.

By use of [Sar¹, Ala¹]angiotensin II and SQ 20881, the present experiments show that chronic one-kidney renal hypertension can indeed be produced despite continuous angiotensin inhibition throughout the acute high renin phase. Furthermore, the intensity of the established hypertension was the same in the dogs receiving angiotensin blockade as in untreated dogs. These results with SQ 20881 show that, although its onset was somewhat delayed, chronic renal hypertension developed without the necessity of acute activation of the renin-angiotensin system. It appears, therefore, that mechanisms other than those specifically induced by activation of the renin-angiotensin system must be importantly involved in the pathogenesis of chronic renal hypertension. On the other hand, the delayed onset of hypertension produced by angiotensin blockade demonstrates that activation of the renin-angiotensin system contributes substantially to the acute phase of renal hypertension. The direct role of the renin-angiotensin system in the genesis of renal hypertension appears to be quite transitory since maximal hypertensive levels were achieved by 5 days after renal artery constriction despite continuous angiotensin II blockade with [Sar¹, Ala¹]angiotensin II.

Comparison of the renal responses to the angiotensin analogue and SQ 20881 suggests that angiotensin blockade was more complete with the converting enzyme inhibitor. With SQ 20881, PRA was markedly elevated throughout the infusion whereas, with [Sar¹, Ala¹]angiotensin II, PRA was only slightly elevated for the first 2 days of the infusion. The decreased PRA during the last 3 days of analogue infusion might reflect an agonistic action at renal receptors; however, the elevated arterial pressure and hypervolemia might also have contributed to the low PRA. Also, PRA was significantly decreased below the normal level during analogue infusion to the normal control dogs; this finding supports the suggestion of an agonistic action. There was no evidence during SQ 20881 infusion of the usual renal sodium retention which occurs after renal artery constriction whereas, with [Sar¹, Ala¹]angiotensin II infusion, sodium retention occurred for 2 days. These findings for sodium excretion and PRA suggest that the intrarenal renin-angiotensin mechanism was still partially operative during administration of the angiotensin analogue. Although the higher (2 μg/kg per min) dose of the angiotensin analogue produced considerably better blockade than did 1 μg/kg per min, the arterial pressure response to renal artery constriction was essentially the same with the two doses of the analogue (Fig. 2). This finding indicates that blockade of the angiotensin II receptors at the peripheral arterioles was nearly complete; nevertheless, under these circumstances chronic hypertension developed and blood pressure was sustained at a high level.

Continuous infusion of SQ 20881, which has no inherent agonistic action, reduced mean arterial pressure by 10 mm Hg before renal artery constriction. After renal artery constriction and during continuous SQ 20881 infusion, mean arterial pressure promptly rose by 10 mm Hg for 4 days and then increased further during the last 2 days of the infusion; altogether, an increase in arterial pressure of 18 mm Hg occurred. These results show that arterial pressure was elevated in these dogs despite excellent angiotensin in blockade, and there was no evidence to suggest that the intrarenal renin-angiotensin mechanisms were left partially intact. Completeness of angiotensin blockade was verified from three lines of evidence. First, administration of 4 μg of synthetic angiotensin I consistently failed to produce a pressor response during the CEI infusion. Second, PRA rose 4- to 5-fold throughout the course of SQ 20881 infusion. This finding indicates successful inhibition of endogenous angiotensin II synthesis and the associated autoregulatory feedback inhibition of renin secretion. Third, throughout CEI infusion, plasma aldosterone did not rise from the basal low levels characteristic of normal sodium replete dogs. Within 24 hours after the end of the CEI infusion, mean arterial pressure increased by 14-18 mm Hg and was chronically sustained at this level. Also, at this time, angiotensin II blockade was no longer effective, as evidenced by restored responsiveness to synthetic angiotensin I. The first two recovery values for PRA of 21.0 and 21.5 ng of angiotensin/ml per 3 hr appeared to be elevated but the changes were not statistically significant.

From these data on angiotensin blockade with SQ 20881, it might be suggested that acute activation of the renin-angiotensin system is important for optimal induction of renal hypertension; arterial pressure did not increase greatly after renal artery constriction until after the CEI infusion was discontinued. However, this interpretation assumes that the SQ 20881 did not affect blood pressure regulation through any mechanism other than the renin-angiotensin system. There is substantial evidence that this is not the case. Angiotensin I-converting enzyme (also known as kininase II) is an enzyme which degrades kinins.21 In addition to its action to inhibit angiotensin II generation, SQ 20881 potentiates bradykinin and its effects.22-24 In the current study, the first 24 hours of SQ 20881 infusion into sodium replete dogs with a normal PRA decreased mean arterial pressure by 10 mm Hg, and the possibility must be considered that this decrease in pressure was mediated by bradykinin. It
should be pointed out that a depressor response to \([\text{Sar',Ala}^8]\text{angiotensin II}\) has not been observed in dogs with a normal PRA,\(^7,6,12,15,25\) so that this depressor response does not appear to have resulted from angiotensin II blockade. Also, the present observations show that continuous SQ 20881 infusion in two unilaterally nephrectomized control dogs and one chronic hypertensive dog decreased blood pressure in two of the three animals; these dogs were in sodium balance and PRA was normal before CEI infusion. In these three dogs, the vasopressor response to bolus injections of bradykinin was markedly enhanced during SQ 20881 infusion. These findings raise the question of a role for bradykinin during SQ 20881 infusion. If bradykinin attenuated the increase in mean arterial pressure by 10–15 mm Hg after renal artery constriction, then the blood pressure response during SQ 20881 infusion and the onset of renal hypertension would be very similar to that observed with the [Sar',Ala']angiotensin II infusion.

The use of the angiotensin II analogue and SQ 20881 in the study of the genesis of renal hypertension has provided results which support the concept that the renin-angiotensin system need never be activated for chronic one-kidney renal hypertension to develop and to be sustained. These findings for one-kidney renal hypertensive dogs have been confirmed\(^2\) by use of the new oral converting enzyme inhibitor. SQ 14225, which was given for 1 day before and 7 days after renal artery constriction. An initial 9 mm Hg depressor response occurred before renal artery constriction in uninephrectomized dogs, and arterial pressure was not significantly elevated after renal artery constriction until the 5th day, after which chronic hypertension occurred. Similar results have been obtained (Freeman et al., manuscript in preparation) in the one-kidney hypertensive rat given SQ 14225 intraperitoneally (80 \(\mu\)g/hr) via an osmotic minipump. Also, in the two-kidney hypertensive rat model, SQ 14225 given intraperitoneally at 80 \(\mu\)g/hr prevented a rise in arterial pressure for 12 days of drug administration (Freeman et al., in preparation); 2 days after the drug was discontinued, blood pressure began to increase and reached a hypertensive level within 6 days. These results for the rat are consistent with the idea that two-kidney hypertension is renin dependent but one-kidney hypertension is renin independent.

These findings for the one-kidney renal hypertensive model raise the question of the importance of salt and water retention and volume expansion. It has been demonstrated that both renal artery constriction\(^2\) and perinephritis\(^26\) produced chronic one-kidney hypertension in uninephrectomized dogs that were severely sodium and volume depleted throughout a 30-day course of study. These observations show, therefore, that salt and water retention and volume expansion are not essential for the development of chronic one-kidney hypertension in the dog. The present data and these considerations on the lack of importance of volume expansion point out the need to search for other renal pressor mechanisms. A promising approach is the recognition of a new hypertensive substance called renopressin described by Skeggs and associates.\(^29\)

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Hydrolase Activities in the Rat Aorta

I. Effects of Diabetes Mellitus and Insulin Treatment

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SUMMARY  Vascular disease in diabetics could arise in part from altered vessel wall catabolism. Specific activities of hydrolases in aortic smooth muscle cells from rats with streptozotocin-induced diabetes were measured. Enzymes included: neutral α-glucosidase, α-mannosidase, and lysosomal N-acetyl β-glucosaminidase, β-galactosidase, cathepsin C, acid α-glucosidase, and acid cholesteryl esterase. After 4, 8, and 11 weeks of diabetes, activities of all enzymes studied were decreased significantly in diabetic vessels, decreases ranging from 15% for cathepsin C to 62% for α-mannosidase. After 3 weeks of diabetes, insulin treatment for 1 week restored enzyme levels to normal. After 7 weeks of diabetes, 1 week of insulin treatment did not restore enzyme levels fully to normal (acid cholesteryl esterase was unchanged); 4 weeks of insulin did. Acid phosphatase and N-acetyl β-glucosaminidase activities were reduced markedly in histochemical studies of diabetic aortas at all time periods and were restored by insulin treatment. Alloxan-induced diabetes gave results similar to those with streptozotocin. Significant decreases of aortic hydrolase activities, including those of lysosomes, occur in experimental diabetes mellitus and could contribute to accumulation of substrates in vascular smooth muscle cells.

EPIDEMIOLOGICAL studies in man have identified diabetes mellitus as one of the risk factors associated with atherosclerosis.1,2 Although an occasional study has focused on the contribution of vessel cell metabolism to this association,3 most emphasis has been given to changes in circulating lipoprotein patterns in diabetes mellitus.1,3,4 A vasculopathy occurs in diabetes mellitus which is characterized by basement membrane thickening in small arteries and arterioles, particularly those of the kidney. Although a genetic basis for this thickening has been proposed, its occurrence in normal kidneys transplanted into diabetic recipients suggests that it may be acquired.5 Based on findings of increased carbohydrate of kidney basement membrane in diabetic man6 and increased levels of the synthetic enzyme, glucosyltransferase, in kidneys of animals with experimental diabetes mellitus, Spiro and Spiro7 proposed that the basement membrane accumulation reflects increased synthesis when hyperglycemia is present.

In addition to biosynthetic studies, investigations of the catabolic machinery in the vessel wall may provide insights into the pathogenesis of vascular disease. For example, lysosomes are affected markedly in atherosclerotic and hypertensive vascular diseases,8-10 and de Duve11 has proposed that lipid accumulation in atherosclerosis may reflect a subtle form of storage disease due to relative deficiency of acid lipase activity in the lysosome. The present studies were carried out to examine the effects of experimental diabetes mellitus on aortic hydrolase activities and to determine how these changes might be related to both the accelerated atherosclerosis and diabetic vasculopathy found in man.

Methods

Male Sprague-Dawley rats (Marland Farms), age 6 weeks and weighing 175-200 g at the outset, were injected with either 10 mg of streptozotocin (Streptozocin; Upjohn) (approximately 55 mg/kg body weight) dissolved in 0.5 ml of 0.9% saline with 0.02 M sodium citrate, pH 4.5, or with 6.7 mg of alloxan (alloxan monohydrate; Sigma) (approximately 40 mg/kg body weight) dissolved in 0.5 ml

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