Renin Secretion in the Chronically Perfused Pulseless Calf

Evidence for Failure of Stimulation by Decreased Pulse Pressure

PERRY M. SHOOR, ULLA M. MARZEC, LEE D. GRIFFITH, FRED S. HAMMILL, RALPH B. DILLEY, AND EUGENE F. BERNSTEIN

SUMMARY To study the influence of arterial pulse pressure on renin release, a chronic pulseless calf preparation was developed using a centrifugal left ventricular bypass pump. After pump implantation and recovery, control measurements of renal vein and arterial plasma renin activity, arterial pressure, and renal artery flow were obtained. The centrifugal bypass pump rate then was increased to capture cardiac output completely, and nine conscious calves were perfused in a nonpulsatile manner (pulse pressure <5-10 mm Hg) for 48 hours. Nonpulsatile perfusion was well tolerated and serum sodium, potassium, creatinine, and blood urea nitrogen were unchanged during bypass. Mean arterial pressure remained relatively constant [117 ± 3 (SE) mm Hg] during bypass, and was not significantly changed from control. Although renal blood flow decreased slightly from control (667 ± 84 ml/min) during the nonpulsatile perfusion period (555 ± 73 ml/min), renin secretion did not increase significantly from control (482 ± 81 ng angiotensin I/ml per hr per min) during the bypass period (531 ± 99). A diurnal cycle of renin secretion was observed during the pulseless perfusions. These data document the lack of any significant stimulatory influence of decreased pulse pressure on renin secretion in a chronic awake calf model.

Ore Res 46:100-107, 1979

ONE of the primary regulators of renal renin release from the juxtaglomerular apparatus appears to be a hemodynamic signal related to changes in adjacent afferent arteriole wall tension (Blaine and Davis, 1971), the major determinant of which is the intraluminal hydrostatic (arterial) pressure. Although decreases in mean perfusion pressure result in stimulation of renin release (Davis and Freeman, 1976), the role of the systolic-diastolic pressure difference (pulse pressure) in regulation of the renin-angiotensin system remains unclear. Studies by Many et al. (1969), Goodman et al. (1976), and Hawthorne et al. (1953) suggest that reduction of pulse pressure alone is a sufficient stimulus for increased renin release. However, others such as Skinner et al. (1964) demonstrated that reduction of pulse pressure without a simultaneous decrease in mean pressure did not result in stimulation of renin release. Most investigations into the importance of pulse pressure in the regulation of renin release have been acute studies, either on isolated organ preparations or in anesthetized animals. The development of a nonpulsatile, centrifugal left ventricular bypass pump for chronic circulatory support (Bernstein et al., 1975) has provided an opportunity to study the acute and chronic effects of nonpulsatile perfusion on renin secretion in an intact unanesthetized animal. Previous studies with this preparation (Johnston et al., 1976) demonstrated no significant change in peripheral plasma renin activity in calves perfused for periods of up to 14 days. The present studies were undertaken to evaluate in more detail the effects of nonpulsatile perfusion on renin secretion using frequent analyses of renin secretory rates as well as plasma renin activity. Although it is difficult to control all the variables that affect secretion in a chronic preparation, data obtained with this model should have direct relevance to the renin release mechanism in the intact animal or person.

Methods

Animal Preparation

After a 2-week quarantine and demonstration of normal hematocrit, white blood count with differential, urinalysis, blood urea nitrogen, and serum creatinine, 16 female Hereford or crossed Hereford-Holstein calves weighing 77-107 kg were fasted for 18 hours and then anesthetized with 4% halothane and oxygen without prior medication. Controlled endotracheal ventilation with oxygen and 1% halothane was maintained with a Bird large animal pressure-cycled respirator. The left common carotid artery and jugular vein were exposed in the neck...
and soft polyvinyl catheters (0.27 cm i.d.) were placed centrally for measurement of aortic pressure and infusion of fluid and medications, respectively. A Foley catheter was placed into the bladder for urine collection.

Response of the Renin Release Mechanism to Renal Artery Constriction and Hemorrhage in the Awake Calf

To document normal responsiveness of the bovine renal renin system to a known hemodynamic stimulus (renal artery constriction) and a mixed stimulus (acute hemorrhage), two calves were anesthetized (as described above), and instrumentation necessary for the collection of renal vein renin samples and measurement of renal artery flow was inserted through a left flank incision using sterile technique. The renal artery and vein were exposed gently and a Silastic catheter (0.062 i.d., 0.095 o.d.) was placed into the renal vein through a small venotomy and positioned peripherally into the renal hilus. A previously calibrated cuff-type electromagnetic flow probe* was placed around the left renal artery, and a hydrostatic vessel occluder was fitted 1 cm peripheral to it. Care was taken to minimize the dissection to limit disruption of the autonomic renal innervation.

The calves were allowed to recover for about 24 hours, and given food† and water ad libitum. On the day after surgery, while in an erect posture (maintained by a sling, harness, and stanchion), duplicate control renal vein and arterial (aorta) samples and renal artery flow and blood pressure measurements were taken 30 minutes apart. Renal artery constriction sufficient to reduce flow by about 40% was produced with the hydrostatic constrictor, and collections were taken 15 and 30 minutes following constriction and again 30 minutes after release of the constrictor. After a subsequent 1-hour stabilization period, the calves were bled during 15 minutes (20 ml/kg). Measurements and sample collections were performed just prior to hemorrhage, at its completion, and 15 minutes later.

Normal Range of Renin Secretion in Awake Calves

Three calves were prepared for the measurement of renin release in a manner similar to that described previously. Following anesthesia, the calves were allowed to recover for 1 day. Then blood pressure and left renal artery flow were measured and samples of renal vein and arterial blood were obtained for plasma renin activity measurement every 4–6 hours for a total of 8 days, and daily mean renin secretion was calculated. During this period, the calves were given a standard feed mixture and water ad libitum and were allowed to lie or stand at will.

* Statham SF 2202 blood flowmeter.
† Calf chow (Ralston-Purina Co.) and alfalfa hay.

Influence of Nonpulsatile Renal Perfusion on Renin Secretion in the Unanesthetized Calf

Following implantation of the Medtronic centrifugal left ventricular bypass pump, nine calves underwent nonpulsatile systemic perfusion. Under general endotracheal anesthesia (halothane), the calves were placed in a lateral position. Operative areas were shaved and prepared with Betadine surgical scrub and antiseptic solution. By sterile technique, polyvinyl catheters were placed in the left internal jugular vein and left carotid artery, and the left thorax was entered through the bed of the 6th rib. After intravenous injection of 5000 U of heparin and 12.5 g of mannitol, the descending thoracic aorta was isolated.

The Medtronic centrifugal blood pump consists of a compact 10 × 9 × 4-cm housing containing the impeller-rotor assembly and a set of flexible polyurethane direct-access cannulas. The 12-mm i.d. inflow cannula is 60 cm in length and has a gentle "J"-shaped recurve 8 cm from the tip to facilitate insertion into the apex of the left ventricle. The 10-mm i.d. return cannula is bonded to a short segment of 12-mm dacron vascular graft for attachment to the descending thoracic aorta. The impeller, baffle, and thrust pad are machined of LTI pyrolytic carbon, and all other blood contact surfaces are coated with a nonthrombogenic polyurethane. The pump is driven by magnetic coupling between the sealed rotor and an external rotating magnet which rotates at a constant speed, variable between 2300 and 6900 rpm, producing an output up to 15 liters/min with a pulse-pressure difference of less than 5–10 mm Hg (transmitted pressure wave resulting from ventricular contraction).

The dacron graft of the pump-return cannula was sutured to the descending aorta in an end-to-side manner. After the pericardium had been opened, the curved portion of the inflow cannula was placed transapically 6 cm into the left ventricular cavity and secured there with a Teflon felt sewing ring. The pump system was primed with normal saline and flushed to remove residual air prior to institution of minimal flow, (2800 rpm; 1–2.4 liters/min). A polyvinyl catheter (0.270 cm i.d.) then was placed in the left ventricle to monitor intracavitary pressure. Pressure catheters were connected to transducers (Statham P23 BB) and the thoracotomy was closed.

Postoperatively, the calves were given 1.25 million units of penicillin G every 4 hours and 1 g of chloramphenicol every 6 hours, intravenously. No further anticoagulants were administered, but the calves were given 10 mg of dipyridimole every 8 hours, intravenously, and a 0.3-g aspirin suppository every 8 hours per rectum. Following surgery, the calves were hoisted into an erect posture with a special sling and placed in a pen and stanchion. After full recovery from anesthesia (average time 3 hours), the endotracheal tube was removed and the calf was allowed to breathe room air. The calves
were given no blood transfusions during or after surgery, but did receive intraoperative infusions of 0.9% NaCl solution. Daily weight and fluid intake and output were recorded. After substantiation of adequate oral intake, intravenous fluids were reduced and the calf was allowed water ad libitum.

To minimize the effect of anesthesia and operative trauma on the renin response, the centrifugal blood pump was maintained at an initial low flow mode for 1 day. During this interval, the calf's heart contributed the majority of the left ventricular output, and aortic pressure and renal artery flow tracings demonstrated normal pulsatile contours. The day after insertion, the pump output was increased to capture fully left atrial return and institute nonpulsatile (NP) systemic perfusion (Fig. 1). Pump flow and aortic pressure tracings were monitored continuously, and pump speed was adjusted to maintain a central aortic pressure trace with less than 5-10 mm Hg pulse pressure.

The calves were maintained in an erect posture (but allowed to rest in their sling) so that orthostatically induced hemodynamic changes would not affect renin output. Calves were fed at the beginning of each 6-hour work shift (2 a.m., 8 a.m., 2 p.m., and 8 p.m.). The laboratory is windowless, and was generally kept well-lighted during the study. Although oral sodium and potassium intake were not controlled, a standardized intravenous protocol was followed, which included 3000 ml of 0.9% NaCl solution during surgery, followed by 5% dextrose in water postoperatively to maintain normal fluid balance. For six calves, all urine output was collected and analyzed for Na⁺ and K⁺, and daily urinary excretion of Na⁺ and K⁺ was calculated for the first 3 postoperative days. The calves tolerated the pump implantation and perfusion periods without difficulty and the experiments were terminated electively.

Blood samples (renal vein and arterial) and measurements (renal artery flow, blood pressure, and cardiac output) were obtained prior to institution of NP bypass and sequentially during the perfusion period. Samples were taken after 30 minutes of NP bypass, at 2-hour intervals for the first 12 hours, and then at approximate 4-hour intervals.

**Laboratory Analyses**

Samples for plasma renin analysis were drawn simultaneously from the renal vein and central aorta and immediately placed in prechilled glass test tubes containing EDTA‡ as an anticoagulant and inhibitor of plasma angiotensinase. After centrifugation for 10 minutes at 2000 g (4°C), the plasma was transferred to capped plastic tubes and frozen at −20°C until analysis. At the time of each sampling, a record was made of blood pressure, pump flow, renal artery flow, time of day, and calf body position.

Plasma renin analyses were performed by radioimmunoassay (angiotensin II⁴¹¹), New England Nuclear). The technique, a modification of the method described by Haber et al. (1969), includes the conversion (angiotensinogen endogenousrenin angiotensin I) of samples at pH 6.0, and duplicate immunoassay of generated angiotensin I at 37° and 4°C to correct for preformed angiotensin I. All of the immunoassays from each experiment were performed at the same time, and the interassay variability (expressed as mean percentage difference (± SE) between duplicate immunoassays of 20 split samples) was 12.0 ± 3.1%. Plasma renin activity was expressed as nanograms of angiotensin I per milliliter per hour of generation at 37°C. Renin secretion was calculated by multiplication of renal plasma flow§ by the renal vein and arterial plasma renin activity difference.

Serum and urine analyses of Na⁺ and K⁺, whole blood hematocrit, blood urea nitrogen, and serum creatinine were determined by standard laboratory methods.

Statistical analysis of changes between control and bypass periods was performed by two-way analysis of variance (Snedecor and Cochran, 1967) using a Wang 600 Series Library Program Volume 139. Multiple regression analysis of data was carried out on a HP 9810A calculator using the 11214A Statistic Analysis System. Statistical significance of coefficients of determinations (r²) and correlation coefficients (r) were determined from a table of 5% points for r (Arkin and Colton, 1963).

**Results**

**Effect of Acute Renal Artery Constriction and Hemorrhage on Renin Response in Awake Calves with Pulsatile Blood Pressure**

Data from calves R-1 and R-2 are presented in Table 1. Renal arterial constriction sufficient to lower renal artery flow by 40% resulted in a several-fold increase in renin secretion by 15 minutes. On release of the constriction, renin secretion decreased to normal by 30 minutes, despite continued...
mild elevations of both renal vein and arterial plasma renin activity. Renal blood flow acutely decreased with constriction but gradually increased during the period of fixed constriction. Acute hemorrhage (20 ml/kg) resulted in decreases of 41% and 49% in mean arterial pressure and renal artery flow, respectively, and was associated with a 4- to 5-fold increase in renin secretion at 30 minutes. Renin release was greater at 30 minutes than at 15 minutes in spite of higher absolute perfusion pressures and renal arterial flows.

### Renin Secretion Rates in Normal (Pulsatile) Awake Calves

The mean arterial blood pressure in these three calves was 112 ± 3 mm Hg during the cumulative 8-day observation period. Mean renal renin secretion was 491 ± 97 ng angiotensin I/ml per hr per min, and mean renal vein and arterial plasma renin activity was 2.12 ± .23 and 1.26 ± .09 mg angiotensin I/ml per hr, respectively.

### Effects of Prolonged Pulselessness on Renin Secretion in the Awake Calf

Of the nine calves in which the Medtronic centrifugal left ventricular bypass device was implanted, the duration of pulseless perfusion (pulse pressure < 5-10 mm Hg) was 24 hours in two, and 2 days in the remainder. Table 2 contains the blood and urine chemistry data measured following pump implantation (pulsatile control) and during the non-pulsatile perfusion period. During the pulseless perfusion, there was no change in hematocrit, blood urea nitrogen, serum creatinine, sodium, or potassium. Urinary volume, sodium, and potassium excretion increased during the 2nd day of NP perfusion.

The calves had fully recovered from the anesthetic during the 20-hour pulsatile observation period, and were eating well. Mean control aortic blood pressure measured prior to institution of pulseless left ventricular bypass averaged 116 ± 4 (se) and did not change significantly during pulse-

---

### Table 1  Renin Response to Renal Artery Constriction and Hemorrhage in Awake Calves during Pulsatile Perfusion

<table>
<thead>
<tr>
<th></th>
<th>Renal artery constriction</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RAC 15 min</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>R-1</td>
<td>116</td>
</tr>
<tr>
<td>MAP</td>
<td>R-2</td>
<td>123</td>
</tr>
</tbody>
</table>

### Table 2  Calf Blood and Urine Chemistry Data

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>9</td>
<td>32 ± 2</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>9</td>
<td>11.9 ± 1.6</td>
<td>13.0 ± 2.3</td>
</tr>
<tr>
<td>Creat (mg/dl)</td>
<td>9</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Na (mEq/liter)</td>
<td>9</td>
<td>132 ± 2</td>
<td>131 ± 1</td>
</tr>
<tr>
<td>K (mEq/liter)</td>
<td>9</td>
<td>3.8 ± 0.2</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vol (ml)</td>
<td>9</td>
<td>3517 ± 633</td>
<td>4110 ± 825</td>
</tr>
<tr>
<td>Na excretion (mEq/day)</td>
<td>6</td>
<td>Not measured</td>
<td>83 ± 43</td>
</tr>
<tr>
<td>K excretion (mEq/day)</td>
<td>6</td>
<td>Not measured</td>
<td>110 ± 17</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

Abbreviations: Hct = hematocrit; BUN = blood urea nitrogen; Creat = creatinine; Vol = volume.
* Day prior to initiation of NP perfusion.
† Expressed/24-hour period.
TABLE 3  Calf Arterial Pressure and Renal Blood Flow during 48 Hours of NP Perfusion

<table>
<thead>
<tr>
<th>Calf</th>
<th>Control</th>
<th>Day 1</th>
<th>Total NP period*</th>
<th>Control</th>
<th>Day 1</th>
<th>Total NP period*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-3</td>
<td>110</td>
<td>106 ± 4</td>
<td>108 ± 2</td>
<td>380</td>
<td>202 ± 20</td>
<td>202 ± 20</td>
</tr>
<tr>
<td>4</td>
<td>116</td>
<td>109 ± 2</td>
<td>109 ± 2</td>
<td>315</td>
<td>298 ± 8</td>
<td>317 ± 10</td>
</tr>
<tr>
<td>5</td>
<td>108</td>
<td>111 ± 2</td>
<td>119 ± 3</td>
<td>1020</td>
<td>640 ± 58</td>
<td>530 ± 54</td>
</tr>
<tr>
<td>6</td>
<td>123</td>
<td>119 ± 2</td>
<td>126 ± 3</td>
<td>800</td>
<td>724 ± 38</td>
<td>719 ± 22</td>
</tr>
<tr>
<td>8</td>
<td>114</td>
<td>114</td>
<td>116 ± 4</td>
<td>1030</td>
<td>939 ± 64</td>
<td>915 ± 45</td>
</tr>
<tr>
<td>10</td>
<td>101</td>
<td>113 ± 5</td>
<td>117 ± 3</td>
<td>130</td>
<td>349 ± 18</td>
<td>345 ± 13</td>
</tr>
<tr>
<td>13</td>
<td>140</td>
<td>116 ± 5</td>
<td>124 ± 4</td>
<td>330</td>
<td>349 ± 18</td>
<td>345 ± 13</td>
</tr>
<tr>
<td>15</td>
<td>113</td>
<td>112 ± 3</td>
<td>112 ± 3</td>
<td>570</td>
<td>626 ± 23</td>
<td>619 ± 14</td>
</tr>
<tr>
<td>16</td>
<td>121</td>
<td>126 ± 3</td>
<td>126 ± 2</td>
<td>630</td>
<td>690 ± 23</td>
<td>749 ± 23</td>
</tr>
<tr>
<td>Mean</td>
<td>116</td>
<td>114 ± 2</td>
<td>117 ± 3</td>
<td>667</td>
<td>564 ± 83</td>
<td>556 ± 73</td>
</tr>
<tr>
<td>SEM</td>
<td>±4</td>
<td>±2</td>
<td>±3</td>
<td>±4</td>
<td>±83</td>
<td>±73</td>
</tr>
</tbody>
</table>

Abbreviation: NS = not significant.

* Total NP perfusion period = 48 hours.
† Mean arterial pressure (mm Hg) ± SEM.
‡ NP perfusion period = 24 hours.
§ Renal artery flow (ml/min) ± SEM.
¶ Compared to control (two-way analysis of variance).

Less perfusion, as seen in Table 3. Left renal artery flow was 667 ± 84 (SE) ml/min (219 ml/min per 100-g kidney) during the control period and decreased only slightly during the 2 days of pulseless perfusion, as shown in Figure 2.

Left ventricular bypass resulted in a decrease in pulse pressure from 45 to about 5 mm Hg with no significant change in mean aortic pressure or renal artery flow. In spite of the acute decrease in pulse pressure, the renin secretory rate did not increase. Data for the duration of the experiment are presented in Tables 4 and 5. The 48-hour pulseless perfusion period has been divided into 6-hour segments, and the mean of data collected during that period for each calf, as well as group-averaged data, are presented. Renal vein plasma renin activity was 3.42 ± 0.81 ng of angiotensin I/ml per hr during control and averaged 4.03 ± 0.86 during the first day and 3.96 ± 0.82 during the entire pulseless perfusion period. Arterial plasma renin activity also did not change significantly from the control level of 2.00 ± 0.56 mg angiotensin I/ml per hr (range, 1.42 ± 0.32 to 2.51 ± 0.54) during bypass.

Table 5 lists the renal renin secretory response to prolonged NP arterial perfusion. There was moderate variability in baseline renin secretory rates between individual calves, both during the control observations and throughout the bypass period. Those calves with higher control secretory rates also had higher secretory rates during pulseless perfusion. Multiple regression analyses failed to reveal a consistent relationship between control renin secretion and serum sodium and potassium. However, a considerable amount (r² = 0.561) of the variability in control renin secretion was inversely related to differences in control renal artery flow and mean arterial pressure (r = 0.75, P between 0.1 and 0.05).

Renin secretion averaged 482 ± 81 mg angiotensin I/ml per hr per min during the control period. Mean secretory rate for the first 24 hours of pulseless perfusion increased slightly by 8% to 524 ± 103, and the mean renin secretory rate during the total bypass period averaged 531 ± 99, which was not significantly different from control.

At the completion of the NP flow bypass period, the left renal artery was narrowed sufficiently in six of the calves to effect an approximate 50% reduction in flow. In each calf there was a prompt increase in renin secretory rate at 15 minutes, which demonstrated the continued responsiveness of the renin release mechanism (Fig. 3). Renal artery narrowing during pulseless bypass produced an increase in renin secretion of similar magnitude to that produced during pulsatile control conditions (Table 1). Although there was no difference in mean renin

---

**Figure 2.** Renal blood flow (EM) and mean arterial blood pressure remained relatively constant during the NP perfusion period.
TABLE 4 Renal Vein Plasma Renin Activity

<table>
<thead>
<tr>
<th>Calf</th>
<th>Control</th>
<th>1-6</th>
<th>7-12</th>
<th>13-18</th>
<th>19-24</th>
<th>25-30</th>
<th>31-36</th>
<th>37-42</th>
<th>43-48</th>
<th>Mean day 1</th>
<th>Mean total NP perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-3</td>
<td>2.78 ≤0.81</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>4</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>5</td>
<td>3.00 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>6</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>7</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>8</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>10</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>13</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>15</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
<tr>
<td>16</td>
<td>3.52 ±1.06</td>
<td>5.99 ±0.37</td>
<td>3.61 ±1.08</td>
<td>2.35 ±0.59</td>
<td>1.90 ±0.68</td>
<td>1.53 ±0.50</td>
<td>1.28 ±0.43</td>
<td>1.04 ±0.36</td>
<td>0.86 ±0.27</td>
<td>0.79 ±0.27</td>
<td>0.69 ±0.26</td>
</tr>
</tbody>
</table>

Abbreviation: NS = not significant.

TABLE 5 Renal Renin Secretion

<table>
<thead>
<tr>
<th>Calf</th>
<th>Control</th>
<th>1-6</th>
<th>7-12</th>
<th>13-18</th>
<th>19-24</th>
<th>25-30</th>
<th>31-36</th>
<th>37-42</th>
<th>43-48</th>
<th>Mean day 1</th>
<th>Mean total NP perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-3</td>
<td>5.94*</td>
<td>5.90†</td>
<td>6.67</td>
<td>5.96</td>
<td>5.97</td>
<td>6.74</td>
<td>4.15</td>
<td>7.46</td>
<td>4.15</td>
<td>6.74</td>
<td>1120 ± 205</td>
</tr>
<tr>
<td>4</td>
<td>5.94*</td>
<td>5.90†</td>
<td>6.67</td>
<td>5.96</td>
<td>5.97</td>
<td>6.74</td>
<td>4.15</td>
<td>7.46</td>
<td>4.15</td>
<td>6.74</td>
<td>1120 ± 205</td>
</tr>
<tr>
<td>5</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>6</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>7</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>10</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>13</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>15</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
<tr>
<td>16</td>
<td>6.05</td>
<td>6.75</td>
<td>5.32</td>
<td>6.08</td>
<td>6.07</td>
<td>5.96</td>
<td>5.96</td>
<td>5.74</td>
<td>4.15</td>
<td>7.46</td>
<td>658 ± 126</td>
</tr>
</tbody>
</table>

Abbreviation: NS = not significant.

Discussion

The initial studies in normal calves demonstrated that the calf renin release mechanism responded to a hemodynamic signal (renal artery constriction) and hemorrhage in a manner similar to that of the more commonly studied canine model. It also demonstrated the rapidity of the renin response and the necessity of evaluation of renin secretion rather than plasma renin activity alone in accurately monitoring renal renin output. Comparable decreases in blood flow secondary to renal artery constriction resulted in similar levels of stimulation of renin release whether flow was pulsatile or pulseless (Fig. 3), confirming the findings of Kolff (1958) showing equal renin response to lower pressure during pulsatile and NP perfusion in acute studies on our anesthetized dog preparation.

The present data demonstrate no significant change in renin secretion during NP bypass in the awake calf. These findings are at variance with some of the previously published studies concerned with the influence of pulseless perfusion on renin release. Although Skinner et al. (1964) demonstrated the calf renin release mechanism responded to normal in each instance.

secretory rates during the control and pulseless perfusion periods, when the data from individual calves were plotted against time of day (or hours of pulseless perfusion, since bypass was initiated at noon), a cyclic pattern of secretion was evident (Fig. 4). On each of the 2 days of bypass, there was an increase in secretory rate just prior to midnight and a fall back to normal levels by midday. This diurnal variation in renin secretion rate was not accompanied by changes in mean artery pressure or renal artery flow (Fig. 2).

At the end of each experiment, the calf was killed and an autopsy performed. The renal arteries and veins were patent and the renal parenchyma grossly normal in each instance.

Table 5 Renal Renin Secretion
Bovine renin secretory response to renal artery constriction during pulseless perfusion. Data from six calves demonstrate a significant increase in secretion with renal artery constriction in the absence of change in pulse pressure.

strated no increase in renin secretion rate with decreases in pulse pressure of up to 50%, Many et al. (1969), using an in-line aortic depulser in dogs, found a 27% increase in renal vein plasma renin activity during a 1-hour period of totally pulseless bilateral renal perfusion. A previous acute study on anesthetized dogs from this laboratory also showed an increase in renal vein renin activity during a 2-hour pulseless perfusion period (Goodman et al., 1976). However, blood pressure decreased by an average of 20% during the perfusion, and could explain part if not all of the renin response, as the intrarenal baroreceptor has been shown to be sensitive to pressure changes of this magnitude.

Kohlstaedt and Page (1940) emphasized the importance of arterial pulse pressure as the regulator of renin release. Pulse pressure was accepted as a significant regulator of both renin release and renal function until Selkurt (1951), Ritter (1952), and Kolf (1958) demonstrated that pulse pressure had no influence on renal blood flow, glomerular filtration, and excretion, provided mean perfusion pressure was kept constant. Additional supportive evidence for this thesis was presented by Skinner et al. (1964), who demonstrated that renin secretion was unaffected by changes in pulse pressure alone, as long as mean perfusion pressures remained equal, and that the renin release mechanism was quite responsive to small (< 10 mm Hg) changes in the level of perfusion pressure.

Although the presence of a renal vascular receptor is no longer in doubt, the specific signal to which it responds is still unclear. Studies using the smooth muscle relaxant papaverine injected intra-arterially have demonstrated inhibition of renin autoregulation, a known afferent arteriolar function, and blockade of the renin response to hemorrhage in the isolated vascular receptor model (Witty et al., 1971). Since stimulation of renin secretion has been demonstrated in the presence of afferent arteriolar dilation, as well as arteriolar constriction, it appears that the receptor responds to a more complex signal than intraluminal pressure per se. Arteriolar wall tension, which is highly dependent on intraluminal pressure but may be modified by other influences, such as arteriolar diameter, sympathetic tone, and intrinsic myogenic factors, may be the predominant factor.

The role of pulse pressure in this multifaceted scheme also is controversial. Following the work of Kohlstaedt and Page (1940), others also demonstrated presumptive evidence of stimulation of renin secretion in response to decreasing pulse pressure. However, all studies that directly measured renin were acute experiments on anesthetized animals or were relatively brief periods of isolated organ perfusion. None measured renal renin secretion rates, but rather examined less sensitive indices, such as peripheral plasma renin activity or blood pressure changes (Huidobro and Braun-Mendez, 1942). The recent development of a pulseless centrifugal left ventricular bypass pump permitted the present examination of the effect of pulseless perfusion on renin secretion in an awake animal over a prolonged period.

Our findings are unlikely to be related solely to interspecies differences, since the calf has been shown to have a responsive renin release mechanism like that of the dog and human. More likely, the conflicting data in the studies previously reported may be due to the lack of hemodynamic stability during acute experiments performed on animals under the influence of anesthetics, or the alterations in central and peripheral hemodynamics, renal blood flow, and plasma renin activity associated with barbiturate anesthesia (McKensie et al., 1967). By allowing the animals to recover...
from anesthesia and the operative procedure for a
day, this potentially troublesome influence has been
minimized in the current experiments. Although
the calves were still in the early postoperative pe-
tiod and therefore not in metabolic equilibrium by
the time NP perfusion was initiated, they were
standing and eating, and hemodynamic measure-
ments were normal. Although sodium metabolism
was not completely controlled, all the calves were
in normal sodium and water balance during the
preoperative quarantine period, and the sodium
load given to calves during both the operative and
bypass periods was controlled.

The daily cyclic variability demonstrated for
renin secretion is intriguing. Careful scrutiny of
laboratory and animal routines has failed to reveal
a pattern of events that could explain the late
evening peaks and afternoon depressions in renin
secretion. However, there is strong evidence for a
similar circadian rhythm in human renin secretion
(Gordon et al., 1966). Circadian rhythms have also
been demonstrated for other renal functions, such
as excretion of sodium, potassium, chloride and
urinary volume, and these variations have been
shown to be independent of input, posture, or other
external influences (Mills, 1966). The circadian var-
iations in renin secretion noted in this study were
not accompanied by variations in blood pressure or
renal artery flow, and the calves were maintained
in an upright posture for the duration of the bypass
period. Although circadian rhythms have been
demonstrated for many biological functions, there
is yet no good understanding of the underlying
biological mechanism or its interface with external
environmental factors.

The circadian pattern of renin secretion added
considerable complexity to the analysis of the influ-
ence of NP perfusion, and prompted a comparison of
the control pulsatile period with the bypass pe-
tiod divided into 24-hour units. Although the con-
trol measurements were taken over a relatively
short time period, the mean renin secretion rate
(482 ± 81 ng angiotensin I/ml per hr per min) was
similar to the mean daily secretion rate obtained
for the three normal pulsatile calves (491 ± 97 ng
angiotensin I/ml per hr per min), which adds con-
siderable credence to the normalcy of the renin
release mechanisms during the control period, just
prior to initiation of NP left ventricular bypass.

References
Arklin H, Colton CR (1963) Tables for Statisticians. New York,
Barnes & Noble, p 155
Bernstein EF, DeLaria GA, Johansen KH, Shuman RL, Stasz P,
Reich S (1975) Twenty-four hour left ventricular bypass with
Blaine EH, Davis JO, Witty RT (1970) Renin release after
hemorrhage and after suprarenal aortic constriction in dogs
without sodium delivery to the macula densa. Circ Res 27:
1081–1089
Blaine EH, Davis JO (1971) Evidence for a renal vascular
mechanism in renin release: New observations with graded
118–125
Davis JO, Freeman RH (1976) Mechanisms regulating renin
release. Physiol Rev 56: 1–56
Goodman TA, Gerard DP, Bernstein EF, Dilley RB (1976) The
effect of pulseless perfusion on the distribution of renal cortical
rhythm in plasma renin activity in man. J Clin Invest 45:
1587–1602
Application of a radioimmunoassay for angiotensin II to the
physiologic measurements of plasma renin activity in normal
Hawthorne DW, Perry SL, Fugate W (1953) Development of
experimental renal hypertension in the dog following reduc-
tion of renal artery pulse pressure without reducing mean
Huidobro F, Braun-Manendez E (1942) The secretion of renin
Johnston GG, Hammill F, Marxec U, Gerard D, Johansen K,
Dilley RB, Bernstein EF (1976) Prolonged pulseless perfusion
Kohlstaedt KG, Page IH (1940) Liberation of renin by perfusion
of kidneys following reduction of pulse pressure. J Exp Med
72: 201–216
Kolff WJ (1958) Discussion of reports on renal factors in hyper-
tension. Circulation 17: 676–682
Mayer M, Giron F, Birtwell WC, Detelning RA Jr, Soroff HS
(1969) Effects of depilation of renal blood flow upon renal
function and renin secretion. Surgery 66: 242–249
171
Ritter ER (1962) Pressure/flow relations in the kidney: Alleged
Selkurt EE (1961) Effects of pulse pressure and mean arterial
pressure modification in renal hemodynamics and electrolyte
and water excretion. Circulation 4: 541–551
Skinner SL, McCubbin TW, Page IH (1964) Control of renin
secretion. Circ Res 18: 64–76
Ames, Iowa, Iowa State University Press, pp 229–338
Witty RT, Davis JO, Johnson JA, Frewitt RL (1971) Effects of
papaverine and hemorrhage on vein secretion in the nonfilter-
ing kidney. Am J Physiol 221: 1666–1671
Renin secretion in the chronically perfused pulseless calf. Evidence for failure of stimulation by decreased pulse pressure.

P M Shoor, U M Marzec, L D Griffith, F S Hammill, R B Dilley and E F Bernstein

_Circ Res._ 1979;45:100-107
doi: 10.1161/01.RES.45.1.100

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/45/1/100

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the _Permissions and Rights Question and Answer_ document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/