Impaired Autoregulation of Blood Flow and Glomerular Filtration Rate in the Isolated Dog Kidney Depleted of Renin

By George J. Kaloyanides, Robert D. Bastron, and Gerald F. DiBona

ABSTRACT

The effect of renin depletion on autoregulation of renal blood flow and glomerular filtration rate was examined in an isolated perfused kidney preparation. Group 1 dogs received a normal-sodium diet, group 2 dogs received deoxycorticosterone acetate (DOCA) and a high-sodium diet, group 3 dogs received DOCA and a sodium-deficient diet, group 4 dogs received a high-sodium diet without DOCA, and dogs in groups 5 and 6 were treated just like dogs in groups 1 and 2, respectively. Renin release was stimulated by decreasing renal arterial pressure to 50 mm Hg. An increase in renal arterial pressure from 100 to 160 mm Hg was associated with an impaired autoregulatory response in kidneys from group 2 dogs and in two kidneys from group 4 dogs; these kidneys exhibited suppressed or absent renin release in response to a decrease in renal arterial pressure. A separate group of experiments demonstrated that DOCA plus a high-sodium diet completely depleted the kidney of renin stores, indicating that decreased renin secretion reflected depletion of renal renin content. Infusing angiotensin II (0.22 ± 0.06 µg/min) into the renal artery of group 2 kidneys did not normalize the autoregulatory response. A decrease in renal arterial pressure from 150 to 50 mm Hg was associated with impaired autoregulation of glomerular filtration rate and renal blood flow in kidneys from group 6 dogs; renin secretion was undetectable in these kidneys. All kidneys from groups 1, 3, and 5 and the remaining six kidneys from group 4 exhibited a normal autoregulatory response and normal renin release. In groups 1-3, zonal blood flow was measured using the radiolabeled microsphere technique. Only in group 2 did a significant redistribution of fractional blood flow from the inner to the outer cortex occur. These experiments demonstrate that renin depletion impairs the capacity of the kidney to autoregulate blood flow and glomerular filtration rate; thus the data are consistent with the hypothesis that the renin-angiotensin system participates in the autoregulatory response.

KEY WORDS

renin secretion  renin-angiotensin system  deoxycorticosterone
arterial pressure  renal vascular resistance  sodium diet
renal blood flow distribution

Autoregulation of renal blood flow refers to the phenomenon whereby renal blood flow remains relatively constant as renal perfusion pressure is varied over the range of 80 to about 180 mm Hg. Constancy of renal blood flow requires changes in renal vascular resistance that parallel changes in renal perfusion pressure. The mechanism by which renal vascular resistance is altered remains a subject of controversy. Thurau and co-workers (1, 2) have proposed that the renin-angiotensin system mediates autoregulation of renal blood flow and glomerular filtration rate through a feedback mechanism involving the macula densa. According to this hypothesis, increasing renal perfusion pressure increases the concentration of sodium reaching the macula densa; in response to this stimulus, the macula densa activates the release of renin which in turn leads to the local formation of angiotensin II. Afferent arteriolar constriction then occurs causing renal blood flow, glomerular filtration rate, and distal sodium concentration to return toward their previous levels. In support of this hypothesis, Thurau and his colleagues have demonstrated that the renin activity of individual glomeruli varies in response to changes in the composition of the fluid perfusing the macula densa (2). Additional support for this hypothesis derives from the studies of Schnermann et al. (3); they have demonstrated that increasing the concentration of...
sodium reaching the macula densa by direct perfusion of the loop of Henle promotes reciprocal changes in the filtration rate of that nephron.

Evidence against this hypothesis includes the well-established fact that renal renin secretion, reflected by renal vein renin activity (4, 5), and angiotensin II formation, reflected by the concentration of this peptide in renal hilar lymph (6), change in a direction opposite to that which would be anticipated if the renin-angiotensin system were mediating the alterations in renal vascular resistance associated with the autoregulatory response. In addition, several groups of investigators have demonstrated that autoregulation persists in the absence of angiotensin II infusions albeit at a higher level of renal vascular resistance (7-9).

In response to these arguments, Thurau et al. (10) have pointed out that measuring renal vein renin activity and, presumably, renal hilar lymph concentrations of angiotensin II may not adequately assess the activity of the renin-angiotensin system at the critical location within the kidney, namely at the afferent arteriole. Similarly, infusing angiotensin II directly into the renal artery may not mimic the effect produced by endogenous angiotensin II formed in the vicinity of the juxtaglomerular apparatus.

If the renin-angiotensin system plays an important role in the autoregulation of renal blood flow, then it follows that depleting the kidney of renin should impair the capacity of the kidney to autoregulate. Two groups of investigators have used such an approach and observed no defect in the autoregulatory response (7, 9). These latter studies would constitute the most compelling argument against the renin-angiotensin system hypothesis were it not for the fact that these investigators failed to verify that the kidney had indeed been depleted of renin.

Because of the critical importance of this point to the argument, we reexamined the effect of renin depletion on autoregulation of renal blood flow in an isolated perfused dog kidney preparation. Renal renin depletion was verified by demonstrating that renin secretion in response to a decrease in renal perfusion pressure was absent and that renal renin content was markedly decreased or absent.

Methods

All studies were performed on mongrel dogs weighing between 15 and 25 kg. The "perfusion dogs" were fed a standard kennel ration. The diet of the "donor dogs" was altered as described later in this paper. On the day prior to the study, the dogs were deprived of food, but water was permitted ad libitum. On the morning of the study, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) with supplemental doses administered as required to maintain light anesthesia. An endotracheal tube was inserted, and respiration was regulated with a Harvard respirator adjusted to maintain arterial pH between 7.35 and 7.45.

The preparation of the isolated kidney was similar to that previously described (11). In brief, a kidney was removed from a donor dog, placed in a receptacle filled with saline maintained at 38°C, and perfused with blood from the femoral artery of a second dog, the perfusion dog. Renal venous blood flowed by gravity into a reservoir from which it was pumped to the perfusion dog's femoral vein. The perfusion dog rested on an adjustable platform; by raising or lowering the platform the hydrostatic pressure between the femoral artery and the isolated kidney could be altered, thereby permitting regulation of the arterial blood pressure of the isolated kidney. Anticoagulation was achieved by infusing heparin; 2 mg heparin/kg was given initially followed by 10 mg/hour for the duration of the study.

After perfusion of the isolated kidney had been established, mean renal arterial pressure was adjusted to 100 mm Hg and mean renal venous pressure was adjusted to 0 mm Hg. A minimum of 45 minutes was allowed for stabilization of renal function; then one of the following six protocols was executed.

In group 1, 15 experiments were carried out on kidneys isolated from donor dogs fed a standard kennel ration; these kidneys were considered to have a normal renin content. In 12 experiments, mean renal arterial pressure was reduced initially to 50 mm Hg and then increased to 100 mm Hg and 160 mm Hg in successive steps at 5-10-minute intervals. At each level of renal arterial pressure, multiple measurements of renal blood flow were made and arterial and renal venous blood samples were collected for renin determinations. In 3 experiments, we only examined the renal blood flow response to raising renal arterial pressure from 100 to 160 mm Hg.

In group 2, 18 experiments were carried out on kidneys isolated from donor dogs injected intramuscularly with 15 mg of deoxycorticosterone acetate (DOCA) in oil daily for an average of 15 days. The diet of these dogs was supplemented with 150 mEq of NaCl and 80 mEq of KCl daily. In these experiments, we restricted our observations to the effect on renal blood flow of raising renal arterial pressure from 100 to 160 mm Hg. In 6 experiments, renal arterial pressure was then maintained at about 160 mm Hg while angiotensin II (Hypertensin, CIBA), 0.25 µg/ml in 0.9% saline, was infused into the renal artery of the isolated kidney at a rate adjusted to reduce renal blood flow to the level observed when renal arterial pressure was 100 mm Hg. After renal blood flow had stabilized at this level, the perfusion pressure was reduced to 100 mm Hg while the infusion of angiotensin II was continued at the same rate. In 5 of the 6 experiments, the sequence of maneuvers was then reversed. The average infusion rate of angiotensin II was 0.86 ± 0.23 ml/min or 0.22 ± 0.06 µg/min.

In group 3, 6 experiments were carried out on kidneys isolated from donor dogs injected intramuscularly with 15 mg of DOCA in oil daily for an average of 19 days. In contrast to group 2 dogs, these dogs were force-fed by gavage a sodium-deficient diet (Nutritional Biochemicals). The sodium content of a nitric acid digest of this diet measured by flame photometry was essentially zero.

In group 4, 8 experiments were carried out on kidneys isolated from donor dogs injected intramuscularly with sodium-deficient diet; these kidneys were considered to have a low renin content. In 6 experiments, mean renal arterial pressure was reduced initially to 50 mm Hg and then increased to 100 mm Hg and 160 mm Hg in successive steps at 5-10-minute intervals. At each level of renal arterial pressure, multiple measurements of renal blood flow were made and arterial and renal venous blood samples were collected for renin determinations. In 2 experiments, we only examined the renal blood flow response to raising renal arterial pressure from 100 to 160 mm Hg.
kidneys isolated from donor dogs who did not receive DOCA but whose daily diet was supplemented with 300 mEq of NaCl for an average of 52 days. In groups 3 and 4, we examined the effect on renal blood flow of raising renal arterial pressure from 100 to 160 mm Hg.

In group 5, eight experiments were carried out on kidneys isolated from dogs fed a standard kennel ration similar to that given group 1 dogs. In these experiments, the perfusion dog received a priming dose of insulin followed by a constant infusion of insulin in 0.9% saline at a rate of 1 ml/min. In contrast to group 1, renal arterial pressure was raised initially to 150 mm Hg. After collecting two 10-minute urine samples along with a midpoint blood sample from the isolated kidney, renal arterial pressure was lowered to 50 mm Hg in 25-mm Hg steps. At each interval, 5 minutes was allowed for renal function to stabilize before two 10-minute urine samples and a midpoint blood sample were collected.

In group 6, seven experiments were carried out on kidneys isolated from dogs injected with DOCA and fed a high-sodium diet identical to that given group 2 dogs. In these experiments, the perfusion dog received a priming dose of insulin followed by a constant infusion of insulin in 0.9% saline at a rate of 1 ml/min. In contrast to group 1, renal arterial pressure was raised initially to 150 mm Hg. After collecting two 10-minute urine samples along with a midpoint blood sample from the isolated kidney, renal arterial pressure was lowered to 50 mm Hg in 25-mm Hg steps. At each interval, 5 minutes was allowed for renal function to stabilize before two 10-minute urine samples and a midpoint blood sample were collected.

Otherwise the experimental protocol was the same as that for group 5.

At the completion of each experiment in groups 5 and 6, simultaneous arterial and renal venous blood samples were obtained while renal arterial pressure was maintained at 50 mm Hg. Similarly, at the completion of each experiment in groups 2-4, renal arterial pressure was reduced to 50 mm Hg for 5 minutes and blood samples were collected for renin assay. Heparinized blood for renin assay was collected in chilled test tubes and immediately centrifuged at 4°C. Plasma renin activity was determined according to the method of Pickens et al. (12). Each pair of arterial and venous samples was assayed in triplicate using three different rats, and the mean value of the three determinations was taken as the renin activity (ng angiotensin II equivalents/ml plasma). Renin release (ng/min) was calculated as the product of renal venous renin activity minus arterial renin activity (Δ renal renin, ng/ml) and renal plasma flow.

Renal renin content was determined in five kidneys from dogs treated with DOCA and a high-sodium diet exactly as described for groups 2 and 6 and in six kidneys from dogs fed a standard kennel ration as described for groups 1 and 5. The renal cortex was dissected, weighed, and extracted for renin according to the method described by Haas et al. (13). Renin substrate was obtained from serum collected 4 days after bilateral nephrectomy of dogs treated for 14 days with DOCA and a high-sodium diet. To remove angiotensinase activity, the serum was acidified with 5N HCl to pH 2.5, maintained at 0°C for 2 hours, and then neutralized with 5N NaOH. In addition, 0.5 mg of phenylmethylsulfonylfluoride was added to each milliliter of serum to inhibit angiotensinase activity; neomycin (0.2 mg/ml) was also added to inhibit bacterial growth. To demonstrate that angiotensinase activity was absent, known quantities of angiotensin II were added to a sample of serum and incubated at 37°C and pH 5.5 for 4 hours; angiotensin II activity was then assayed in the rat. In nine experiments the mean recovery was 104 ± 4%. To determine renal renin content, a sample of cortical extract was added to 2 ml of serum and incubated at 37°C and pH 5.5 for precisely 10 minutes in the case of extracts from normal kidneys and for 4 hours in the case of extracts from kidneys treated with DOCA. Immediately following the incubation period, the test tubes were plunged into a mixture of Dry Ice and acetone to interrupt the reaction. Angiotensin II activity was then assayed in the rat as described earlier in this paper. Renal renin content was expressed as the quantity of angiotensin II activity generated per hour of incubation per gram of kidney cortex.

Distribution of renal cortical blood flow was determined in groups 1-3 by the radiolabeled microsphere technique. Microspheres approximately 15μ in diameter labeled with either 14C or 3H were suspended in 20% dextran. With renal arterial pressure at 100 mm Hg and again after raising renal arterial pressure to 160 mm Hg, 1-2 x 10⁶ microspheres in a volume of 0.1-0.2 ml were injected slowly into the renal arterial catheter against the direction of flow about 15 cm from the kidney. This injection technique did not cause detectable changes in renal blood flow. Moreover, in preliminary studies, we established that the distribution of microspheres injected in this manner was not significantly different from that seen following percutaneous left ventricular microsphere injection. The requirement of anticoagulation in our studies prohibited the routine use of the latter technique. At the termination of the study, two to four coronal slices approximately 8 mm thick were cut from the kidney. A rectangular wedge 8–10 mm wide aligned parallel with the cortical-papillary axis was sectioned from each coronal slice. The cortical thickness was measured with calipers, and an appropriate tissue slicer was selected for sectioning the cortex into four slices of equal thickness. The slices were placed in plastic vials and weighed; the radioactivity of each slice was then assayed in a gamma well counter. The counts per gram per cortical zone were determined for each wedge of tissue and expressed as a percent of the total counts for all four zones. Changes in distribution of fractional cortical blood flow were inferred from changes in the percent distribution of microspheres between the zones; no corrections were made for differences in the absolute volumes of the four zones. Absolute renal blood flow per cortical zone was determined from the product of renal blood flow per gram of kidney weight and fractional zonal blood flow.

Renal arterial and venous pressures of the isolated kidney were monitored with Statham 23AA and 23Db pressure transducers, respectively, connected to the arterial and venous catheters about 8 cm from the kidney. Systemic arterial and venous pressures were similarly monitored from catheters in the femoral artery and vein. Recordings were made with a Beckman dynograph. Renal blood flow was measured directly by timing the flow from the renal vein. Renal plasma flow (RPF) was calculated according to the formula RPF = RBF x (1 – 0.95PCV) where RBF equals renal blood flow and PCV equals packed cell volume measured by a microhematocrit centrifuge. Glomerular filtration rate was determined from the clearance of inulin.

The autoregulation index was defined as ΔRBF/ΔP₉ A over the perfusion pressure interval from 100 to 165 mm Hg, where P₉ A equals renal arterial pressure. The efficiency of autoregulation was calculated from the formula of Semple and deWardener (14): (RBF₁ – RBF₂)/RBF₂ – (P₉ A₁ – P₉ A₂)/P₉ A₁. A ratio of zero indicates perfect autoregulation, and a ratio of one indicates absence of autoregulation. Renal vascular resistance was calculated as P₉ A/RBF and expressed in peripheral resistance units (PRU) of mm Hg/ml min⁻¹. Student’s t-test was used to evaluate the difference...
of paired data within each group and mean data between groups (15). The data in the text, tables, and figures are expressed as means ±1 S.E.

Results

Figure 1 depicts the changes in renal blood flow, Δ renal renin, and renin secretion in the isolated kidneys of group 1 in response to changes in renal arterial pressure. A significant decrease in Δ renal renin and in renin secretion was observed as renal arterial pressure was increased from 50 to 100 mm Hg. These parameters showed a further slight decrease as pressure was elevated to 163 mm Hg, and autoregulation of renal blood flow was evident. Renal vascular resistance increased from 0.36 to 0.61 and 0.95 PRU as renal arterial pressure was raised from 50 to 100 and from 100 to 163 mm Hg. These experiments confirm the previously demonstrated dissociation between renal vascular resistance and renin release (4, 5).

Figure 2 depicts the mean change in renal blood flow per mean change in renal arterial pressure for groups 1 and 2. In group 1, renal blood flow increased from 173 ± 13 to 184 ± 14 ml/min as renal arterial pressure was raised from 99 ± 1 to 163 ± 2 mm Hg. The mean autoregulation index for the group was 0.17 ± 0.04 ml/min mm Hg⁻¹. According to the formula of Semple and de-Wardener (14), this value represents an autoregulation efficiency of 91% (autoregulation efficiency expressed as a percent is derived by subtracting the ratio [RBF₁ - RBF₂]/RBF₁ + [P₁ - P₂]/P₁ from 1 and multiplying by 100). In contrast, renal blood flow of group 2 increased from 216 ± 16 to 272 ± 22 ml/min as renal arterial pressure was elevated from 100 ± 1 to 161 ± 2 mm Hg. The mean autoregulation index for group 2 was 0.96 ± 0.14 ml/min mm Hg⁻¹, a value significantly greater than that observed in group 1 (P < 0.001); this value reflects the fact that the efficiency of autoregulation in group 2 was reduced to less than 58%.

Figure 2 also illustrates that at the lower pressure of 100 mm Hg absolute renal blood flow in group 2 was greater than that in group 1 (P < 0.01) and reflects the fact that control renal vascular resistance in group 2 was less than that in group 1 (P < 0.05). In response to the rise in renal arterial pressure, renal vascular resistance increased to a greater extent in group 1 than it did in group 2 (P < 0.01).

The kidneys in group 1 were obtained from donor dogs fed a standard kennel ration, whereas those in group 2 were taken from donor dogs fed a high-sodium diet and given DOCA to deplete their kidneys of renin. To test for renin depletion, renin secretion was stimulated by decreasing renal arterial pressure to 50 mm Hg. The results are summarized in Table 1. In group 1, the mean Δ renal renin was 10.1 ± 2.9 ng/ml and the mean renin secretion was 967 ± 303 ng/min. In group 2, Δ renal renin averaged 0.2 ± 0.2 ng/ml and renin
<table>
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<th>Renin secretion (ng/ml)</th>
<th>Renin secretion (ng/min)</th>
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<tr>
<td>1</td>
<td>10.1 ± 2.9</td>
<td>967 ± 303</td>
</tr>
<tr>
<td>2</td>
<td>0.2 ± 0.2</td>
<td>11 ± 18</td>
</tr>
<tr>
<td>3</td>
<td>9.3 ± 3.6</td>
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<td>4a</td>
<td>8.3 ± 2.8</td>
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</tr>
<tr>
<td>4b</td>
<td>-0.2</td>
<td>-47</td>
</tr>
<tr>
<td>5</td>
<td>16.7 ± 5.1</td>
<td>2152 ± 704</td>
</tr>
<tr>
<td>6</td>
<td>-1.1 ± 0.6</td>
<td>109 ± 55</td>
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See text for explanation of groups.

secretion averaged 11 ± 18 ng/min. Both group 2 values are significantly less than those obtained for group 1 (P < 0.001) and not significantly different from zero (P > 0.4).

In a separate group of experiments, renal renin content was determined in five kidneys from dogs treated with DOCA and a high-sodium diet similar to that given group 2 dogs. Renal renin content measured 13 ± 5 ng angiotensin II/hour g⁻¹ kidney cortex. In contrast, renal renin content of kidneys from dogs fed a standard kennel ration measured 3753 ± 711 ng angiotensin II/hour g⁻¹ kidney cortex. These data indicate that treating dogs with DOCA and a high-sodium diet for 15 days depletes their renal stores; thus, the data justify the conclusion that the absence of renin secretion in response to a decrease in renal arterial pressure reflects renal renin depletion.

In six experiments in group 2, angiotensin II was infused into the renal artery to determine whether this vasoactive agent could restore the autoregulatory response. The results are illustrated in Figure 3. Step 1 depicts impaired autoregulation as renal arterial pressure was raised from 100 to 160 mm Hg. In step 2, angiotensin II was infused directly into the renal artery at an average rate of 0.22 ± 0.06 μg/min to reduce renal blood flow toward the level that would have been expected if perfect autoregulation had occurred. In several experiments, we overshot this mark and established a mean renal blood flow slightly less than the control level. At step 3, renal arterial pressure was reduced to the control level of approximately 100 mm Hg. If angiotensin II had restored the autoregulatory response, the decrease in renal blood flow from the level established in step 2 would have been relatively small. The change in renal blood flow that followed the reduction in renal arterial pressure paralleled the change in renal blood flow observed in step 1 prior to the infusion of angiotensin II. These data indicate that, although angiotensin II increases the level of renal vascular resistance, it does not normalize the autoregulatory response. In

![Figure 3](http://circres.ahajournals.org/)}
five experiments, these maneuvers were repeated in the reverse order; the changes in renal blood flow were virtually identical to those illustrated in Figure 3.

In group 3, the kidneys were obtained from donor dogs chronically treated with DOCA as in group 2; in contrast to group 2, group 3 dogs were fed a sodium-deficient diet to examine the effect of DOCA per se on renin release and autoregulation. As can be appreciated from Figure 4, treatment with DOCA alone did not impair the autoregulation of renal blood flow. The autoregulation index for group 3 was $0.13 \pm 0.02$ ml/min mm Hg$^{-1}$, a value significantly lower than that for group 2 ($P < 0.001$) but not significantly different from that for group 1 ($P > 0.5$). Efficiency of autoregulation was approximately 93%. Control renal vascular resistance in group 3 was not significantly different from that in group 2 ($P > 0.2$), but, similar to the findings for group 1, renal vascular resistance increased to a greater extent than it did in group 2 ($P < 0.01$). Renal renin secretion and $\Delta$ renal renin in group 3 were significantly greater than they were in group 2 ($P < 0.05$) but not significantly different from the values observed in group 1 ($P > 0.4$). Thus, group 3 experiments indicate that the absence of renin release and the impairment of the autoregulatory response in group 2 kidneys are not due to DOCA per se but require the presence of adequate sodium in the diet. The lack of suppression of renal renin release by DOCA in the absence of salt loading is in agreement with the findings of Robb et al. (16) in the dog and Goodwin et al. (17) in the rat but at variance with the findings in the rat studies of Christlieb et al. (18), who concluded that DOCA suppresses renal renin release through a direct effect.

Group 4 experiments were undertaken in an attempt to induce renal renin depletion without using DOCA, a technique previously used by Thu-rau (19). The effect of chronic salt loading on the autoregulatory response is illustrated in Figure 5. In six experiments (solid symbols) referred to as group 4a in Tables 1 and 2, autoregulation remained intact as judged by an autoregulation index of $0.20 \pm 0.07$ ml/min mm Hg$^{-1}$, which represents an autoregulation efficiency of 90%. In two experiments (open symbols) referred to as group 4b in Tables 1 and 2, autoregulation was significantly impaired. Of particular interest is the fact that the six kidneys exhibiting normal autoregulation also exhibited normal renal renin secretion. In contrast, the two kidneys in group 4b with impaired autoregulation did not release renin when renal arterial pressure was reduced.

In groups 5 and 6, we examined the effect of reducing renal arterial pressure on glomerular filtration rate and renal blood flow. The results are illustrated in Figure 6. In group 5 (normal-sodium diet), glomerular filtration rate decreased from $36.7 \pm 1.9$ to $32.3 \pm 1.8$ ml/min ($P < 0.001$) as renal arterial pressure was reduced from 150 to 100 mm Hg. Decreasing perfusion pressure from 100 to 75 mm Hg caused a proportionately greater decrease, indicating that over this range autoregulation of glomerular filtration rate was less efficient, a finding similar to that reported for the intact kidney (20). In group 6, glomerular filtration rate decreased in a linear manner from $42.8 \pm 3.6$ to $32.1 \pm 3.5$ ml/min ($P < 0.001$) as renal arterial pressure was reduced from 150 to 100 mm Hg. When perfusion pressure was lowered to 75 mm Hg, a sharper decline in glomerular filtration rate was observed; this fall probably signifies that the afferent arteriole became maximally dilated as pressure was lowered from 100 to 75 mm Hg, resulting in a fall in glomerular capillary pressure in parallel with the decrease in renal arterial pressure. The fact that renal vascular resistance reached its nadir at 75 mm Hg in group 6 (Fig. 7) supports the conclusion that the afferent arteriole was maximally dilated at this pressure level. The absolute as well as the percent change in inulin clearance in group 6 over the interval of 150 to 100 mm Hg was significantly greater than that in group 5. The change in inulin clearance was $-10.8 \pm 1.3$ ml/min.
Effect of increasing renal arterial pressure on renal blood flow of the isolated kidney in group 4. These dogs received a high-sodium diet but no DOCA. The open symbols refer to the two kidneys in which renin release was not detected in response to a decrease in renal arterial pressure.

in group 6 and −4.4 ± 0.8 in group 5 (P < 0.005); the percent change was −25.8 ± 3.5% in group 6 and −11.9 ± 2.1% in group 5 (P < 0.005). Similarly, the change in inulin clearance per change in renal arterial pressure over the same interval was 0.087 ± 0.016 ml/min mm Hg⁻¹ in group 5 and 0.200 ± 0.024 ml/min mm Hg⁻¹ in group 6 (P < 0.005). These data indicate that the capacity to autoregulate glomerular filtration rate was significantly impaired in group 6 compared with that in group 5.

Figure 6 also illustrates that the capacity to autoregulate renal blood flow was similarly im-

Summary of Hemodynamic Data in Groups 1–4

<table>
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<tr>
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<th>$P_{na}$ (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>RVR (mm Hg/ml min⁻¹)</th>
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<tr>
<td>Group 1</td>
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<td>Group 4a</td>
<td>102 ± 1</td>
<td>161 ± 2</td>
<td>206 ± 13</td>
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$P_{na}$ = renal arterial pressure, RBF = renal blood flow, and RVR = renal vascular resistance. See text for explanation of groups.
paired in group 6. As renal arterial pressure was reduced from 150 to 75 mm Hg, a linear decrease in renal blood flow occurred followed by a steeper decline as renal arterial pressure was lowered from 75 to 50 mm Hg. In contrast, renal blood flow was well maintained in group 5 as renal arterial pressure was lowered from 150 to 100 mm Hg. However, with further reductions in renal arterial pressure to 75 and 50 mm Hg, the slope of the curve became slightly steeper, indicating that the autoregulatory range had been exceeded. Over the range of 150 to 100 mm Hg, the autoregulation index was 0.36 ± 0.14 ml/min mm Hg⁻¹ for group 5 and 1.59 ± 0.23 ml/min mm Hg⁻¹ for group 6 (P < 0.001). Autoregulation efficiency over the same interval was 82% for group 5 and 42% for group 6.

Figure 7 depicts the change in renal vascular resistance as renal arterial pressure was reduced from 150 to 50 mm Hg. In group 5, renal vascular resistance decreased in a linear manner over the entire interval. It also decreased in a linear manner in group 6 as renal arterial pressure was reduced from 150 to 75 mm Hg. However, renal vascular resistance did not decrease any further as renal arterial pressure was reduced to 50 mm Hg, suggesting that the renal vascular bed was maximally dilated when renal arterial pressure was reduced to 75 mm Hg. There was no significant difference in renal vascular resistance between the two groups when renal arterial pressure was reduced to 50 mm Hg. The slopes of the lines describing the change in renal vascular resistance over the interval of 150 to 75 mm Hg were significantly different (P < 0.001).

Table 1 shows data for Δ renal renin and renin secretion for the two groups. In contrast to the high renin secretion observed in group 5, no renin secretion was detected in kidneys of group 6. Renin secretion in group 5 was also higher than that observed in groups 1, 3, and 4. The reason for this difference might be related to the protocol employed in group 5. The reduction in renal arterial pressure over the range of 75 to 50 mm Hg was sustained for 30 minutes in group 5 and, therefore, may have resulted in a more intense stimulus for renin secretion or allowed more time for the renin secretory response to reach its maximum than occurred in groups 1–4 in which arterial pressure was abruptly lowered to 50 mm Hg for only 5 minutes.

Figure 8 illustrates the effect of increasing renal arterial pressure from 100 mm Hg to 160 mm Hg on the intrarenal distribution of blood flow in groups 1–3. No significant change in the fractional distribution of intrarenal blood flow was detected in either the normal-sodium group (group 1) or the DOCA-treated, low-sodium group (group 3). In both groups, autoregulation of renal blood flow was intact. In contrast, a significant redistribution of blood flow from the inner to the outer cortex was observed in the DOCA-treated, high-sodium group (group 2). The percent of microspheres in zone C4 decreased from 17.4 ± 1.3 to 13.9 ± 1.5% (P < 0.025) in response to the rise in renal arterial pressure, whereas the percent of microspheres in zone C1 increased from 32.1 ± 1.5 to 36.8 ± 1.9% (P < 0.005).

Changes in absolute renal blood flow distribution to the four cortical zones are summarized in Table 3. Total renal blood flow per gram kidney weight increased in all groups in response to raising renal arterial pressure from 100 to 160 mm Hg; however, the increase in the DOCA-treated, high-sodium group (group 2) was significantly greater than that in the other two groups (P < 0.01). The increase in renal blood flow per gram kidney weight in the DOCA-treated, high-sodium group (group 2) was distributed to zones C1–C3, with the largest increment occurring in C1. No change in renal blood flow per gram kidney weight occurred in C4. Although a slight increase in renal blood flow per gram kidney weight to zones C1 and C2 was also seen in the normal-sodium group (group 1), the magnitude of the increase was considerably less than that in the DOCA-treated, high-sodium group (group 2) (P < 0.01).

Discussion

The present experiments were designed to examine the role of the renin-angiotensin system in the autoregulation of renal blood flow and glomerular
filtration rate. If the renin-angiotensin system plays a major role in mediating autoregulation, then renal renin depletion should impair the autoregulating response. In a review of this subject, Thurau (19) stated that this situation was indeed the case, but he did not provide any details.
RENIN DEPLETION AND AUTOREGULATION

To test this hypothesis, we used an isolated perfused dog kidney preparation, which affords several advantages over other techniques for studying autoregulation. Since the kidney is completely isolated, the influence of renal nerve activity is eliminated. In contrast to most other studies of autoregulation (4–8, 21–23), this technique allows the examination of the response to a standard pressure stimulus over a uniform pressure range and excludes the possibility that extrarenal factors related to aortic constriction, vagotomy, carotid sinus denervation, or carotid occlusion influence the response.

In an earlier study (11), we reported the functional characteristics of this preparation including the fact that the isolated kidney exhibits autoregulation of both renal blood flow and glomerular filtration rate. Recently, we demonstrated that the isolated kidney exhibits appropriate changes in renin release in response to ureteral occlusion and changes in renal perfusion pressure (24).

The present experiments demonstrated that, compared with kidneys having a normal renal renin content, kidneys depleted of renin by DOCA and salt loading or by salt loading alone exhibited marked impairment of autoregulation of renal blood flow and glomerular filtration rate. Moreover, impaired autoregulation of renal blood flow in renin-depleted kidneys was evident whether renal arterial pressure was increased or decreased.

Our findings are in agreement with those of Thurau (19) and Brech et al. (25) but at variance with those of Gagnon et al. (9) and Belleau and Earley (7). Gagnon and his colleagues detected no impairment of autoregulation in animals maintained on a high-sodium diet for 5 weeks and therefore interpreted their data as evidence against Thurau’s renin-angiotensin hypothesis for autoregulation of renal blood flow and glomerular filtration rate. However, these authors reported no measurements of renin secretion or renal renin content and thus failed to establish that renin depletion had been achieved. In our group 4 experiments, we also attempted to induce renin depletion by chronic salt loading without DOCA administration, a technique used by Thurau (19). In view of the well-known inverse relationship between sodium intake and plasma renin activity (26), we were somewhat surprised that sodium loading for 7 weeks caused renal renin depletion in only two of eight kidneys. Nevertheless, in both instances autoregulation was impaired. The remaining six kidneys exhibited normal renin release and a normal autoregulatory response.

The reason renin depletion was not induced in all eight kidneys is not clear. Thurau (19) maintained his dogs on a high-sodium diet for an average of 8 weeks, which is only slightly longer than the 52 days of high-sodium diet employed in our group 4 experiments. However, it is not clear from his paper whether the level of daily sodium intake was similar to or greater than that used in the present experiments. Dogs exhibit the capacity to rapidly excrete an oral sodium load (27). Since our dogs usually ingested their sodium load within the first 8 hours, it may be that they rapidly excreted the high-sodium intake and for a significant part of the day were in normal sodium balance. If suppression of renin synthesis is related to continuing positive sodium balance, as occurs in animals treated with sodium and DOCA, then it is possible that a high-sodium diet alone may not provide a sufficiently sustained stimulus related to positive sodium balance to maintain inhibition of renin synthesis.

In any event, the group 4 experiments indicated that chronic sodium loading for up to 7 weeks may not be sufficient to cause renal renin depletion; this finding may explain why Gagnon et al. (9) found no impairment in the renal autoregulatory response of their animals. Belleau and Earley (7) reported a normal autoregulatory response in dogs that were maintained on a high-sodium diet for 4–9 weeks and injected with 5–10 mg of DOCA in oil intramuscularly daily or on alternate days. Although it seems likely that such treatment would have depleted the kidney of renin, these investigators did not establish this point by measuring renal renin content or renin secretion in response to stimulation. Recently Brech et al. (25) have reported findings similar to our own. In their study, sodium loading for 4–6 weeks did not abolish the renal autoregulatory response and did not suppress renin secretion, whereas salt loading plus DOCA administration did impair the autoregulatory response in association with absent renal vein renin activity. In addition, Brech et al. (25) found that renal renin content was decreased by DOCA and a high-sodium diet.

An alternative explanation for the disparity between our results and those of Gagnon et al. and Belleau and Earley may be that the response of the isolated kidney to renin depletion differs from that of the intact kidney. To resolve this question, the effect of documented renin depletion on autoregulation in the intact kidney needs to be reexamined.

It should be noted that in no instance in the present experiments was the autoregulatory re-
sponse completely abolished. Autoregulation efficiency was 58% in group 2 and 42% in group 6. Thus, some autoregulatory mechanism was still operating so that renal vascular resistance changed in a direction appropriate to the change in renal arterial pressure. The observation in group 6 that glomerular filtration rate did not decrease in a linear manner over the lower pressure range suggests that part of the resistance change occurred across the afferent arteriole. Whether this residual autoregulatory capacity reflects incomplete depletion of renal renin or some other mechanism normally operating in conjunction with the renin-angiotensin system remains uncertain.

The results of our experiments are internally consistent in that isolated kidneys depleted of renin did not autoregulate blood flow and glomerular filtration rate normally. Several factors in addition to renal renin depletion might account for this effect. First, it is possible that the impaired autoregulatory response might be a nonspecific effect related to the initial level of renal vascular resistance rather than to the renal renin depletion per se. For example, to maintain blood flow constant in response to a given change in perfusion pressure, the cross-sectional area of a dilated vessel would have to be altered to a greater extent than that of a constricted vessel to achieve the same change in resistance. If this fact were the explanation for impaired autoregulation, then the slopes of the renal blood flow and renal vascular resistance curves of renin-depleted kidneys should parallel those of normal kidneys. As can be seen in Figures 6 and 7, the slopes are divergent. The fact that increasing renal vascular resistance in group 2 by infusing angiotensin II did not restore the autoregulatory response also argues against this possibility.

In addition, in subsequent studies, we have observed little effect of vasodilators on the isolated kidney’s capacity to autoregulate blood flow (28).

It is also possible that DOCA plus salt loading alters renal vascular reactivity by a mechanism unrelated to the renin-angiotensin system. To establish this possibility would require demonstrating a normal autoregulatory response in the presence of renin depletion. In no instance thus far have we observed a normal autoregulatory response in a renin-depleted kidney. However, we have yet to examine the effect of renal renin depletion induced by other techniques such as potassium loading.

Finally, the possibility remains that the altered autoregulatory response is a direct consequence of renal renin depletion. The question then arises whether renal renin release and angiotensin formation modulate changes in renal vascular resistance as proposed by Thurau (1) or whether renin plays a permissive role in that autoregulation is impaired in its absence. The major argument against Thurau’s hypothesis is that renin release assessed by measurements of renal vein renin activity changes in a direction opposite to that which would be predicted if the renin-angiotensin system were mediating the changes in renal vascular resistance (4, 5). A similar dissociation was observed in our experiments. The observation of Baille et al. (6) that angiotensin II concentrations in renal lymph increase in response to decreases in renal perfusion pressure also argues against Thurau’s hypothesis. Nevertheless, it is possible that renal vein renin activity and renal lymph concentrations of angiotensin II may not reflect the activity of the renin-angiotensin system at the juxtaglomerular apparatus.

Britton (29) has proposed a hypothesis for the autoregulation of renal blood flow that reconciles these apparent contradictions. He postulates that renin located in granules of the juxtaglomerular apparatus can be released either into the lumen of the efferent arteriole or into the cytoplasm of the juxtaglomerular cells. Transport of renin from granule to cytoplasm is mediated by a sodium-dependent carrier that is regulated by the macula densa cells in response to changes in distal tubule sodium delivery. Cytoplasmic renin is postulated to be the rate-limiting factor in regulating afferent arteriolar tone by interacting at the luminal membrane with angiotensinogen and converting enzyme to form bound angiotensin II. According to this hypothesis, a decrease in renal arterial pressure would result in a decrease in sodium delivered to the macula densa which in turn would inhibit transport of renin from granules to cytoplasm of afferent arteriolar cells and result in a decrease in afferent arteriolar tone. At the same time, renin would be available for release directly into the lumen presumably of the efferent arteriole where it could not interact with specific receptors of the afferent arteriole but could exert its systemic effects. Although this theory provides a unifying hypothesis for data previously considered to be contradictory, direct evidence in its support is lacking.

In addition to predicting that renal renin depletion would impair the autoregulatory response, the renin-angiotensin hypothesis for autoregulation of renal blood flow also predicts that renal renin depletion would have a greater effect on outer cortical blood flow than it would on inner cortical.
blood flow due to the fact that a graded distribution of renin exists within the renal cortex with the superficial nephrons being rich in renin and the juxtamedullary nephrons having relatively little renin (30, 31). To test this hypothesis, we measured the distribution of renal blood flow with the radiolabeled microsphere technique. No significant change in the fractional distribution of microspheres was detected in groups 1 and 3 in which autoregulation was intact, although a slight increase in absolute renal blood flow to zones C₁ and C₂ did occur in group 1. In group 2, the impaired autoregulatory response was associated with a significant increase in both fractional and absolute blood flow to the outer cortex. That the increase in renal blood flow to the outer cortex represents a functional change and not an artifact due to streaming of microspheres is supported by another study (28) in the isolated kidney in which increasing renal blood flow with vasodilators caused redistribution of blood flow from the outer to the inner cortex. The latter finding is similar to that observed by Stein et al. (32) in the intact dog kidney and, as noted by these investigators, is opposite to that which would be predicted if streaming were an important variable influencing microsphere distribution.

Conversely, the renin-angiotensin hypothesis for the autoregulation of renal blood flow also predicts that in the normally autoregulating kidney an increase in medullary flow should occur in response to a rise in renal perfusion pressure, since the juxtamedullary nephrons are relatively poor in renin and hence should not regulate blood flow as effectively as the outer cortical nephrons (19). In groups 1 and 3, we found no evidence that blood flow to the juxtamedullary nephrons had increased. In fact, the data indicate a tendency for blood flow to be distributed toward the outer cortex in response to a rise in renal perfusion pressure.

McNay and Abe (23) also examined the effect of alterations in perfusion pressure on distribution of renal blood flow. Although the experimental design and methods employed by these investigators differed from those used in the present study, they found a significant pressure-dependent effect on blood flow distribution with an increase in distribution to the inner cortex occurring with a reduction in renal perfusion pressure. These changes are opposite to those predicted by the renin-angiotensin hypothesis. It is obvious that, if the renin-angiotensin system plays a major role in mediating the autoregulatory response, our understanding of this mechanism is far from complete.

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GEORGE J. KALOYANIDES, ROBERT D. BASTRON and GERALD F. DIBONA

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