Renal Intracortical Blood Flow Distribution, Function, and Sodium Excretion in Unanesthetized Dogs following Vena Caval Ligation

By Robert A. Gutman and Robert L. McRae

ABSTRACT

We studied the renal function and the intrarenal blood flow of nine dogs whose thoracic inferior vena cava had been previously ligated (caval dogs) and nine other dogs. Following preparatory surgery which included placement of a left atrial catheter, a femoral artery catheter, and bilateral ureteral catheters, the caval dogs gained an average of 2.1 kg of fluid weight, whereas the normal dogs gained no weight. Although neither the caval dogs' blood pressure (114 ± 7 vs. 120 ± 4 mm Hg) nor their inulin clearance (0.64 ± 0.06 vs. 0.79 ± 0.06 ml/min g⁻¹ kidney weight) was significantly reduced, their estimated renal blood flow (CVR[(1-hematocrit)]) was considerably lower (2.30 ± 0.24 vs. 3.25 ± 0.15 ml/min g⁻¹). During the clearance study, the caval dogs' excretion of sodium (79 ± 18 vs. 158 ± 17 μEq/min) and their fractional clearance of sodium (2.0 ± 0.4 vs. 3.4 ± 0.5%) were reduced. Studies with microspheres failed to demonstrate a selective decrease in blood flow. However, comparison studies of nine other dogs (five caval and four normal) demonstrated that microsphere results were less reproducible in caval dogs than they were in normal dogs. We have concluded that reduced blood flow is the only consistent alteration of renal function in this edematous animal model and that previous suggestions of altered distribution are not supported by these studies.

KEY WORDS

inulin
renal clearance
para-aminohippurate
edema
microspheres
ureteral catheterization

Barger (1) has suggested that the pattern of distribution of renal blood flow influences the renal excretion of sodium. He has reported that "sodium retention occurs when blood flow is largely distributed to the inner cortical and outer medullary region and that natriuresis ensues when blood flow is increased to the outer cortical region." This conclusion was based on studies of experimental heart failure (1) and diuretic-induced natriuresis in dogs (2). These and subsequent studies in man and animals (3-10), which have also demonstrated this relationship, have used the radioactive inert gas (¹³³Kr or ¹³³Xe) method (11, 12). This method is indirect, but it has the advantage that it is applicable to man and is reasonably reproducible under most circumstances. However, more direct assessments of alterations of the pattern of distribution of intracortical renal blood flow have frequently failed to support the conclusions of the washout technique (13). The most frequent of these direct methods uses nucleotide-impregnated carbon microspheres of a size (15 ± 5μ in diameter) that lodges in the glomerular tufts so that the microspheres can be detected in tissue slices (14, 15). In a number of experimental animal studies in which increased renal blood flow has been observed or expected, the results of the microsphere method are directionally different from those of similar experiments with xenon (13, 16-19). The results of studies of intrarenal blood flow in response to ureteral obstruction also vary depending on which method is employed (20, 21). There is one model, dog hemorrhagic shock, for which the results of the two methods are concordant (22-24).

The present study was designed to test the theory of selective reduction of outer cortical blood flow distribution in a chronic sodium-retaining model; the microsphere method was used. Dogs with a previously ligated inferior vena cava (caval dogs), a traditional model of sodium retention (25), have been studied with the xenon method (10, 26). The first of these studies (10) used acute constriction, and found a reduction of outer cortical blood flow. The second study (26) used chronic caval dogs; however, the results were difficult to interpret because of changes to the kidney caused by the necessary renal artery catheter.

Methods

Standard renal clearance measurements of the individual kidneys of nine normal dogs and nine caval dogs were carried out while microspheres were injected into the left atrium to evaluate the relationship, if any, of
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blood flow distribution to renal function and sodium excretion. Nine additional dogs (four normal and five caval) were studied under similar conditions but without ureters and femoral artery catheters. In these dogs, we injected two groups of microspheres (153Sr-labeled and 144Ce-labeled spheres) to evaluate the reproducibility of the distribution analysis. All studies were performed on mongrel dogs weighing between 8 and 20 kg. The dogs were awake, lying quietly under minimal sedation.

SURGICAL PREPARATION

The first 18 dogs were prepared 4–5 days prior to the study under sterile conditions using sodium pentobarbital anesthesia. Two Silar catheters (2.5 mm, o.d., Extracorporeal Medical Specialties) especially prepared for ureteral catheterization were inserted retroperitoneally in the lumen of each ureter. These catheters were led subcutaneously to the back of the dog where they were protected from trauma by a nylon protective jacket. A femoral artery catheter was implanted, led to the same site, filled with heparin solution, and capped. Next, a right thoracotomy was done. The middle lobe of the lung was held gently, exposing a pulmonary vein. A small flexible soft polyvinyl catheter (0.36 mm, i.d., and 0.72 mm, o.d., Michael V. Kudravets) was threaded into the left atrium, secured, filled with heparin solution (10,000 units/ml), and capped; the end was then placed in a subcutaneous pocket for easy recovery at the time of the study. Through the same thoracotomy incision with no further dissection, the thoracic inferior vena cava was partially ligated with umbilical tape in those dogs designated as caval dogs. The thoracotomy was closed under negative pressure to promptly reinflate the lungs. The 9 dogs studied for reproducibility of radioactive microsphere distribution were prepared in the same fashion except that femoral artery and ureteral catheters were not placed. Following surgery, the dogs were given penicillin (100,000 units, im) and streptomycin (0.5 g, im) for 2 days. They were walking and eating the next day. To assure that the ureteral catheters had adequate flow for at least a few hours each day, 1 liter of 0.9% saline was given intravenously on each of the postoperative days. On this regimen, the normal dogs did not gain weight, but the caval dogs gained an average of 2.1 kg and developed obvious ascites.

RENAL FUNCTION STUDY (18 DOGS)

The day prior to the study of renal function, drinking water was removed, and the dogs received 5 units of pitressin tannate in oil. We used morphine (15–20 mg, im) to sedate the dogs and diazepam (Librium) (2.5–5 mg, im) as required to maintain the dogs in apparent comfort. The room was kept quiet, darkened, and warm. The dogs were gently restrained on their left sides but were able to raise their heads.

Cardiovascular phenomena and responses of such dogs are qualitatively different from those seen under sodium pentobarbital anesthesia and are apparently normal. The blood pressure is lower; the heart rate is slower and shows sinus irregularity. In preliminary studies of similar dogs with chronically implanted magnetic flow probes in the renal artery, normal Herring-Breuer reflexes, which are rarely seen under sodium pentobarbital anesthesia, were observed. Under these conditions, the dogs lay quietly and responded normally to patting and gentle stimulation. The femoral artery catheter was connected via a Statham gauge to a calibrated Sanborn polygraph to record mean blood pressure. The left atrial catheter was recovered by making a small skin incision overlying the end of the catheter.

Saline (0.9%, 3 ml/min, iv) was kept running throughout the study. Inulin and para-aminohippurate (PAH) were infused at a rate of 1 ml/min in a concentration designed to maintain blood levels at 25 mg/100 ml and 2 mg/100 ml, respectively. This infusate contained aqueous pitressin in a concentration adjusted to administer 0.5 munits/min. After 45–60 minutes of equilibration, the first of three 20–30-minute collection periods was begun. Urine was collected from each indwelling ureteral catheter, and its flow rate was accurately measured. Blood was collected from the indwelling femoral artery catheter. During the second of these periods, approximately 1,000,000 radioactive carbonized microspheres (15 ± 5μ, 5–10 μc, suspended in 10% dextran with Tween 80, 3M Company) were slowly injected into the left atrium via the chronically implanted catheter; this injection was followed by a 2–3-ml flush of saline.

REPRODUCIBILITY STUDY (9 DOGS)

Five caval dogs and four normal dogs were sedated and restrained in the same manner described for the radioactive microsphere studies. On the third day after surgery, 153Sr-labeled microspheres were given; 2 or 3 days later, 144Ce-labeled microspheres were used.

PREPARATION OF RENAL TISSUE FOR MICROSPHERE ANALYSIS

The dogs were killed after the study, and sections of the kidneys were prepared for gamma radiation detection by a modification of the method described by Stein et al. (18). Just prior to death, under anesthesia, the femoral artery catheter was advanced caudad until the tip was upstream from the renal arteries; 20 ml of India ink was then injected. After death, the kidneys were removed, stripped of their capsule, and fixed in 10% Formalin. Four 3–6-mm thick sagittal slices were taken by hand from near the hilum. Each kidney slice was the source of three approximately rectangular prisms of tissue cut so that the curves of the surface and the corticomedullary junction were minimized and nearly parallel. Under a dissecting scope, using a graduated eyepiece redicle, four equally thick sections of glomerular cortex were taken and arbitrarily identified as zone 1 (the outermost portion) through zone 4 (the innermost portion). Thus, 12 representative samples (four slices, three prisms from each, each cut into four layers) of each layer were usually cut. These samples were grouped, placed in a tared counting tube, weighed and counted on a triple-channel auto-gamma counter (Beckman Co.). The usual weight of tissue counted was 150–500 mg. The usual count rate was over 2,000 counts/min. The efficiency of the counter was such that this count rate indicates over 500 spheres were present in each counting vial. The counts per gram of tissue were calculated for each isotope for each layer and totaled. The fraction of counts per gram in each layer of tissue is reported. The kidneys from the nine dogs in the second study were handled identically. Simultaneous counting was done with an energy discriminator; less than 40% of
the $^{85}$Sr counts was present in the $^{141}$Ce channel and there was negligible contamination of the $^{85}$Sr channel by the $^{141}$Ce. Counts were corrected by precisely measuring the interference with appropriate standards. Counts in the $^{141}$Ce channel exceeded the $^{85}$Sr counts.

Laboratory determinations of inulin and PAH were done by automated techniques (27, 28). Serum protein was estimated by refractometry. Hematocrit was determined in clinical microhematocrit tubes. Serum and urine sodium and potassium determinations were done by flame photometry. Osmolality determinations were done by freezing-point depression. Clearances of inulin, PAH, and osmolality and excretion rates of sodium and potassium were calculated for each kidney by a standard formula: $T_c H_2O = C_{osm} - \text{urea flow rate}$. Clearances of inulin were expressed per gram of kidney weight. Clearances of osmoles, sodium, and potassium and tubular reabsorption of water were expressed as fractions of glomerular filtration rate.

Results

COMPARISON OF RENAL FUNCTION IN AWAKE CAVAL AND NORMAL DOGS

The caval dogs all gained fluid weight from the time of surgery to the time of study (16.2 ± 0.8 to 18.3 ± 1.0 kg). The weight of the normal dogs did not increase. Although serum protein concentration was not measured at the time of surgery, it is unlikely that it was initially different in the two groups. At the time of the study, the plasma protein concentration was significantly lower in the caval dogs than it was in the normal dogs (3.7 ± 0.2 vs. 5.2 ± 0.1 g/100 ml, $t = 7.27, P < 0.001$). There was no difference in mean blood pressure (120 ± 4 vs. 114 ± 7 mm Hg, normal vs. caval). The glomerular filtration rate (GFR) may have been lower than normal in the caval dogs, but the difference did not achieve statistical significance (Table 1). However, the estimated renal blood flow ($C_{pAH}/(1 - Hct)$ g$^{-1}$ kidney weight) was 29% lower on the average in the caval dog; this difference was highly significant (Table 1). Not only was the sodium excretion rate ($U_{Na}V$) lower, but also the proportion of filtered sodium which was excreted ($C_{Na}/GFR$) was decreased (Table 1). The excretion rate of potassium ($U_{K}V$) was slightly higher; however, the fractional excretion of potassium ($C_{K}/GFR$) was the same (Table 1). Under the influence of vasopressin and during a mild sodium diuresis, the tubular reabsorption of sodium was lower in the caval dogs than it was in the normal dogs. This somewhat surprising finding is not explained by a difference in clearance of osmoles ($C_{osm}$). Although the relationship between $T_c H_2O$ and $C_{osm}$ was not examined in detail, $C_{osm}$ ranged from 2 to 14 ml/min. At all levels, $T_c H_2O$ was generally lower, and actual free water excretion was noted from one kidney from each of four caval dogs.

COMPARISON OF MICROSPHERE DISTRIBUTION

In spite of the difference in renal function, no trends or significant differences were noted in the intracortical distribution of radioactive microspheres in normal dogs compared with caval dogs (Table 1, Fig. 1).

### TABLE 1

<table>
<thead>
<tr>
<th>Zone of kidney</th>
<th>Normal</th>
<th>Caval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (%)</td>
<td>31 ± 1</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>2 (%)</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>3 (%)</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>4 (%)</td>
<td>22 ± 1</td>
<td>24 ± 3</td>
</tr>
</tbody>
</table>

Both groups had received 1 liter of 0.9% NaCl daily since surgery. The weight of the normal dogs did not change, but the caval dogs showed a significant gain averaging 2.1 kg. They all developed ascites, and their plasma protein concentrations were significantly lower than they were in normal dogs (3.7 g/100 ml vs. 5.2 g/100 ml). GFR = glomerular filtration rate, $C_{pAH}$ = clearance of para-aminobiphenyl, $Hct =$ hematocrit, $FF =$ filtration fraction, $U_{Na}V =$ urinary sodium excretion rate, $C_{Na} =$ sodium clearance, $U_{K}V =$ urinary potassium excretion rate, $C_{K} =$ potassium clearance, $C_{osm} =$ clearance of osmoles, and $T_c H_2O =$ tubular reabsorption of water.

* $P < 0.001$.

† $P < 0.05$.

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NINE DOGS WERE SPECIFICALLY STUDIED FOR EVALUATION OF THE REPRODUCIBILITY OF THE MICROSPHERE DISTRIBUTION. THE DIFFERENCE BETWEEN THE DUPLICATE VALUES OF FRACTIONAL ZONE DISTRIBUTION IN THE TEN KIDNEYS (FIVE DOGS) FROM CAVAL DOGS WAS SIGNIFICANTLY GREATER THAN THE DIFFERENCE OF DUPLICATE VALUES IN THE NORMAL DOGS (7.3 ± 5.6 vs. 2.5 ± 1.8, t = 2.237, P < 0.05). THIS INCREASED VARIABILITY OF DATA WAS REFLECTED IN ALL FOUR ZONES. NO TREND IN DIRECTION WAS NOTED IN DATA FROM THE FIRST TO THE SECOND INJECTION OF MICROSHERES. THE DATA WERE CALCULATED WITHOUT REGARD TO SIGN BECAUSE NO CONSISTENT DIRECTIONAL CHANGE WAS EXPECTED OR OBSERVED. HOWEVER, THE DISPERSAL OF THE DATA (sd) AROUND THE MEAN DIFFERENCE WAS ALSO GREATER IN THE CAVAL DOGS, SUGGESTING THAT THE TWO POPULATIONS WERE DIFFERENT. FIGURE 2 GRAPHICALLY REPRESENTS THE RELATIVELY POOR REPRODUCIBILITY IN THE CAVAL DOGS.

FIGURE 2

Reproducibility of fractional distribution of microspheres to the kidneys of normal and caval dogs showing higher variability of results in the caval dogs.

REPRODUCIBILITY OF MICROSPHERE DISTRIBUTION

Nine dogs were specifically studied for evaluation of the reproducibility of the microsphere distribution. The difference between the duplicate values of fractional zone distribution in the ten kidneys (five dogs) from caval dogs was significantly greater than the difference of duplicate values in the normal dogs (7.3 ± 5.6 vs. 2.5 ± 1.8, t = 2.237, P < 0.05). This increased variability of data was reflected in all four zones. No trend in direction was noted in data from the first to the second injection of microspheres. The data were calculated without regard to sign because no consistent directional change was expected or observed. Moreover, the dispersal of the data (sd) around the mean difference was also greater in the caval dogs, suggesting that the two populations were different. Figure 2 graphically represents the relatively poor reproducibility in the caval dogs.

Discussion

This study was designed to provide further information on the relationship of intracortical blood flow distribution to chronic extracellular volume expansion and sodium retention. The chronic caval dog of Davis and Howell (25) shares several important features of sodium-retaining diseases of man. This preparation has a well-maintained glomerular filtration rate, a modest reduction of renal plasma flow, and an expanded extracellular volume. Thus the mechanism of sodium retention may be different in the acute caval dog and the dog in hemorrhagic shock in which glomerular filtration rate is low and extracellular volume is not expanded. Two previous studies of the intracortical blood distribution of chronically sodium-retaining dogs (26, 29) share the possible dual disadvantages of anesthesia and renal artery catheterization. Anesthesia may blunt the response of total renal blood flow to other stimuli (30). This blunting alone may explain why we found that chronic caval dogs had reduced PAH clearance whereas Kaloyanides et al. could not (31). The presence of an indwelling arterial catheter may influence the function and the flow of the kidney under study. Newsome et al. (26) have found a reduced glomerular filtration rate of the cannulated kidneys of caval dogs. Mowat et al. (32) and we (33) have seen renal blood flow instability in dogs during inert gas washout studies. Although such large changes of blood flow are undoubtedly not the experience of every laboratory doing gas washout studies, more subtle changes may occur.

The present study used unanesthetized normal and caval dogs without a renal artery catheter. The dogs without caval ligation did not gain weight and therefore probably did not experience an expansion of extracellular volume. In contrast, the caval dogs did gain weight; they developed ascites, their serum protein concentration was diluted, and their extracellular volume was expanded. Even during the study, these caval dogs were actively retaining administered sodium. This retention resulted from an increased fractional reabsorption of sodium and perhaps from a slight reduction in glomerular filtration rate, although the difference was not statistically significant. The apparent reduction of T₃H₂O in the caval dogs is not readily explainable but has been previously reported in caval dogs during osmotic diuresis (34).
In these dogs and under these conditions, we observed no consistent alteration of microsphere distribution within the glomerular cortex. In the presence of the observed marked reduction of estimated renal blood flow, \((C_{PAH}/[1-Hct])\) in caval dogs, we have interpreted this finding as indicating a uniform reduction of renal blood flow from surface to juxtamedullary cortex in caval dogs. Other possibilities exist. The reduced clearance of PAH could indicate reduced tubular secretion of organic acids or a change in the distribution of blood favoring the juxtamedullary cortex. The first possibility cannot be ruled out but there are no previous data or suggestions for it. The second possibility seems unlikely in view of the lack of altered distribution of microspheres. Although there appears to be no consistent variation of the pattern of blood flow to the glomerular cortex in caval dogs, the examination of the reproducibility of duplicate observations suggests that there is less consistent flow distribution in these dogs than in normal dogs. This instability of blood flow is perhaps analogous to that described in cirrhotic man by Epstein et al. (8) and may account for the difference in results between the two previous studies of intrarenal blood flow in caval dogs (10, 26).

We therefore conclude that in chronic unanesthetized caval dogs something other than redistribution of intracortical renal blood flow distribution accounts for the reduction of sodium excretion and the enhancement of renal tubular sodium reabsorption. Under certain circumstances and perhaps in man such changes occur. The findings of a reduced proportion of the first component of the xenon washout curve in sodium-deprived man (3) and in cirrhotic man (7–8) may indicate such a relationship. That interpretation is however uncertain because of the difficulty in reproducing the results of xenon washout with studies by microspheres (13). Nevertheless, one must acknowledge the support for this concept from micropuncture studies of rats which indicate that the outer cortical nephron filtration rate is more responsive to changes in sodium intake than is whole kidney glomerular filtration rate (35, 36). It is also conceivable that more complex alterations of distribution of flow are partially responsible for modulation of sodium excretion and that these alterations would be reflected by changes in inert gas washout pattern and not by simple analysis of changes in microspheres within arbitrary layers of the cortex (37). Finally, it must be noted that in these dogs a reduction of total renal blood flow in the absence of a significant alteration of glomerular filtration rate may alter peritubular capillary blood protein concentration and thus oncotic pressure and peritubular hydrostatic pressure so as to favor increased uptake of tubular fluid. Thus, these data are consistent with recent theories of modulation of sodium excretion (38).

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