Measurement of intrarenal anatomic distribution of krypton-85 in endotoxic shock in dogs

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RENAL BLOOD FLOW DISTRIBUTION has been calculated by means of the disappearance rate and the volume of distribution of radioactive gas. As described by Thorburn et al. (16), 85Kr (dissolved in saline) is injected into the renal artery. A scintillation counter detects the rate of decreasing radioactivity in the kidney. Cortical, juxtamedullary, and medullary flow rates have been calculated (1, 12, 16) by a subtractive process, which separates the logarithmic components of the multiexponential washout curve.

Several investigators have reported renal blood flow compartments somewhat different than those measured by 85Kr washout (5, 14, 15). Slotkoff et al. (14) reported difficulty in relating the various components of the 85Kr washout curve to specific intracortical areas as measured with radioactive microspheres. Mowat et al. (10) suggested that graphical or mathematical separation of the washout curves into components does not necessarily reflect natural flow dynamics.

However, other investigators have used 85Kr during various experimental conditions. Carriere et al. (1) reported decreased outer cortical blood flow but relatively stable inner cortical and outer medullary flow during prolonged hemorrhagic hypotension in the dog. Passmore and Baker (12) confirmed this observation, but indicated a return of outer cortical flow during the late irreversible stages of hemorrhagic shock when the vascular tone deteriorates.

Since renal hypofuction is common in all forms of shock, one purpose of this study was to determine if renal blood flow redistribution is also encountered in endotoxic shock. Immediately after intravenous administration of a lethal dose of endotoxin, there is a marked decrease in cardiac output and blood pressure, caused by pooling of blood primarily in the portal venous system (17). After the initial decline, blood pressure usually increases again, sometimes almost to control values, but eventually declines, terminally resulting in death. Release of adrenal catecholamines may be partially responsible for the secondary increase in blood pressure (11).

Injected endotoxin also results in an immediate decrease in glomerular filtration rate, renal plasma flow, and urine flow (3). Hinshaw et al. (3) also noted renal ischemia as a prominent feature of acute oliguric renal failure consequent to endotoxic shock. By using angiographic and histologic studies of kidneys from dogs that had died in endotoxic shock, Kawarada et al. (6) reported that renal cortical ischemia may be somewhat ameliorated by administration of steroids or phenoxybenzamine.

We have devised a method, utilizing the endotoxic shock model, to examine the anatomic distribution of radiokrypton compartmental washout. Our work demonstrates the 85Kr washout from certain renal vascular beds by the direct measurement of the washout for each anatomically unique area of the kidney. This method avoids mathematical fractionation of a washout curve into its exponential components. Evidence is presented for renal blood flow redistribution in endotoxic shock.

METHODS

Animal preparation. Acute experiments were performed on two groups of mongrel dogs of both sexes weighing 10–26 kg. Short-term anesthesia during intubation was provided by intravenous Pentothal Sodium, and anesthesia was maintained during the experiments.
ANATOMIC DISTRIBUTION OF KRYPTON-85 IN ENDOTOXEMIA

by Metofane (methoxyflurane) gas to a level at which the blink reflex was abolished. Both Metofane and oxygen were delivered to the intubated dogs via an inhalation anesthesia apparatus. The dogs were fasted 12-24 h but given water ad libitum before the experiment.

A left flank incision was made inferior to the rib cage to expose the perirenal area. A curved 25-gauge needle (attached to a polyethylene catheter) was inserted into the lumen of the renal artery for $^{85}$Kr injection. Strings were put in place to tie the renal artery and vein and surrounding renal fat. After surgery, the dogs were allowed to stabilize for at least 10 min. The wound was closed to prevent drying of the kidney. Approximately 200 $\mu$Ci (0.2 ml) $^{85}$Kr/10 lb dog wt (dissolved in saline) were injected via the renal arterial catheter and the injection was followed by a saline flush. Injection and flush were timed and consistently took 5 s. The renal artery, vein, and fat pads were tied and the kidneys excised at 10, 25, 45, 65, 95, and 125 s after the beginning of the injection (time zero). Each kidney was then placed in a freezing bath of acetone and Dry Ice for at least 10 min.

Samples of isotope were counted on a regular basis to determine the exact injected dose in counts per minute. At the end of each experiment, the kidney was weighed in order to determine the exact dose of isotope injected per gram of kidney tissue.

**Tissue preparation.** Each frozen kidney was cut midhorizontally with a band saw to produce a fan-shaped slab of tissue (Fig. 1). Each slab was cut into basic anatomical regions. Tissue $A_1$ is the outer cortex, tissue $A_2$ is the inner cortex plus outer stripe of the outer zone of medulla (Fig. 1). The tissue pieces were kept frozen by being dipped frequently into the Dry Ice and acetone solution, and were weighed on a Rolfer-Smith balance, placed in a test tube on ice, and covered with melted paraffin to prevent isotope escape from the tissue. The paraffin hardened immediately and from that time the radioactivity levels of the samples were very stable, as was shown when the tissue pieces were counted several times over a 1-day period. The samples were counted for gamma emission in a Nuclear-Chicago automatic gamma counter.

Initially, to determine whether $^{85}$Kr was lost from tissue during the paraffin-imbedding procedure, some pieces of kidney tissue were immediately placed in test tubes containing Dry Ice and acetone and counted. Then the still-frozen piece of kidney tissue was removed from the acetone and Dry Ice and covered with melted paraffin, as described above, and recounted. No significant loss of radioactivity was attributable to the paraffin-imbedding process.

The specific activity for each piece of renal tissue was graphed on the ordinate of a semilogarithmic scale with time (after $^{85}$Kr injection) on the abscissa. A line was statistically fitted to the data for each described tissue type. The equation $y = Ae^{bx}$ was found to be the best equation representing this line. Several other equations were tried but the high correlation coefficient of this equation made it the preferred choice. This is the standard equation used to describe the washout of radioactive material from a vascular bed (7, 16). A Pearson $r$ coefficient was used to determine the correlation between $\ln y$ and $x$. $P$ is a test of whether or not the slope is different from 0 ($b \neq 0$). The slope of that line was considered to be proportional to the $^{85}$Kr washout rate from that tissue type. The half time of the radioactivity loss was calculated from that line and used to calculate nutrient flow in each type of renal tissue.

The flow in any one component as determined by the slope of the line representing that component can be calculated as: flow (ml/min per 100 g) $= 0.693 \times 100/t_{1/2}$, where $t_{1/2}$ is the length of time required for the radioactivity in the component to decrease by half its original value, and 0.693 is $\log_2$ of 2. The points at which each of the components intersected with the ordinate was taken to be the $y$-intercept value and was used to calculate the quantity of radioactivity initially distributed to each of the component tissue types. The derivation of these relationships has been published previously (16).

Diagram

**Endotoxic shock.** The above experiment was also performed on dogs in endotoxic shock. The dogs were injected intravenously with a lethal dose (3 mg/kg) of E. coli Bacto lipopolysaccharide B (Difco Laboratories). Approximately 30 min later, when blood pressure recovery seemed maximum, $^{85}$Kr was injected into the renal artery and the kidneys were excised as in the control group. Kidneys were excised at 15, 35, 65, and 95 s after zero time. Otherwise the experimental procedures were as described for the control group.

**RESULTS**

The calculated flow in the outer cortical tissue ($A_1$) was 462 ml/min per 100 g in the control study but only 122 ml/min per 100 g during shock ($P < .001$) (Fig. 2). The amount of radioactivity initially distributed to $A_1$ tissue (y intercept) in the control group was significantly greater (5.1 counts/min per mg) than that of the shock group (3.54 counts/min per mg) ($P < .001$). Flow in inner cortical ($A_2$) tissue (Fig. 3) was also significantly less in the shock animals (166 ml/min per 100 g) than in the controls (396 ml/min per 100 g) ($P <
However, the initial radioactivity distributed to this tissue was essentially the same in both groups (4.37 vs. 4.76 counts/min per mg in controls).

Flow in the inner stripe of the outer zone of medullary tissue was essentially the same in both groups (130 and 134 ml/min per 100 g). However, the quantity of radioactivity initially distributed to this tissue was significantly greater in the shock group (2.196 vs. 1.26 counts/min per mg in controls) (P < .001) (Fig. 4).

**DISCUSSION**

The present study was undertaken to compare renal blood flow distribution during control conditions with that found during endotoxic shock and to measure 85Kr washout from specific renal tissue zones. These objectives were accomplished by the dissection of kidney anatomy and the determination of radioactivity for each specific tissue zone at specific time intervals after 85Kr injection. In control experiments, Thorburn et al. (16) used an external scintillation detector and subtractive methods to separate four renal washout compartments. They reported flow rates of 472 and 132 ml/min per 100 g of tissue for components I and II, respectively. In previous trials in our laboratory, we found in a series of eight dogs that when they were determined by external scintillation counting and the subtractive process, components I and II appeared to be fused approximately 30 min after endotoxin injection. This single component had a flow rate of 182 ± 33 (± SE) ml/min per 100 g as compared to the two components of 475 ± 45 and 81 ± 8 ml/min per 100 g that this group of eight dogs had during control conditions. It seemed that the single fused component (182 ± 33 ml/min per 100 g) might represent flow in a large proportion of the kidney, as was reported in previous studies of hemorrhage. Carriere et al. (1), Passmore and Baker (12), and Lachance et al. (8) have reported component fusion as determined by external scintillation counting methods for dogs in hemorrhagic shock. In the present study, which reveals more information about flow in specific renal tissue zones than was previously known, we found flows of 122, 166, and 134 ml/min per 100 g in zones A₁, A₂, and B, respectively. Although direct comparison may be difficult, it seems that these three flow rates would have been inseparable in previous washout studies and that they represent essentially a single rate of washout from a large proportion of the kidney.

**Tissue A₁** is outer cortex and includes the following vessels: interlobular arteries, glomerular tufts, afferent arterioles, efferent arterioles, and peritubular capillaries, and this tissue is recognized grossly because of its granular appearance (Fig. 1). **Tissue A₂** consists of inner cortex and the medullary rays, which belong to the outer stripe of the outer zone of the medulla. Vascular elements include the peritubular capillaries, efferent
anatomic distribution of krypton-85 in endotoxemia

endotoxic shock
slope = -0.0188 cpm/mg/sec
flow = 134 ml/min/100 g
r = -0.89
p < .001
intercept = 2.196 cpm/mg

control
slope = -0.024 cpm/mg/sec
flow = 130 ml/min/100 g
r = -0.89
p < .001
intercept = 1.26 cpm/mg

FIG. 4. Radioactivity levels for tissue B (inner stripe of outer zone of medulla) from control dogs and dogs in endotoxic shock as determined by tissue sampling. Each symbol represents an individual arterioles, arcuate arteries and veins, and a small population of glomerular tufts and afferent arterioles. Anatomically, this area is between the outer cortex and the bright red of the inner stripe of the outer zone of the medulla. The efferent arteriole of the juxtamedullary glomeruli provides the vasculature of the medulla.

In the control group, when the flow in tissue A1 was compared to that of tissue A2 it was found that the slopes of the two lines were not significantly different. Slotkoff et al. (14) compared intracortical distribution of radioactive microspheres to inert gas washout. They reported that component I of the washout probably represented total cortical flow. Our studies support that possibility, but components A1 and A2 did have significantly different y intercepts (P < .05), which indicates that there may have been more radioactivity initially distributed to the outer cortex. Slotkoff et al. (14) and Stein et al. (15) used radioactive microspheres to study the intracortical distribution of preglomerular blood flow and also found more radioactivity distributed to the outer cortex than to the inner during control conditions.

In our endotoxin-injected group, the intercept for component A1 was significantly smaller than in the control group (P < .001), whereas the intercepts for component A2 were the same in both groups. Therefore, it seems that a relatively greater fraction of the cortical blood flow perfused the inner cortex during shock. This agrees with the hemorrhage findings of Slotkoff et al. (14) and Stein et al. (15). Our initial distribution data for components A1 and A2 agree with that reported from microsphere studies, as was expected, since injected microspheres would measure the initial distribution of cortical blood flow. Our data reveal marked reduction of flow in the outer cortex and, therefore, indicate a blood flow redistribution during shock similar to that reported by Carriere et al. (1) and Passmore and Baker (12). In the present study it is impossible to specify the cause of this redistribution. However, hypotension, vasoconstriction, or chemical mediators may play a role.

Blood flow through the inner stripe of the outer zone of the medulla (tissue B) was not significantly different between the control group and the shock group in spite of the expected vasoconstriction and hypotension of the endotoxic shock. The absence of difference suggests that the control of blood flow in this area is different from that in the cortex. The flow rate in our B component is very similar to the values reported for flow in this area of the kidney in hemorrhage studies by other investigators (1, 8, 12). The significantly greater value for the intercept in the endotoxin group indicates that more radioactivity was initially distributed to this vascular bed (tissue B) and, therefore, it may actually increase in size during shock. This finding also agrees with the previous studies (1, 8, 12). Furthermore, the flow rates for tissues A1, A2, and B were all quite similar, and, therefore, could explain the fusion of components I and II during shock reported in those previous studies (1, 8, 12). Our technique provided better delineation between cortical and medullary zones than autoradiography, which probably explains why the flow rate of inner
stripe outer zone tissue was specifically limited to that area and did not include the inner cortex, as was previously reported (1, 8, 16), from autoradiographic studies.

Apparently the $^{85}$Kr that entered the inner stripe of the outer zone of the medulla (B) saturated the tissue rapidly, since radioactivity levels in this region reached a peak 5 s after injection. This finding is somewhat unusual because some deterrent to isotope penetration by the countercurrent mechanism of the vasa recta (9) would be expected to keep the $^{85}$Kr out of this region of the kidney for a longer period of time.

Our studies indicate that the flow in the inner stripe of the outer medullary zone is controlled separately from that of the cortex. The distinctive nature of the blood vessels in this area has previously been described (2, 4, 13), and it seems possible that there could be a certain specific flow rate for this area that would be quite different from that for the inner cortex. Jones and Herd (4) have done some excellent studies using serial autoradiographs obtained by freezing and slicing kidneys at various intervals after $^{85}$Kr injection. They found the initial distribution of $^{85}$Kr to be similar to that reported in previous studies (16). The disappearance of radioactivity from the cortex was as rapid as would be expected when the rate of blood flow through the area is considered. Their studies also revealed a set of peritubular capillaries in the outer layer of the medulla that rises directly from the juxtamedullary efferent arterioles. The flow through these capillaries could be separate from and not too susceptible to the countercurrent exchange of the true vasa recta.

The present technique seems to be theoretically sound and gives more information about the intrarenal circulation than either the inert gas washout or microsphere technique, but requires a large series of animals. We have reported individual flow rates from specific renal tissue zones during control conditions and in a group of dogs subjected to endotoxic shock. In addition, we are reporting significant redistribution of intrarenal blood flow during endotoxic shock in dogs.

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