Countercurrent Diffusion in the Renal Cortex of the Rabbit

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SUMMARY. Evidence was obtained for countercurrent diffusion of 14C-n-butanol in the rabbit renal cortex. After injection into the renal artery of perfused, isolated rabbit kidneys, concentrations of both 14C-butanol and tritiated water in the initial samples collected from the renal venous outflow exceeded those of 125I-albumin when the rate of perfusion was low. When flow was increased, the 125I-albumin curve was shifted to earlier times relative to the other labels. These observations confirm the existence of arteriovenous diffusion of 14C-butanol and tritiated water in the kidney. In a second set of experiments, 14C-butanol and tritiated water were infused for 10 or 60 seconds into the renal arteries of anesthetized rabbits, and the kidneys were then removed and frozen in liquid nitrogen. Cores were cut from the kidney surface and sectioned in a cryostat, and the ratio of 14C-butanol to tritiated water was calculated at increasing depths in the cortex. This ratio rose from 0.54 ± 0.03 (SEM) at the surface to 0.98 ± 0.07 at 3 mm beneath the surface in kidneys perfused for 10 seconds. This gradient was less steep after 60 seconds of perfusion. The early appearance of 14C-butanol relative to tritiated water in the renal venous outflow and delayed equilibration of 14C-butanol in the outer renal cortical tissue are consistent with counter-current diffusion. It is suggested that this exchange may occur between adjoining interlobular arteries and veins in the cortex and may contribute to high carbon dioxide tensions found near the renal surface.

ARTERIOVENOUS diffusion of oxygen in the kidney was first reported 25 years ago by Levy and Sauceda (1959). Since then, renal arteriovenous diffusion has been found for hydrogen gas (Aukland et al., 1963; Aukland and Berliner, 1964), tritium gas, krypton gas, deuterated water (Perl and Chinard, 1968), alcohols (Chinard et al., 1968), heat (Aukland, 1967; Roed and Aukland, 1969), and carbon dioxide (Effros and Nioka, 1983). The site of solute and gas exchange has not been defined, but recent observations of high CO2 tensions in structures near the surface of the kidney have led to the suggestion that there may be countercurrent diffusion of CO2 produced in the cortex from interlobular veins into adjoining interlobular arteries (Dubose et al., 1978, 1979; Sohtell and Karlmark, 1976; Gennari et al., 1982; Maddox and Gennari, 1983). Unfortunately, the interpretation of carbon dioxide tensions within the kidney is complicated by uncertainties concerning local pH, metabolic CO2 production, and formation of CO2 from HCO3~ by acid secretion in the tubules. Use of an alternative indicator which would not be influenced by those variables is therefore desirable. In the present study, we have investigated the transit of 14C-butanol through the rabbit kidney and its distribution in the cortex and found that this indicator does seem to be subject to countercurrent exchange in this tissue.

Methods

Transit Time Studies

Three sets of experiments were conducted in this study. In the first set, the renal venous outflow patterns of 125I-albumin, 14C-butanol, and 3H2O were compared, following injection into perfused rabbit kidneys. Five albino New Zealand rabbits weighing 2.9 ± 0.6 (SD) kg were anesthetized with 15 ml of a 10 mg/ml solution of pentobarbital in isotonic saline, and a kidney was removed from each and placed on a moist dish maintained at 37°C. Catheters were placed in the renal artery and vein, and the blood was flushed from the kidney with a pH 7.4, 37°C red cell-free solution containing 50 g albumin, 2.1 g (30 meq/liter) NaHCO3; 0.2 g MgSO4; 1.5 g glucose, and 5000 U heparin in each liter of Ringer’s lactate solution. The kidney then was perfused at between 0.4 and 6.0 ml/min with this perfusate. Next, 0.1 ml of the perfusate containing 7 μCi 125I-albumin [labeled by the procedure of McConahey and Dixon (1966)], 12 μCi 14C-n-butanol (6.44 mCi/mmol), and 12 μCi 3H2O (New England Nuclear) was rapidly injected into the renal arterial catheter and serial samples of the venous outflow were collected at between 0.4-second and 2.2-second intervals. The catheter volume between the point of injection and the renal artery was 0.05 ml. The corresponding volume between the renal vein and collector tubes was 0.9 ml. Outflow samples (0.2 ml) were placed in 10 ml of scintillation fluid (ACS, Amersham) and counted in automated β- and γ-counters. Isotope counts were corrected for cross-over and back-ground. Outflow concentrations of each indicator were divided by the amounts injected to yield ‘fractional concentrations’ which were then plotted against time (see Fig.
1). The initial areas under the curves were calculated up to and including the time at which the 125I-albumin curve reached peak values. The initial areas of the 14C-butanol and 3H2O curves were divided by those of 125I-albumin to yield "upslope ratios" plotted against flow rate (see Fig. 2).

In Vivo Tissue Distribution Studies

In the second set of experiments, kidneys were exposed at laparotomy in 10 rabbits anesthetized as described above. A thin wall 25-gauge needle was placed in the renal artery of one kidney, and 14C-butanol and 3H2O in perfusate (see above) were infused into the renal arterial blood flow for either 10 seconds (five rabbits) or 60 seconds (five rabbits). Renal blood flow was determined with an electromagnetic flow meter (Narco Bio-Systems, Inc.) and averaged 20 ± 5.4 ml/min (s.d.), and arterial blood pressure averaged 97/63. The kidneys then were excised rapidly and mounted in a plastic jar containing an embedding solution of resins and polyols (OCT, Miles Lab.) which was then immersed in liquid nitrogen. The frozen kidneys were placed in a cryostat at −20°C, and 1-cm diameter cores were cut from the surface with a cork borer. The cores were resealed with more OCT and sliced at 40-μm intervals to a depth of 3 mm. Microscopic examination indicated that these sections were derived exclusively from the cortex. Consecutive groups of five slices were placed together in glass scintillation vials and digested overnight at 50°C in 0.3 ml of water and 1 ml of a mixture of quaternary amines (Protosol, New England Nuclear). Ten milliliters of ACS scintillation fluid were added to each vial, and the samples were counted in an automated ^-counter. Where necessary, nonradioactive tissue slices were added to the vials to ensure equal quenching, as determined by external quench monitoring, and indicator concentrations were divided by the concentration in the infusate.

Although recirculation after 10 seconds of infusion was minimal, concentrations of 14C-butanol in the initial samples of perfusate collected from the renal vein exceeded those of 3H2O, which, in turn, exceeded those of 125I-albumin (Fig. 1). As flow was increased, the 125I-albumin curve shifted toward relatively earlier times, and the initial concentrations of 125I-albumin now exceed those of the diffusible indicators.

Tissue Equilibration Studies

In the third set of experiments, five kidneys were immersed in 10 ml of the perfusate used in the infusion studies containing 14C-butanol and 3H2O. The solution was pumped over the surface of the kidneys for 120 minutes to encourage cortical equilibration with the indicators. Thereafter, they were frozen, cored, sliced, and processed as described above.

Binding of 14C-butanol to albumin was determined by placing 4.0 ml of the 5 g/dl albumin solution used in the transit time studies into a regenerated cellulose dialysis bag. The bag then was bathed in a tube with 10 ml of a solution containing all of the same ingredients except albumin. 14C-Butanol was placed in the outer solution, and the distribution of 14C-butanol between the two solutions was determined after 15 hours of mixing at 37°C. A value of 14% was calculated for binding to albumin under these conditions.

Results

Transit Time Studies

At low rates of perfusion, concentrations of 14C-butanol in the initial samples of perfusate collected from the renal vein exceeded those of 3H2O, which, in turn, exceeded those of 125I-albumin (Fig. 1). As flow was increased, the 125I-albumin curve shifted to a relatively earlier time compared to both 14C-butanol and 3H2O. These observations are summarized in Figure 2 in which the upslope ratios of 14C-butanol and 3H2O are plotted against the rate of perfusion. The upslope ratios of both of the diffu-
FIGURE 2. The changes in the relative initial positions of $^{125}$I-albumin and the diffusible indicator curves are summarized for five experiments. Perfusion rates in ml/min per gram of kidney tissue were increased or decreased, and values of the upslope ratios (see report) were calculated. Upslope values of $^{14}$C-butanol exceeded those of $^3$H$_2$O, and both sets of ratios decreased with flow (means and standard errors are shown, $P < 0.01$ ANOVA, Tukey tests).

Tissue Distribution Studies

Following a 10-second infusion of $^{14}$C-butanol and $^3$H$_2$O, concentrations of the former relative to the latter indicator were distinctly less in the outer portion of cortical tissue (Fig. 3). The ratio of $^{14}$C-butanol to $^3$H$_2$O rose from $0.55 \pm 0.03$ (SEM) to $0.98 \pm 0.07$ at a depth of 2.8 to 3.0 mm. This delay in the delivery of $^{14}$C-butanol to the outer cortex was less apparent after 60 seconds of infusion. The ratio of $^{14}$C-butanol to $^3$H$_2$O rose from $0.65 \pm 0.06$ to $0.86 \pm 0.04$ over the same distance. The linear regression equations at 10 and 60 seconds were

$$\frac{^{14}C}{^3H}_{10\text{seconds}} = 0.147 \text{ (depth)} + 0.548, \quad r = 0.987$$
$$\frac{^{14}C}{^3H}_{60\text{seconds}} = 0.074 \text{ (depth)} + 0.692, \quad r = 0.875.$$ 

The slopes of these lines were significantly different at the $P < 0.01$ confidence level.

Tissue Equilibration Studies

After 2 hours of in vitro exposure to $^{14}$C-butanol and $^3$H$_2$O, concentrations of the former indicator exceeded those of the latter by 10% in the outer cortex (Fig. 3). No significant trend in relative concentrations of these indicators with tissue depth was observed. The regression line shown in Figure 3 is

$$\frac{^{14}C}{^3H}_{120\text{minutes}} = 0.017 \text{ (depth)} + 1.052, \quad r = 0.39.$$ 

Discussion

The indicator transient studies of the perfused kidneys described above lend new support to the concept that $^{14}$C-butanol and $^3$H$_2$O can diffuse directly from renal arterial to venous vessels. At low perfusion rates, early venous concentrations of these diffusible indicators actually exceeded those of $^{125}$I-albumin, suggesting that they had left the arterial vessels and reached nearby venous sinks. As flow was increased, the $^{125}$I-albumin curves moved to earlier times relative to those of $^{14}$C-butanol. This shift reflects the fact that the time required for transport of the diffusible indicators through the kidney is influenced less by intravascular transport than that required by $^{125}$I-albumin, which is presumably transported from arteries to veins within the renal vasculature.

The relative positions of the $^{125}$I-albumin and diffusible indicators can be expressed in terms of the upslope ratios of the areas under the $^{14}$C-butanol and $^3$H$_2$O curves to that of $^{125}$I-albumin curve. These areas are calculated from the appearance times to the peak of the $^{125}$I-albumin curves. Increases in the flow resulted in a relatively more rapid delivery of $^{125}$I-albumin in the renal vein, and upslope ratios declined, as indicated in Figure 2. It should be emphasized that, had the rate of diffusion of butanol and $^3$H$_2$O out of the vessels been limited by endothelial permeability or red cell or plasma binding, the upslope ratios would have increased at higher flows. Shortening of transit times in the exchange vessels would have reduced the opportunity for diffusion out of the renal vessels (Crone, 1963; Bassingthwaithe, 1974).

Although $^{14}$C-butanol reached the renal veins more rapidly than $^3$H$_2$O, equilibration of $^{14}$C-butanol with the outer cortex with constant infusions...
of these indicators was slower for $^{14}$C-butanol than $^{3}$H$_2$O. These two observations suggest that there was more exchange of $^{14}$C-butanol than $^{3}$H$_2$O between adjoining arterial and venous vessels radiating to the surface of the renal cortex. As expected, when the infusion was prolonged from 10 seconds to 60 seconds, equilibration of $^{14}$C-butanol relative to $^{3}$H$_2$O became more complete. With the passage of time, concentrations of $^{14}$C-butanol presumably became more uniform along the arterial and venous vessels in the renal cortex. By 60 seconds, aortic concentrations of both indicators had increased to approximately one-fourth of those calculated in the renal arterial blood. This increase was due to recirculation, and presumably contributed somewhat to the disequilibrium still found at 60 seconds, since the increasing arterial concentration $^{14}$C-butanol would tend to equilibrate more rapidly with the deeper portions of the cortex. In other words, had recirculation been avoidable, the difference between the 10- and 60-second infusion studies would have been even greater. To be sure that the differences in $^{14}$C-butanol relative to $^{3}$H$_2$O found at different depths in the cortex were not due to differences in tissue solubility, kidneys were superfused for 120 minutes with these indicators, and tissue concentrations were calculated. As indicated in Figure 3, the $^{14}$C:$^{3}$H ratio became relatively uniform, and $^{14}$C-butanol concentrations exceeded those of $^{3}$H$_2$O by about 10%. The decreasing ratios of $^{14}$C-butanol to $^{3}$H$_2$O found in the more superficial slices of cortex after 10 or 60 seconds of perfusion indicate that countercurrent diffusion of $^{14}$C-butanol has occurred in the renal cortex. It is quite likely that countercurrent diffusion of $^{14}$C-butanol also occurs in the medulla, but flow is more rapid in the cortex, and a greater fraction of the label is delivered to the cortex than to the medullary tissues. It is therefore likely that cortical countercurrent diffusion contributes in a significant manner to the early appearance of $^{14}$C-butanol observed in the isolated kidneys perfused at low flow rates.

The solubility of $^{14}$C-butanol in lipid membranes presumably is responsible for higher tissue concentrations than $^{3}$H$_2$O at 120 minutes. Chinard et al. (1969) found that the olive oil-to-water distribution coefficient of $^{14}$C-butanol is 368 times as great as that of $^{3}$H$_2$O. The ability of $^{14}$C-butanol to penetrate tissues more rapidly than $^{3}$H$_2$O has also been observed in a variety of other tissues (Overton, 1899; Collander, 1954; Wright and Diamond, 1969; Effros, et al., in press) and presumably is related to its solubility in lipids of the cellular membranes. Tissue concentrations of $^{14}$C-butanol relative to $^{3}$H$_2$O in the kidney slices presumably would have been even greater were it not for the fact that 20% more butanol distributes into red cells than in plasma (Chinard et al., 1969), and 14% of the plasma butanol is bound to protein (see Methods).

Although $^{3}$H$_2$O equilibrates more rapidly with the more superficial cortex than $^{14}$C-butanol, some countercurrent exchange of $^{3}$H$_2$O is not unlikely, since diffusion bypass of this indicator is also evident in the transient experiments. Differences in the arrival of iodoantipyrine, tritiated water, and microspheres in the renal cortex were related to a variety of factors, including countercurrent exchange, by Clausen et al. (1979). However, in preliminary experiments, we found that this indicator emerged from the renal vein more slowly than $^{3}$H$_2$O, making it less suitable than $^{14}$C-butanol for these experiments.

Although these experiments indicate that there is countercurrent exchange of a lipophilic solute in the renal cortex, they do not define the vessels responsible for this process. Unlike the capillaries (vasa recta) of the medulla, the peritubular vessels of the cortex are arranged in a complex and relatively random network which arises from efferent arterioles at various depths within the cortex (Beeuwkes et al., 1981). It has been observed that tubular fluid flow in the subcapsular cortex is frequently oriented in a manner counter to capillary flow (Steinhausen et al., 1970), but the multiple sites of supply and drainage of the cortical capillary bed and its intricate architecture would tend to preclude a coherent countercurrent flow in this capillary-tubular network that could account for the progressively slower rate of butanol equilibration as the cortical surface is approached.

In contrast to the capillary bed, the interlobar, arcuate, and interlobular arteries of the renal cortex closely parallel one another to the surface of the kidney. In some species, including dogs and cats, much of the venous drainage of the outer cortex flows through veins which drain to the kidney surface (Beeuwkes et al., 1981). In contrast, the venous drainage of rats, rabbits, mice, and guinea pigs is uniformly centripetal and opposite to the centrifugal flow of arterial blood. This arrangement seems ideal for countercurrent exchange of lipophilic solutes, but the thickness of the vascular walls of even the small interlobular arteries and veins may effectively block such diffusion. Evidence for exchange of $^{18}$O across arterial and/or arteriolar walls has been obtained in the hamster cheek pouch and cremaster muscle (Duling and Berne, 1970), the retina of rhesus monkeys (Ernest, 1977), and cat lungs (Conhaim and Staub, 1980). Similar exchange of lipophilic solutes may therefore be possible in interlobular renal arteries and veins.

By using two indicators with different lipid solubility, it was possible to show that the arrival of the more lipid soluble indicator ($^{14}$C-butanol) in the superficial cortex is delayed by what is presumed to be countercurrent diffusion. This observation complicates interpretation of single isotope studies of regional renal blood flow with $^{85}$Kr (Thorburn et al., 1963). For example, reports that superficial cortical flows decrease disproportionately as renal blood
flow falls (Carriere et al., 1966) may reflect countercurrent exchange of $^{85}$Kr which would tend to be exaggerated when flow decreases.

It is tempting to relate these observations concerning $^{14}$C-butanol to the distribution of respiratory gases in the renal cortex. Carbon dioxide is produced in the cortex both by metabolism and by the secretion of acid and dehydration of bicarbonate in the renal tubules. However, only the former mechanism results in net CO$_2$ production. The CO$_2$ produced by metabolism may enter the renal venous vessels and diffuse into adjoining arterial vessels, thereby tending to keep superficial CO$_2$ tensions relatively high. In contrast, oxygen arriving in the interlobular arteries would tend to diffuse into adjoining veins in a manner that would decrease oxygen tensions near the renal surface. These gradients would become more pronounced at lower renal blood flows. However, the opportunity for such arteriovenous exchange would tend to be limited by the relatively high affinity of carbon dioxide and even more of oxygen for blood relative to the renal tissue. Because oxygen concentrations in red cells are much greater than those in the plasma and renal vessel walls, there would be a greater tendency for this gas to remain intravascular in transit through the cortical tissues. This could explain why tissue PO$_2$ near the surface is not low (Leichtweiss et al., 1969; Aukland and Krog, 1970), even though CO$_2$ tensions seem to be high in this region.


INDEX TERMS: Countercurrent diffusion • Renal vasculature • Diffusion bypass

References


Supported by National Institutes of Health Grant HL 28255. Address for reprints: Richard M. Effros, M.D., Harbor-UCLA Medical Center, A-15, 1000 West Carson Street, Torrance, California 90509.

Received April 26, 1984; accepted for publication July 12, 1984.
Countercurrent diffusion in the renal cortex of the rabbit.
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Circ Res. 1984;55:463-467
doi: 10.1161/01.RES.55.4.463

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4371

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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