Renal tubular dynamics in the intact canine kidney

LILACH O. LERMAN, MARTIN RODRIGUEZ-PORCEL, PATRICK F. SHEEDY, II, and J. CARLOS ROMERO

Department of Physiology and Biophysics, and Department of Radiology, Mayo Clinic and Foundation, Rochester, Minnesota, USA

A technique capable of non-invasive characterization of the tubular fluid dynamics (transit times and changes in fluid concentration) in different nephron segments could shed light on the relationship between renal hemodynamics and tubular function. The present study was undertaken to investigate the capability of electron beam computed tomography (EBCT), a high temporal and spatial resolution scanner, to assess changes in renal tubular dynamics. We have previously demonstrated the feasibility [1–4] and reproducibility [5] of measuring *in vivo* global and regional renal volume, perfusion, and blood flow with EBCT in both animals and humans. However, the ability to non-invasively follow changes in contrast concentration using longer scanning sequences offers the possibility of recording its intra-tubular passage [6].

In the present study we performed sequential renal scanning with EBCT, using appropriate scanning duration and time intervals to monitor the transit of a single contrast medium bolus through both the vascular compartment and different nephron segments. Transit times and the change in contrast concentration in the various nephron segments were quantified and utilized to assess the degree of fluid (accompanied by electrolytes) reabsorption or excretion in a particular tubular segment. Moreover, regional perfusion of blood was simultaneously calculated from the same time-density curves.

Methods

This study was performed according to institutional animal care and use guidelines. Seventeen mongrel dogs were anesthetized (sodium pentobarbital, 30 mg/kg i.v.), intubated, and ventilated with room air. A femoral artery was cannulated, and a PE 240 catheter advanced via the abdominal aorta to the level of the renal arteries, for measurement of renal perfusion pressure with a pressure transducer (Statham P23ID, Gould, Hato Rey, Puerto Rico) before and after each study. The femoral vein was similarly cannulated for administration of fluids and additional anesthesia, and an infusion of saline (1 to 2 ml/min) into the intravenous catheter was initiated. This was followed by insertion of a #8F Rodriguez catheter into the mid-thoracic descending aorta via the left carotid artery for contrast media injections.

In 6 of the dogs (Group A, body wt 19 to 20 kg) one kidney was also exposed with a subcostal incision and an electromagnetic flow

probe (Carolina Medical Electronics, King, NC, USA) placed around the renal artery for continuous RBF monitoring. In the remaining 11 dogs (Groups B and C) an additional PE 200 catheter was inserted in the urinary bladder for urine collection.

Following surgical preparation, each animal was transferred and positioned in the EBCT (Imatron C-100; Imatron Inc., South San Francisco, CA, USA) scanning gantry. After a one hour recovery period, baseline EBCT studies were performed in each animal using the contrast medium iopamidol (Isovue®-370; Squibb Diagnostics, Princeton, NJ, USA). Following a 30-minute recovery period EBCT studies were repeated.

In 6 dogs (Group A) the second study was performed using ethiodol (Savage Laboratories, Melville, NY, USA). This is an ethiodized oil radio-opaque diagnostic agent whose emulsion represents an intravascular non-filterable marker, since it has a stable particle size which passes freely through microcirculation but not through openings between endothelial cells [7].

In 11 additional dogs (Groups B and C) urine was collected for 10 minutes before and after each EBCT study for measurement of urinary flow rate and osmolarity. In addition, 15 minutes before the second study, each dog received either an 8-second femoral venous injection of 3.5 mg/kg of furosemide (Group B, $N = 7$) or saline vehicle (Group C, $N = 4$). In Group B urinary volume losses consequent to furosemide administration were replaced by intravenous Ringer lactate.

EBCT scanning sequence

Each study was performed during respiratory suspension at end-expiration. For the study of perfusion and tubular dynamics, one mid-hilar tomographic level was localized in the left kidney [2] and scanned in the high resolution, single-slice (6 mm thick) flow mode. Forty consecutive scans were performed immediately after a bolus injection (0.5 cc/kg over 1 second) of the contrast medium into the aortic catheter. The first twenty scans were performed at the rate of 1 scan/0.6 seconds for six seconds, 1 scan/second for another four seconds, 1 scan/two seconds for another four seconds and 1 scan/2.5 seconds for four seconds, and the last 20 images were obtained at five seconds intervals for 100 seconds, which brought total scanning time to 124 seconds. The scanning sequence used for the first 20 scans was designed to sample rapid intravascular density changes [2], whereas the last 20 scans were used primarily to follow intratubular density changes. Since washout of lisamine green from the distal tubules is almost complete after 108 seconds [8], this period of time was sufficient for detection of density changes occurring at earlier nephron segments. Each dog received assisted ventilation in between scans during the last 20 scans.

In Groups B and C each flow study was immediately followed by a volume study, performed in the high resolution mode, as previously described [1]. To minimize the potential effects of contrast on renal hemodynamics, additional contrast medium was not administered for this study; hence, individual cortical and medullary volumes and blood flows were not separately quantified [2].

Following completion of the studies the dog was killed with Sleepaway® (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA). Urinary volume obtained in each of the 10-minute control periods was measured in a graduated cylinder, and urinary osmolarity was measured using a Micro Osmometer (Precision Systems).

Data analysis

Regions of interest (ROI) were selected in the cross-sectional images from the aorta, whole kidney, renal cortex, outer medulla, and inner medulla [2, 9]. In groups B and C the cortex was further subdivided into two equidistant concentric zones, defined as outer and inner cortex. The computer then generated for each ROI distinctive time-density curves, describing the change in tissue density consequent to transit of contrast in that region.

Perfusion. Perfusion (ml blood/cc tissue/min) was calculated from the first peak of the time-density curve obtained in each ROI, reflecting the passage of contrast through the vascular compartment, using the algorithm [2]:

$$\text{Perfusion} = \text{Peak height of tissue curve}$$
$$\times \text{Area under aortic curve}^{-1} \times 60$$

Renal volume. Renal volume was calculated from each volume study by identifying and manually tracing the renal contours on each tomographic level. The areas within these regions were then summed and multiplied by the slice thickness to yield renal volume [1].

Renal blood flow. RBF was calculated as the product of whole kidney perfusion and the corresponding renal volume [2].

Transit times. Each peak observed following the vascular phase in the time-density curves obtained in each renal region was analyzed separately. Transit time was calculated as the difference between appearance and disappearance times of each peak (first inflection and deflection, respectively).

Contrast concentration. The area enclosed under each tubular and aortic curve (between the first inflection and deflection of each curve, respectively) was calculated using a data analysis/graphics computer program. The ratio of the area under each peak to that under the aortic curve [10] was then utilized to assess the process of concentration (or dilution) of contrast in each nephron segment relative to pure blood (%).

Results

Distinctive time-density curves were obtained from each of the renal regions. Qualitatively similar and reproducible curves were obtained in each dog with the use of the filterable contrast media (iopamidol) under all experimental conditions. Density peaks corresponding to transit through the cortical (Fig. 1A) and medullary (Fig. 1B) vascular compartments were recorded shortly after contrast appearance in the aorta. However, from then on it was possible to characterize the systematic appearance of distinc-
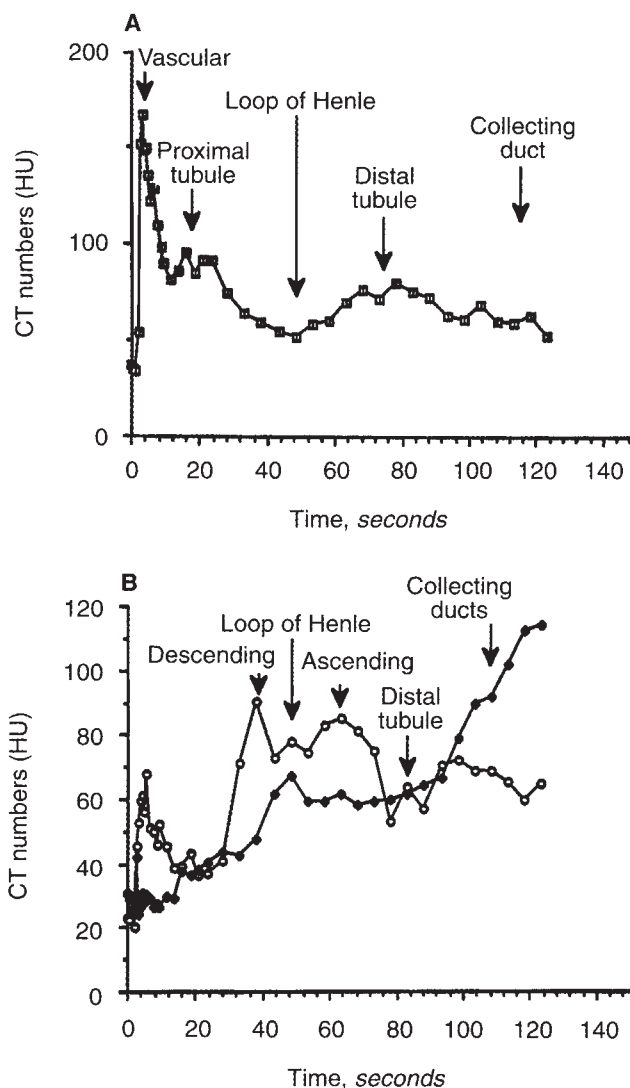

Fig. 1. *Time-density curves describing change of contrast concentration in the various nephron segments contained (A) in the renal cortex, and (B) in the outer (O) and inner (◆) medulla. The labels correspond to transit of contrast through a particular tubular segment.*

tive peaks corresponding to transit of contrast medium through specific segments of the renal tubules (Fig. 1).

In group A the second series of time-density curves was recorded using the non-filterable contrast medium ethiodol. In this group, regional renal perfusion, RBF, and mean arterial pressure, were similar during the two sets of EBCT studies with the use of the two different contrast media ($P > 0.05$). Nevertheless, although the time-density curves obtained with ethiodol showed the vascular portion, they invariably lacked the typical subsequent peaks obtained from the renal cortex, outer medulla, and inner medulla when iopamidol was used.

In Group B, prior to baseline studies two dogs were observed to be producing dilute urine at a high rate compared to the rest of the group, probably due to repeated flushing of the aortic catheter with inadvertently large amounts of saline, leading to diuresis. These two dogs were excluded from analysis, and Group B therefore included only 5 dogs.

Table 1. Renal tubular transit times and relative contrast media concentration (mean ± SEM) as measured with electron beam computed tomography in Group B ($N = 5$) in the various nephron segments

	Tubular segment						
	Proximal	Descending	Henle loop	Ascending	Distal	OM CD	IM CD
Transit times *seconds*							
Baseline	18.1 ± 2.2	26.4 ± 3.7	42.8 ± 5.5	21.0 ± 2.8	63.3 ± 3.6	41.0 ± 3.4	55.4 ± 4.4
Furosemide	33.3 ± 7.0[a]	32.7 ± 6.0	38.8 ± 5.0[a]	11.3 ± 1.6[a]	44.1 ± 2.0[a]	33.0 ± 2.7[a]	48.7 ± 3.9
Contrast concentration *%*							
Baseline	0.6 ± 0.1	1.2 ± 0.2	3.3 ± 0.5	1.1 ± 0.1	2.0 ± 0.3	2.3 ± 0.3	9.4 ± 1.8
Furosemide	1.4 ± 0.2[a]	1.3 ± 0.2	1.4 ± 0.2[a]	0.6 ± 0.1[a]	1.8 ± 0.0	1.5 ± 0.2[a]	2.3 ± 0.6[a]

Measurements were obtained at baseline and following administration of furosemide (3.5 mg/kg). Abbreviations are: OM, outer medulla; IM, inner medulla; CD, collecting duct.

[a] $P < 0.05$ compared to baseline.

Renal perfusion (global and regional), volume, and blood flow, as well as mean arterial pressure, were similar at baseline in groups B and C. In Group C, cortical and outer medullary perfusions increased slightly during the second experimental period (+28.4%, $P = 0.01$, and +28.2%, $P = 0.02$, respectively), due to contrast effect in volume-replete animals [11]. There was no difference in outer compared to inner cortical perfusion in either experimental period. Inner medullary perfusion, RBF, renal volume, and mean arterial pressure remained unchanged.

In Group B, perfusion of the whole kidney and renal cortex increased significantly following furosemide administration (from 3.88 ± 0.85 to 5.44 ± 0.85, $P = 0.001$, and from 5.24 ± 1.07 to 7.94 ± 1.14 ml/min/cc, $P = 0.0001$, respectively). However, there was no difference in outer compared to inner cortical perfusion either before (5.38 ± 1.26 vs. 4.96 ± 0.69 ml/min/cc, respectively, $P = 0.518$) or after (8.57 ± 1.29 vs. 7.55 ± 1.16 ml/min/cc, respectively, $P = 0.147$) furosemide administration, and there was no difference in the relative change in perfusion between them. The increase in total cortical perfusion in Group B was significantly greater than in Group C ($P = 0.026$). Inner medullary perfusion in Group B, on the other hand, was found to decrease significantly (from 0.86 ± 0.11 to 0.60 ± 0.06, $P = 0.005$) following furosemide administration. Both RBF and renal volume increased in Group B (from 215.5 ± 21.3 to 380.5 ± 34.5 ml/min, and from 59.3 ± 5.9 to 71.8 ± 3.5 cc, respectively, $P < 0.005$), but no change was observed in mean arterial pressure.

In Group C a small, insignificant increase in urinary flow rate, associated with a significant decrease in urinary osmolarity (−26.7 ± 5.7%, $P = 0.04$), resulted probably from administration of contrast media [11]. In group B, though, an increase in urinary flow rate and decrease in urinary osmolarity (+1730 ± 533%, $P = 0.01$, and −70 ± 4%, $P = 0.004$, respectively) that followed furosemide administration were considerable, and significantly more pronounced than in Group C ($P = 0.01$ and $P = 0.00005$, respectively).

EBCT-derived measurements of transit times through the various nephron segments, as well as the intratubular concentration of contrast media relative to aortic blood, were at baseline similar between Groups B and C. No change in either tubular transit times or intratubular contrast concentration was observed in Group C during the second EBCT study.

However, in Group B furosemide induced a +80.5 ± 19.2% ($P = 0.03$) prolongation of the contrast transit time through the cortical proximal tubule (Table 1). In the inner medullary loop of

Henle and in the outer medullary ascending limb of Henle's loop, on the other hand, transit times were significantly shortened (−9.4 ± 3.3%, $P = 0.037$, and −44.7 ± 7.6%, $P = 0.01$, respectively). Similarly, transit times were significantly shortened in the cortical distal tubule and in the outer medullary collecting duct (−28.6 ± 2.6%, $P = 0.006$, and −19.1 ± 4.5, $P = 0.016$, respectively). In the inner medullary collecting duct in Group B, transit time showed a trend for shortening by −11.7 ± 4.6% ($P = 0.09$), a change which was significantly different from the +4.2 ± 7.3% increase in transit time through the inner medullary collecting duct observed in Group C ($P = 0.033$).

In addition, dynamic changes in intratubular contrast concentration have also been observed in Group B during the second EBCT study (Table 1). In the proximal tubule, where transit time was prolonged following furosemide administration, the contrast medium was also concentrated by +121.2 ± 16.5% compared to baseline ($P = 0.003$). However, from the inner medullary loop of Henle and distally the intratubular contrast medium was relatively diluted, as reflected in decreased concentration relative to aortic blood (Table 1). Considerable dilution has initially taken place in the inner medullary loop of Henle, where the contrast concentration decreased by −56.5 ± 7.1% ($P = 0.011$), followed by a −45.5 ± 11.6% fall in the outer medullary ascending limb ($P = 0.012$). In the cortical distal tubule there was also a trend for dilution of contrast (−18.5 ± 5.3%, $P = 0.07$), whereas in the more distal nephron segments (that is, outer and inner medullary collecting ducts) this process was again marked (−33.7 ± 4.6, $P = 0.007$, and −74.1 ± 5.8%, $P = 0.018$, respectively).

The change in intratubular contrast concentration in both groups correlated well with the commensurate change in urinary osmolarity ($r = 0.79$, $P < 0.05$).

Discussion

This study demonstrates a novel methodology which may be very useful to investigate, in the intact kidney, changes in tubular dynamics that occur at physiological and pathological situations. We found that the transit of a bolus of filterable contrast medium, recorded by variable-sequence scanning of the kidney with EBCT, can be utilized to depict the ultra-function of the different nephron segments. This technique was validated by illustrating the disappearance of renal time-density curves when a non-filterable contrast medium was used instead. Furthermore, furosemide, a specific loop diuretic, brought about the expected

shortening of transit time and dilution of intratubular fluid in most nephron segments distal to the loop of Henle.

Radiographic contrast agents behave similar to inulin in the sense that they are removed from circulation primarily via glomerular filtration, and are not secreted or reabsorbed by the renal tubules [12]. Their transit time in the kidney is dependent on renal function and is relatively constant under normal conditions of hydration and sodium intake; the changes in their concentration, determined by the progressive reabsorption of the glomeruli ultrafiltrate, should also exhibit a standard pattern. These features enable routine clinical examinations such as excretory urography. Moreover, the externally detected concentration of radiographic, iodinated contrast agents is linearly related to the measured attenuation, thus enabling quantitative measurements.

These combined qualities of radiographic contrast media and the high spatial and temporal resolution of EBCT provided the means by which a bolus of filterable contrast medium could be followed and quantified during its transit via the different vascular and tubular segments. With the use of a non-filterable contrast medium in Group A, the only discernible change was the disappearance of the peaks succeeding the vascular curve, which had been observed in the same dogs with the use of iopamidol. This implies that these curves had been derived from intratubular contrast. Renal perfusion, blood flow, or perfusion pressure did not play a role in this difference since they were similar in both studies.

As expected, the average transit time through the different nephron segments measured in Group B at baseline and in Group C remained fairly constant, and very close to those seen in studies where tubular fluid movement was monitored by administration of another inulin-like substance, lisamine green [8]. Similarly, contrast concentration showed a progressive, more than 5-fold increase (from 0.6 to 3.3) from the proximal tubule to the bend of the loop of Henle, an area of high osmolality. In contrast, in the late segment of the ascending loop of Henle and early distal tubules, constituting the diluting portion of the nephron, relative dilution of the contrast medium was observed.

The efficacy of EBCT to characterize changes in tubular dynamics produced by alterations in fluid reabsorption was studied by the administration of furosemide. The effects of furosemide on renal hemodynamics included an increase in RBF and cortical perfusion [13, 14], which was greater in Group B than C, and may be prostaglandin-mediated [15]. This renal vasodilation was associated with a decrease in inner medullary perfusion, as previously described [16]. On the other hand, our results do not support furosemide-induced intracortical redistribution of blood flow [17], since no significant differences in perfusion between the inner and outer cortex has been observed. Furosemide has also led to an increase in renal volume, as known to occur during diuresis [18], but no change in renal perfusion pressure.

The effects of furosemide on intratubular fluid dynamics depend on the site of the nephron segment. The most immediate consequence of the furosemide inhibition of sodium chloride transport in the thick ascending limb of the loop of Henle would conceivably be dilution of intratubular contents and elevation of tubular fluid volume in this tubular segment. This would increase intratubular pressure, thus facilitating the flow of tubular fluid (that is, shortening transit times) from the thick ascending limb to the distal part of the nephron and, at the same time, would decrease the pressure gradient between the proximal tubule and

distal segment of Henle's loop [19], thus prolonging the proximal tubular transit time. These effects were indeed observed in our study (Table 1).

Administration of furosemide also induced changes in contrast concentration consistent with the alterations observed in transit times. Retention of sodium chloride and fluid in tubular lumen, starting at the loop of Henle, resulted in a higher delivery of fluid to the distal tubules and collecting ducts and in contrast dilution. In the proximal tubule, on the other hand, slower transit has most probably facilitated a twofold increase in the concentration of the contrast medium, reflecting an increase in fluid reabsorption.

Except for various renal factors, transit times recorded through the kidney would also depend on technical considerations such as characteristics of the indicator bolus and the administration routes. Furthermore, future development of mathematical algorithms to strip the partially overlapping tubular curves may enhance the accuracy of our calculations. Nonetheless, transit times measured through the various nephron segments generally agree with those previously reported [8], and contrast concentration usually followed predictable patterns. These measurements are also expected to be species-dependent to some extent, as renal anatomy and regional tubular composition may vary among species.

In summary, we evaluated a new method to study renal hemodynamics and intratubular fluid dynamics using a single injection of filterable contrast medium and EBCT scanning. This procedure may allow elucidation of the coupling of renal circulation with tubular function in the *in vivo*, intact kidney, and help assess the characteristics of tubular fluid concentration and flow as indices of renal function *in vivo*.

Reprint requests to Lilach O. Lerman, M.D., Ph.D., Department of Physiology and Biophysics, Mayo Clinic and Foundation, 200 First Street SW, Rochester, Minnesota 55905, USA. E-mail address: Lerman. Lilach@Mayo. Edu

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