Contrast ultrasonography of the kidney: a new method for evaluation of renal perfusion in vivo

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ABSTRACT Assessment of the effects of pharmacologic agents on renal blood flow (RBF) is clinically important in many disease states, including hypertension and congestive heart failure. However, because of the complexities of RBF, quantitation in vivo has been technically difficult. This study demonstrates the utility of ultrasound imaging of the kidney combined with injection of a sonicated radiocontrast solution (Renografin-76) for the assessment of regional renal blood flow. The technique uses a suspension of uniform microbubbles (diameter 4.4 ± 2.8 μm), which when injected directly into the descending aorta are distinctly visualized by renal ultrasound. Five dogs were studied. Catheters were placed in the descending aorta for injection of sonicated Renografin and in the renal artery for drug infusions. Data were collected before and during intrarenal artery infusions of bradykinin and norepinephrine. Total RBF was measured by electromagnetic flowmeter. Video density time curves were generated for comparable segments of the outer renal cortex and fit to a monoeponential decay curve. This allowed calculation of the mean exponential decay index (t₁/₂). An increase in t₁/₂ paralleled decreased renal perfusion (i.e., longer washout of contrast material). The opposite was true for a decrease in t₁/₂. Bradykinin increased RBF from 134 ± 26 to 249 ± 19 ml/min (p < .01 vs control), and norepinephrine decreased RBF from 130 ± 25 to 51 ± 17 ml/min (p < .01 vs control). When compared with control data, bradykinin resulted in a 29% decrease in t₁/₂ (3.81 ± 1.09 vs 2.72 ± 1.04 sec; p < .01 vs control), whereas norepinephrine prolonged t₁/₂ by 88% (3.92 ± 1.25 vs 7.36 ± 2.29 sec; p < .01 vs control). There was an inverse linear relation between total renal blood flow and t₁/₂ values. Thus renal contrast ultrasonography is a new technique that allows repetitive real-time ultrasound imaging of blood flow within the kidney of the intact animal. This technique allows quantitative assessment of renal perfusion as well as assessment of the effects of pharmacologic agents on RBF.


DISURBANCES in renal blood flow (RBF) are frequent sequelae of derangements in cardiovascular physiology. This is particularly true in patients with systemic hypertension or low cardiac output states. The ability to assess RBF serially in vivo would allow one to answer many questions regarding the efficacy of clinical management decisions. For example, does the increased cardiac output associated with long-term treatment of congestive heart failure with vasodilators or positive inotropic agents result in improved perfusion of the kidney? If so, is there a preferential increase in perfusion of the renal cortex or medulla?

Many techniques, including use of microspheres, transit renography, hydrogen electrodes, heat clearance, and radiography have been developed in an attempt to quantitate global as well as regional RBF.①④ To date, no method to determine regional renal blood flow has proved reliable, accurate, and easy to perform in vivo. This study introduces the technique of renal contrast ultrasonography performed in conjunction with injections of small amounts of sonicated Renografin-76 (meglumine diatrizoate and sodium diatrizoate).
The resultant injectate consists of a solution containing microbubbles that are smaller than red blood cells and can therefore pass the capillary bed in an unhindered manner. These microbubbles act as individual reflectors and are the source of the ultrasound contrast effect. This technique is similar to contrast echocardiography, a widely used method for delineating intracardiac structures, assessing valvular competence, and most recently, evaluating myocardial perfusion.9–16

Methods

Surgical preparation and instrumentation. Five mongrel dogs (22 to 35 kg) were anesthetized with 30 mg/kg sodium pentobarbital. After endotracheal intubation, room air ventilation was maintained with a Harvard volume respirator. After intubation, a left flank incision was made and the left kidney and the renal artery were exposed. An electromagnetic flow probe (Narco) was placed around the left renal artery for measurement of total RBF. The electromagnetic flow probe was connected to a flowmeter (Carolina Medical Electronics). Zero values were recorded at the beginning of each experiment by briefly occluding the distal renal artery.

Ultrasound imaging (Hewlett-Packard, Andover, MA) was performed with a 5 MHz transducer with the animal in the right lateral decubitus position. The transducer was attached to a stand to allow stable ultrasound imaging from the outer surface of the left kidney. A No. 7F multipurpose catheter (Cordis) was advanced in a retrograde fashion from the right femoral artery and positioned under fluoroscopic guidance into the descending aorta at the level of the left renal artery. This catheter was used to measure systemic arterial pressure (Statham transducer Model P23DBO) and for bolus injections of sonicated microbubbles (Renografin-76). A No. 7F right Judkins catheter (Cordis) was advanced in a retrograde manner through the left femoral artery and positioned under fluoroscopy into the left main renal artery. This catheter was used for intrarenal artery infusion of either bradykinin or norepinephrine. The right femoral vein was canulated with a large-bore catheter and used for continuous fluid hydration with 0.9% NaCl at 0.2 ml/kg/min to maintain a total diuresis of approximately 1 to 2 ml/min.

Experimental design. Baseline data were obtained by injecting a bolus of sonicated Renografin-76 while the two-dimensional ultrasound image of the kidney was recorded on videotape. The sonicated Renografin-76 was injected through the No. 7F multipurpose catheter positioned in the descending aorta just above the renal artery bifurcation. Two separate sets of baseline data were recorded.

Thirty minutes after completion of baseline data collection, 0.25 μg/kg/min bradykinin was infused into the left renal artery through the right Judkins catheter. After renal artery blood flow had increased at least 25% over its baseline value, a repeat bolus of sonicated Renografin-76 was injected while the two-dimensional ultrasound image was recorded. Thirty minutes after completion of the bradykinin infusion, new baseline recordings were obtained. This was followed by a 10 min infusion of norepinephrine, 0.5 μg/kg/min, into the renal artery. Once renal blood flow had decreased by at least 25% compared with baseline values, sonicated Renografin-76 was injected and renal ultrasound recordings were obtained. All injections of sonicated Renografin-76 were standardized by means of a volume- and pressure-controlled, electrocardiogram-gated power injector (Medrad, Mark IV). Renal blood flow, aortic systolic and diastolic pressures, and heart rate were monitored at all times. Drugs were always infused in the above-mentioned order.

Echo contrast agent preparation. The microbubble solutions used as echo contrast agents were prepared in a standardized manner.9,10 A 10 ml syringe was filled with 8 ml of sterile Renografin-76 and placed ½ inch into the horn portion of the sonicator unit (Model 220 Heat Systems/Ultrasonics of Plainview, NY). With the energy settings at 50% duty (180 W/cm), the Renografin-76 was sonicated for 30 sec. Immediately after the energy was turned off, the plunger was reinserted into the syringe and the microcavitated solution was injected into the multipurpose catheter. Assessment of the size of the microbubbles was performed in a manner previously described.9,10 The average diameter obtained was 4.4 ± 2.8 μm by laser analysis.17

Figure 1 demonstrates serial recordings of a single bolus injection of Renografin-76 given before any drug infusions. Baseline conditions are represented in frame 1. After the bolus injection, the contrast material is seen in the interlobar arteries (frames 2 and 3) before reaching the renal cortex (frame 4). The last two frames represent echocontrast washout (frames 5 and 6).

Data analysis. Each dog served as its own control. The paired t test was used to compare data. A p value < .05 was considered statistically significant. The contrast renal ultrasound images were digitized with an off-line computer (Quantic 1200, Bruce Franklin, Inc. Bellevue, WA). An edge-detection algorithm permitted rapid, accurate detection of the cortical borders throughout the injection cycle.14 The outer cortex of the kidney was then divided radially into 32 equal regions. Each of these individual regions was analyzed by a videodensitometric technique in which mean pixel intensity was plotted against time. A typical example of an intensity (gray-level) curve obtained during an injection of Renografin-76 microbubbles is shown in figure 2, A. Initially a relatively flat baseline gray intensity is noted, followed by a rapid rise corresponding to the opacification of the renal cortex induced by the bolus injection of Renografin-76. This is followed by a decay slope and return to near baseline intensity. A semilog plot of intensity (gray level) vs time (seconds) for these data is shown in figure 2, B. After the peak, the logarithm of intensity (gray level) decreases linearly with time (r = −.96). This suggests that the best fit for the decay slope portion of each curve is represented by a monoequation function. Therefore a monoequation function was used to describe the decay slope portion of each curve. From these data, individual decay rates (t½) were calculated. A linear least-square fit of the natural logarithm of the intensity minus the preinjection background was used, with t½ being proportional to the negative reciprocal of the slope of least fit. The t½ values (seconds) were then used as measures of renal perfusion for the remainder of the analysis.

Results

Hemodynamics. Control data acquired before infusions of bradykinin and norepinephrine were similar for heart rate as well as aortic systolic and diastolic pressures. Intrarenal infusions of bradykinin and norepinephrine as well as intra-aortic injections of sonicated Renografin-76 did not alter these variables (table 1).

Renal blood flow. Control renal blood flow was 134 ± 26 ml/min before infusion of bradykinin and 130 ± 25 ml/min before infusion of norepinephrine. Intrarenal infusion of bradykinin resulted in an 85% rise in RBF (134 ± 26 vs 249 ± 19 ml/min; p < .01 vs...
control), and infusion of norepinephrine resulted in a 62% decrease in RBF (130 ± 25 vs 51 ± 17 ml/min; p < .01 vs control).

Exponential decay index. Data representing the correlation between total RBF and mean exponential decay (t½) for all conditions of each experiment are represented in figure 3. Under control conditions the t½ was 3.81 ± 1.09 sec before bradykinin and 3.92 ± 1.25 sec before norepinephrine. When compared with control, bradykinin resulted in a 29% decrease in t½ (3.81 ± 1.09 vs 2.72 ± 1.04 sec; p < .01 vs control), whereas norepinephrine prolonged t½ by 88% (3.92 ± 1.25 to 7.36 ± 2.29 sec; p < .01 vs control). There was an inverse linear relationship between absolute renal blood flow and t½ values. Figure 4 displays superimposed intensity curves obtained from a representative animal under control conditions as well as during infusions of bradykinin and norepinephrine. For all conditions, there was a brisk rise from baseline to peak contrast effect and subsequent decay slope. It was the decay slope of each curve that differed for all conditions. When compared with control curves, the decay slope became steeper during infusion of bradykinin and shallower during infusion of norepinephrine.

This corresponded to increased and decreased blood flow, respectively.

Discussion

Although it is widely accepted that glomerular filtration rate and tubular function are influenced by changes in total and regional RBF, the mechanisms regulating intrarenal blood flow distribution remain incompletely understood. This is due in part to the limitations of current measuring techniques.1-5 During the past few years a wide variety of techniques have been developed in an attempt to better understand the renal circulation.1-5 However, because of methodologic problems associated with each technique, there currently exists no "gold standard" for repetitive non-invasive quantitation of global as well as regional RBF. This is particularly true when invasive procedures need to be kept to a minimum, as when RBF is studied in humans. Numerous animal studies have used radioactive microsphere methods for assessment of RBF.8,18-20 Particular problems associated with this technique include making the spheres small enough to avoid streaming but large enough to avoid transglomerular shunting.8,18-20 In addition, actual tissue must

FIGURE 1. Serial recordings of a single bolus injection of Renografin-76 under control conditions. Baseline ultrasound images before microbubble injection are represented in frame 1. After the bolus injection of sonicated Renografin-76, contrast material is seen in the interlobar arteries (frames 2 and 3), before reaching the renal cortex (frame 4). Frames 5 and 6 represent contrast washout.
be excised to quantitate organ perfusion. Other techniques such as gas washout enable multiple noninvasive RBF measurements over extended periods of time. However, this technique is limited by the inability to assess spatial-temporal relationships within the kidney.\textsuperscript{21-23} Contrast renal ultrasonography is a new technique that allows serial and repetitive high-resolution, real-time tomographic ultrasound assessment of RBF, permitting quantitative imaging of RBF as well as assessment of the effects of pharmacologic agents on renal perfusion. Using contrast ultrasonography we were able to assess total RBF with electromagnetic flowmeters as a standard reference. Future investigations will require the study of regional kidney perfusion (videodensitometric analysis of individual anatomic renal compartments) with appropriate reference standards designed to validate the regional variations in flow.

\begin{table}
\centering
\caption{Heart rate and systemic blood pressure responses to intrarenal infusions of bradykinin and norepinephrine}
\begin{tabular}{|c|c|c|}
\hline
 & \textbf{Bradykinin} & \textbf{Norepinephrine} \\
 & \textbf{(0.25 \textmu g/kg/min)} & \textbf{(0.5 \textmu g/kg/min)} \\
\hline
HR (bpm) & 121 \pm 9 & 121 \pm 9 & 121 \pm 12 & 119 \pm 10 \\
\textbf{P} (mm Hg) & 149 \pm 7 & 144 \pm 10 & 150 \pm 7 & 151 \pm 6 \\
\textbf{P} (mm Hg) & 114 \pm 8 & 112 \pm 11 & 117 \pm 7 & 116 \pm 6 \\
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\end{tabular}
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\end{table}

\textbf{Methodologic considerations.} To interpret the data presented in this study, several issues should be addressed. First, Kremkau et al.\textsuperscript{24} and Meltzer et al.\textsuperscript{11} have shown that the source of the echo contrast effect is gaseous microbubbles. Variability in microbubble size and stability could potentially alter RBF as well as generate axial streaming, transglomerular shunting, and trapping within arterioles and glomeruli, thereby distorting the accurate assessment of renal perfusion.\textsuperscript{3, 5, 8, 25, 26} To minimize this variability, sonication was used to generate small uniform microbubbles. This method has yielded reproducible and stable microbubbles of fairly uniform size (i.e., 4.4 \pm 2.8 \mu m) by laser analysis.\textsuperscript{9, 17} Prior studies have demonstrated the ability of these sonicated microbubbles to pass unhindered through the capillary circulation.\textsuperscript{9, 10} Thus, with the sizes of the microbubbles used in this study, transglomerular shunting should not have constituted a major problem.\textsuperscript{22, 23} Mild transglomerular trapping can

\begin{figure}
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Graph of total renal blood flow (ml/min) vs mean exponential decay (t/2) for all conditions of individual experiments. C\textsubscript{BK} = control, before bradykinin infusion; C\textsubscript{NE} = control, before norepinephrine infusion; BK = bradykinin; NE = norepinephrine. Numbers correspond to the individual set of experiments.}
\end{figure}
explain the fact that the decay portion of each curve only returned to near-baseline intensity. RBF remained unchanged during the intra-aortic injections of sonicated Renografin-76. These data are similar to the results obtained from a previous study in which coronary blood flow in dogs was unaltered by intra-aortic injections of various echogenic contrast agents. The microbubble stability has been previously established in vitro. In addition, we have shown that sonicated microbubbles are stable enough to cross the lungs of dogs, thereby providing evidence for stability in vivo. This was further corroborated by our current observation that sonicated Renografin-76 microbubbles injected at the level of the renal arteries were visualized in the inferior vena cava.

Second is the issue of data analysis. Because of the heterogeneity of different anatomic renal compartments, “washout” curves are composed of several monoexponential components (multiexponential). Therefore, in this study video intensity vs time curves were generated only for comparable segments of the outer renal cortex. This anatomic area has a more homogeneous blood flow distribution when compared with other renal compartments and may therefore explain the monoexponential-like behavior of the computer-generated “washout” curves. For each condition, RBF was assessed comparing video intensity vs time curves. Each of the analyzed curves showed the characteristic pattern of stable gray scale values under control conditions, a peak in gray scale level associated with the injection of Renografin-76, and a subsequent fall to near-baseline intensity in gray scale during the decay phase. This decay portion of the curve was fit to a monoexponential function to derive a $t_1/2$ value. From our data, it is the $t_1/2$ that appears to be a reliable measure of perfusion. Bradykinin increased RBF and shortened $t_1/2$, whereas norepinephrine decreased RBF and prolonged $t_1/2$. An inverse linear relationship between absolute RBF and $t_1/2$ was obtained in each individual experiment. To extrapolate absolute RBF values from this new method, it is necessary to know the relation between video intensity and number of microbubbles per milliliter of blood. Studies in vitro have revealed that at bubble concentration less than 1500/ml, concentration is directly proportional to echoreflective properties.

Finally, there is the issue of safety. Recently, Gillam et al. have reported that repeated injections of hand-agitated Renografin-saline mixture (microbubbles 11.5 $\pm$ 6.6 $\mu$m) cause transient disturbances of myocardial function without histologic evidence of myocardial, renal, or cerebral injury. These changes in left ventricular function were probably caused by transient interruption of the microcirculation due to the relatively large size of the microbubbles used in their study. In our study, sonication was used to generate small microbubbles of uniform size that can traverse the capillary circulation in an unhindered fashion. Although no extensive pathologic studies of kidneys perfused with sonicated microbubbles have been made, it appears that this is a safe, reliable technique for assessing renal tissues before human studies can be initiated.

**Clinical implications.** Ultimately, the clinical usefulness of this technique relies on the potential of imaging RBF in humans in a real-time, serial, noninvasive manner. This would potentially allow quantitative assessment of the distribution of renal perfusion as well as assessment of the effects of pharmacologic agents of RBF. It is currently possible to visualize the kidneys in humans by means of ultrasonography without direct access to the organ. Renal ultrasonography combined with the recent development of microbubbles that will safely traverse the pulmonary circulation should permit assessment in vivo of renal perfusion in humans by means of a contrast agent injected into a peripheral arm vein.

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