Renal cortical and medullary blood flow responses to altered NO-availability in humans

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Abstract

The objective was to quantify regional renal blood flow in humans. In nine young volunteers on a controlled diet, the lower abdomen was CT-scanned and regional renal blood flow determined by positron emission tomography (PET) scanning using $\text{H}_2\text{H}^{15}\text{O}$ as tracer. Measurements were performed at baseline, during constant intravenous infusion of nitric oxide (NO) donor (glyceryl nitrate), and after intravenous injection of NO synthase inhibitor (L-NMMA). Using the CT-image, the kidney pole areas were delineated as volumes of interest (VOI). In the data analysis, tissue layers with a thickness of one voxel were eliminated stepwise from the external surface of the VOI (‘voxel peeling’), and the blood flow subsequently determined in each new, reduced VOI.

Blood flow in the shrinking volumes of interest (VOIs) decreased as the number of cycles of voxel peeling increased. After 4-5 cycles, blood flow was not reduced further by additional voxel peeling. This volume-insensitive flow was measured to be $2.30 \pm 0.17 \text{ ml} \cdot (\text{g} \cdot \text{min})^{-1}$ during the control period; it increased during infusion of glyceryl nitrate to $2.97 \pm 0.18 \text{ ml} \cdot (\text{g} \cdot \text{min})^{-1}$ ($p<0.05$) and decreased after L-NMMA injection to $1.57 \pm 0.17 \text{ ml} \cdot (\text{g} \cdot \text{min})^{-1}$ ($p<0.05$). Cortical blood flow was $4.67 \pm 0.31 \text{ ml} \cdot (\text{g} \cdot \text{min})^{-1}$ during control; unchanged by glyceryl nitrate, and decreased after L-NMMA ($3.48 \pm 0.23 \text{ ml} \cdot (\text{g} \cdot \text{min})^{-1}$, $p<0.05$).

In conclusion, PET/CT scanning allows identification of a renal medullary region in which the measured blood flow is (i) low, (ii) independent of reduction in the VOI, and (iii) reactive to changes in systemic NO supply. The technique seems to provide indices of renal medullary blood flow in humans.
Introduction

In the kidney, blood flow in the cortex and medulla is regulated independently (3, 7, 22). Only a small fraction (~10%) of total renal blood flow enters the renal medulla. However, the regulation of this flow is important because it seems to play a key role in the regulation of tubular function, sodium excretion, fluid volume control, and ultimately blood pressure regulation (3, 11, 24). Studies of renal medullary blood flow are complicated by the complex vascular anatomy of the region (1, 29, 30). The medulla is perfused from efferent arterioles of the inner cortical or juxtamedullary nephrons. These vessels form the descending vasa recta (DVR). At the border between the inner and outer medulla, vascular bundles are formed containing both the DVR and ascending vasa recta (AVR). The DVR and AVR are in close apposition favouring efficient equilibration by counter-current exchange. This arrangement helps to preserve the cortico-medullary concentration gradients of sodium chloride (NaCl) and urea necessary for urinary concentration, but also influences the kinetics of flow tracers.

Measurement of regional renal blood flow can be approached experimentally by several distinct methods. Videomicroscopic measurement of red blood cell velocity is a highly reliable method, but limited to single surface vessels of the cortex of the surgically exposed kidney or to vasa recta of the surgically exposed papilla. Laser-Doppler flowmetry has been used extensively in anesthetized as well as conscious animals, although absolute flows cannot be obtained. The advantages of the method are that repeated measurements can be performed in the same region, and that the use of several probes allows measurements to be obtained simultaneously at different depths in the parenchyma (29). However, neither of these two methods can be used in humans as they are highly invasive.

Quantitative Positron Emission Tomography (PET) is a functional imaging technique that among other things can provide estimates of regional blood flow non-invasively in selected organs by mathematical modelling of the kinetic behaviour of a radiotracer, e.g., $H_2^{15}$O. It is considered the
method of choice for estimations in humans of regional cerebral blood flow (31) and regional myocardial blood flow (10).

Only few studies have been undertaken to apply this method to the quantification of overall renal blood flow (RBF) in humans. These have shown that it is feasible to assess RBF by PET/CT in humans, and that dynamic images from the abdominal aorta can be used as arterial input function (2, 13, 18, 28). The latter is important as it reduces the invasiveness of the method since the need for arterial blood samples is eliminated. Measurements of cortical and medullary blood flow do not appear to be available. CT-scans provide precise anatomical localization of structures and organs, in theory making identification of renal cortex and medulla possible.

Nitric oxide synthases (NOS) are a family of enzymes that catalyze the production of the powerful vasodilator NO from L-arginine. Chronic intrarenal infusions of the NOS inhibitor L-NAME cause selective reduction of renal medullary blood flow in rats (25, 27). Infusion of the NOS substrate L-arginine increases renal medullary blood flow in spontaneous hypertensive rats (21). Taken together, these animal studies suggest that altered NO availability is particularly important in the medulla, but also contributes to regulation of cortical perfusion.

In the present study we have assessed blood flow in the medullary region of human subjects by PET/CT. Pharmacological manipulation of NO availability was attempted in order to produce positive and negative changes in renal medullary blood flow. The aim was to examine if such changes in medullary blood flow could be detected by PET/CT.

Materials and methods

Subjects

Experiments were carried out in nine healthy male volunteers aged 22-31 years weighing 79.6 ±7.3 kg (mean ±SD) with a body mass index of 23.4 ±2.1 kg m⁻². All subjects gave written informed consent. The study was approved by the local ethics committee (file no. S-2008060) and was performed in full compliance with the Declaration of Helsinki. Prior to the study, a clinical examination was performed in each subject. None of the subjects provided any medical history that
suggested major illness or showed any signs of renal disease, arrhythmia, diabetes or hypertension, and all denied use of any kind of medication for two weeks prior to or during the study. All subjects were normotensive and had normal plasma sodium, potassium, and creatinine concentrations.

**Experimental protocols**

Both the absolute values of regional renal blood flow and the changes in response to alterations in NO availability can be expected to be related to sodium balance. Therefore, four days prior to the investigation the subjects were placed on a standardized diet containing 150 mmol NaCl day$^{-1}$ prepared by the kitchen for special diets at the university hospital. The volunteers were allowed to drink water as desired. They agreed not to exercise on the day before the experiment. To assess compliance with the prescribed diet, 24-h urinary sodium excretion (07.00-07.00 h) was measured on the day prior to investigation.

Before the experiment, the subjects were awoken at 06.30 h. At 06.45 h they consumed a light standardized breakfast. At 07.00 h, urine samples were collected, and at 07.10 h, they were taken to the laboratory. They were weighed and placed in the supine position on the PET/CT scanner bed for the duration of the experiment. An intravenous catheter (Venflon Pro 20GA, Becton Dickinson, Helsingborg, Sweden) was placed in a dorsal vein of the left hand for infusions and bolus injections. Cardiac output (CO) and systemic vascular resistance (SVR) were measured non-invasively by impedance cardiography (PhysioFlow PF-03, Manatec Biomedical, Macheren, France). Briefly, the impedance cardiograph measures stroke volume based on changes in thoracic impedance and heart rate (HR), allowing calculation of CO. Autocalibration is based on the subject’s characteristics (age, height, body mass and systolic/diastolic blood pressure at rest). The mean difference between the CO values obtained in normal subjects at rest by the direct Fick and PhysioFlow methods has been found to be negligible (0.07 L min$^{-1}$) (6). Brachial blood pressure was measured every 10 min by an automatic oscillometric monitor (Colin Press-Mate BP-8800, ViCare Medical A/S, Birkerod, Denmark).
After 30 min of equilibration, PET/CT-measurements were carried out in 2D using a GE Discovery VCT PET/CT-scanner (GE Healthcare, Milwaukee, WI, USA) (27).

A low-dose helical CT-scan (64 slices) was acquired using a standard CT protocol with a transaxial scan field of view of 70 cm. Data was reconstructed with a field of view of 50 cm, matrix size of 512x512 (pixel size 0.98 mm) and a slice thickness of 3.75 mm using filtered back projection and noise filtered with a standard GE CT-filter. Subsequently, an injection of $\text{H}_2\text{^{15}O}$ was given intravenously (1000 MBq in 5 ml of saline as a rapid bolus flushed in with 20 ml of saline), and the first dynamic 4-min PET-scan (baseline data) was performed.

The dynamic PET scans (23 frames; 12x5 s + 6x10 s + 3x20 s + 2x30 s) were acquired with 47 images per frame spaced by 3.27 mm, and covering an axial FOV (field of view) of 157 mm. The scan FOV was 70 cm. Attenuation correction was performed based on the CT-scan. The PET images were reconstructed as 128X128 matrices of 5.47 mm pixels transaxially, and a slice thickness of 3.75 mm, using iterative method OSEM (ordered-subset expectation maximization; 2 iterations, 20 subsets) and filtered with a 2D- Gaussian-filter (Full Width at Half Maximum at 4.29 mm) resulting in an isotropic resolution of approximately 5 mm.

Next an infusion of glycercyl nitrate (Glyceryl Nitrat, 5 mg/ml, Amgros, Copenhagen, Denmark) was started in a dose of 0.3 $\mu$g kg$^{-1}$ min$^{-1}$. Due to considerable interindvidual differences in sensitivity to glycercyl nitrate (8), the dose was increased stepwise until each subject had an increase in HR of some 10% (dose range 0.3 – 0.9 $\mu$g kg$^{-1}$ min$^{-1}$); HR was used as a surrogate marker of vasodilatation. Following second injection of $\text{H}_2\text{^{15}O}$, the second PET-scan (glyceryl nitrate) was performed. When this scan was completed, the glycercyl nitrate infusion was stopped. The pharmacological half-life of glycercyl nitrate is approximately 2 min. After the HR had returned to preinfusion levels, a bolus injection of L-NMMA was given intravenously (L-NMMA acetate, 3 mg kg$^{-1}$, Clinalfa Basic, Bachem Distribution, Weil am Rhein, Germany). In other studies, this dose has been found to be an effective vasoconstritor in humans (16). The final PET-scan (L-NMMA) was performed 5 min after the L-NMMA injection.
**Side effects of medication**

During the infusion of glyceryl nitrate, 7 out of the 9 subjects reported unwanted side effects in the form of headache, tension in shoulders, and slight nausea. None of the subjects experienced any side effects after the L-NMMA bolus. Unscheduled termination due to side effects did not occur.

**Data analysis**

The scanner software automatically co-registers the PET and CT scans. Before image processing, all scans were inspected to check alignment between the PET and CT images, see Fig. 1 for a representative image.

*Renal medulla:* Data were analyzed stepwise as follows: Via the CT-scan image, the kidney poles were identified, and the longitudinal axis was defined as the line between them. Planes perpendicular to the longitudinal axis at points ¼ of the interpolar distance from the poles defined the intrarenal limits of the original volumes of interest (OVOI). The OVOI was drawn using ‘Display’ software (open-source program Display, Montreal Neurological Institute, McGill University, Montreal, Canada, http://www.bic.mni.mcgill.ca/~david/). Tissue layers with a thickness equivalent to one PET voxel (3.27 mm) were eliminated from the outer cortical part of the VOI in a stepwise fashion starting with the most superficial layer (‘voxel peeling’), see Fig 2a-d. Following the removal of each layer, the blood flow of the remaining part of the VOI was calculated (see below).

*Renal cortex:* Using the same procedure as above the OVOI was defined. A tissue layer with a thickness equivalent to one voxel was eliminated from the outer cortical part of the OVOI, resulting in a reduced VOI. This was then subtracted from OVOI using MINC tools. With this approach an outer cortical layer with a thickness of 1 voxel was identified (Fig. 3), and the blood flow in this layer calculated.

**Processing of PET-data**

Assuming tracer clearance via the urine to be negligible, the blood flow was calculated based on the Kety-Schmidt one-compartment model (20). Curve fitting was performed by use of
both a one-compartment and a two-compartment model. Results generated by use of the one-compartment model are reported because this procedure consistently gave the best fits. The fitting program yields the kinetic parameter $K_1$ which is indicative of tracer clearance from blood to tissue; this was then corrected for an extraction fraction of 0.85 to obtain blood flow (15). The tracer concentration in the blood of the abdominal aorta was used as arterial input function. A small region of interest (approximately 5 voxels) was defined in the centre of the aorta on the summed PET-image where all of the 23 dynamic image frames were converted to one static image. This was done in all transaxial slices with the exception of the most cranial and most caudal slice, giving a total of 45 slices. This method has been shown to be a suitable alternative to arterial blood sampling (13, 19).

Statistical analysis

All values are presented as mean ± standard error of the mean (SEM). Comparisons were performed by one-way analysis of variance (ANOVA) for repeated measures. In case of significant differences, post hoc Dunnett’s tests were performed. Statistical calculations were performed with GraphPad Prism (GraphPad Software, San Diego, USA). Differences were considered significant at $p<0.05$.

Results

For all nine subjects, the rates of sodium excretion on the day prior to the experiment were similar, 150 ± 12 mmol/24 hours, indicating compliance with the dietary protocol.

Systemic hemodynamics

The main cardiovascular response parameter was HR. In the baseline setting this was stable at 63.2 ± 3.3 beats per min. During the glyceryl nitrate infusion it increased 14% ± 3 to 74.5 ± 3.4 beats per min ($p < 0.001$). After the L-NMMA injection, HR decreased to 49.8 ± 3.5 ($p < 0.001$) (Fig. 4a). Mean arterial blood pressure (MABP) decreased by 8% ± 2 ($p < 0.01$) on glyceryl nitrate and increased 8% ($p < 0.01$) on L-NMMA (Fig. 4b). Cardiac output was unchanged during glyceryl nitrate, and decreased 27% ± 2.19 ($p < 0.01$) after L-NMMA (Fig. 4c). As expected, the infusion of glyceryl
nitrate caused a decrease in systemic vascular resistance (10% ± 4, \( p < 0.05 \)), and the injection of L-NMMA was followed by an increase (20% ± 3, \( p < 0.001 \)) (Fig. 4d). Infusion of the NO-donor glyceryl nitrate caused significant systemic vasodilation, the opposite effect was observed after bolus injection of the NOS-inhibitor L-NMMA.

Renal medulla

The initial blood flow in the OVOI including cortex and medulla was 4.39 ± 0.18 ml/g tissue/min (Fig. 5). The blood flow of the VOI decreased as the number of cycles of voxel peeling increased. After 4-5 cycles of peeling, the flow in the reduced VOI became insensitive to additional peeling (Fig. 5). This volume-insensitive flow was 2.30 ± 0.17 ml/g tissue/min at baseline and increased to 2.97 ± 0.18 ml/g tissue/min (\( p < 0.05 \)) during infusion of glyceryl nitrate. The bolus of L-NMMA caused a decrease to 1.57 ± 0.17 ml/g tissue/min (\( p < 0.05 \), Fig. 6a).

Renal cortex

In the outermost voxel layer of the kidney, the flow was 4.67 ± 0.31 ml/g tissue/min (Fig. 6b). It was unaltered during infusion of glyceryl nitrate (4.66 ± 0.53 ml/g tissue/min). The bolus of L-NMMA was followed by a decrease to 3.48 ± 0.23 ml/g tissue/min (34% ± 19, \( p < 0.05 \)).

Discussion

The primary aim of the study was to establish a method for measuring human renal cortical and medullary perfusion. Apparently we have attained this objective. However, any study attempting to measure renal medullary blood flow in humans is faced with two major problems. First, there is no other method to which the results can be compared, and second, the highly complex vascular anatomy which provides excellent conditions for countercurrent exchange, raises questions about the validity of the simple algorithms traditionally used for calculation of flows.

The validity of the present data is best assessed by simultaneous measurements carried out with the present method and another independent method capable of quantification of local blood flow, e.g. tissue perfusion by laser Doppler devices. Such a comparison is not without difficulties;
firstly, laser Doppler flowmetry does not provide absolute values of flow, but only relative changes (7, 24, 34), and secondly, the method is invasive to an extent which makes it unfeasible for use in humans. Nevertheless, laser Doppler flowmetry in an appropriate large experimental animal with a kidney structure similar to man, e.g. the pig, seems to be the preferable choice for validation. At present we do not have access to facilities allowing for simultaneous renal laser Doppler flowmetry and PET/CT scanning in large experimental animals; therefore, this issue remains to be addressed by future experiments.

In anesthetized pigs, comparison of total renal blood flow determination by ultrasound and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) has been performed recently (23). In this study, an ultrasound probe was placed on the renal vein through a laparotomy and the animals were investigated while still anesthetized. After recording of morphological images, an intravascular contrast agent (gadofosveset trisodium) was administered prior to the determination of total and regional renal blood flow. The results show that total renal blood flow values can be estimated by DCE-MRI within a range of 60 to 170 ml/min/100 cm³ tissue can be assessed with an error of less than 10%. Calculations of the regional blood flow values were based on the flow distribution assessed by DCE-MRI and the total renal blood flow obtained with the ultrasound probe. The renal medullary blood flow obtained with this method (approximately 0.90 ml/min/cm³ tissue) were lower than those found in the present study (some 2.3 ml/min/cm³ tissue). However, a number of differences between the study of Ludemann et al. (23) and ours may account for this discrepancy, notably their use of anesthetized, laparotomized pigs, an artificial contrast agent, and the combination of MRI and ultrasound measurements. In contrast, we investigated conscious, healthy humans by use of labelled water and PET scanning.

The interference of the vascular counter current arrangement of the renal medulla is another potential problem. The question of diffusion - to a disturbing extent within the appropriate time frame - of labelled water from the descending to the ascending vasa recta is not easily addressed. In
an organ without counter current structures, an analogous problem would arise in case of shunting of blood from the arterial to the venous vessels (a-v shunting), thereby providing a mismatch between the arterial input function and the tracer activity curve within the specific tissue in question. In the present situation, tracer diffusion from descending to ascending vasa recta will affect the results in a way similar to a-v shunting, but here it is possible to address the issue by measuring the blood flow in the individual voxel layers. If significant shunting occurred, then the flow values in the outer medulla should be spuriously high while the values of the remaining smaller VOIs would be falsely low. Therefore the profile of the measured values of perfusion in individual voxel layers is interesting. Generally, if quantified in single shells with a thickness equivalent to one voxel, the tissue perfusion pattern was characterized by a continuous decrease from a high level of cortical flow to levels of much smaller flow in the medullary region (Fig. 7). This smooth decrease does not provide conclusive evidence, but is consistent with lack of significant effect of tracer diffusion. It may be argued that if the perfusion drops abruptly at the cortico-medullary border, then the effects of sizeable tracer diffusion will be a smoothing of this transition and therefore the measured flow pattern will be similar to that seen in Fig. 7. This issue seems worth addressing in future experiments by multiple, simultaneous measurements of local blood flow at various depths. However, this seems to necessitate the use of relatively large experimental animals in which several laser Doppler flow probes can be placed at various depths in the kidney without significant disturbance of organ function.

Current notions of the mechanisms responsible for sodium balance involve changes in renal medullary blood flow (3, 7). Long-term arterial BP regulation seems to be the result of multiple BP feedback loops operating in parallel (4, 12). In one of these feedback loops, blood volume is the regulated variable. Blood volume is a monotone function of sodium intake (5, 9, 33), and the mechanisms controlling sodium homeostasis are important, therefore, for long-term control of arterial BP. The present method seems to provide a tool by which the relation between blood volume and renal medullary blood flow can be investigated in humans.
**Perspectives**

We have developed a new method for measurement of blood flow in a well defined area in the medullary region of the kidney. Taking anatomical and functional evidence together, it is reasonable to assume that this tissue predominantly represents the renal medulla. Future studies should include validation of this method by simultaneous investigations of local renal blood flow by PET/CT and Doppler methods in experimental animals, e.g., in pigs as well as an attempt to include an anatomical definition of the renal medulla as well. Fully validated, the method could become a valuable instrument in the analysis of normal homeostasis as well as the pathophysiology of diseases possibly involving the renal medullary circulation, e.g. essential hypertension. Acutely, sodium excretion may be increased by an order of magnitude without any change in arterial pressure (26, 32, 35) and chronically there is no clear cut relation between sodium intake and arterial blood pressure (9, 14, 17); with the present method, it is possible to address the role of renal medullary circulation in the regulation of sodium excretion without changes in arterial pressure. Furthermore, the possible changes in renal medullary flow associated with essential hypertension can be addressed.

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**Conflict of interest**

None declared
Figure legends

Fig. 1.
Summed PET image co-registered to CT-scan. Scale bar indicates counts per second in the PET image.

Fig. 2.
Representative CT-image of the kidney from one subject. (a) Red area: the lower kidney pole region defined as the original volume of interest (OVOI). (b) A superficial tissue layer with a thickness equivalent to one PET voxel (3.27 mm) was eliminated from the OVOI to obtain a new reduced VOI. (c) The subtraction of a tissue layer of one voxel was repeated to obtain a further reduced VOI (OVOI minus two voxel layers). (D) Another voxel layer was eliminated to obtain a further reduced VOI (OVOI minus three voxel layers). This process was repeated as long as technically possible.

Fig. 3.
Representative CT-image of the kidney from one subject. (a) Red area: the lower kidney pole region defined as the original VOI (OVOI). (b), One voxel layer was subtracted to obtain the reduced VOI. (c) The reduced VOI was subtracted from the OVOI, leaving only the most superficial voxel layer.

Fig. 4.
Hemodynamic parameters during the study. (a) Heart rate, (b) mean arterial blood pressure, (c) cardiac output, and (d) systemic vascular resistance. Statistically significant differences from baseline values: *P<0.05, **P<0.01 and ***P<0.001.
Fig. 5.

Regional renal blood flow plotted as a function of the number of voxel layers peeled off the original VOI (OVOI). Statistically significant differences from the value in the OVOI: ***P<0.001.

Fig. 6.

Regional renal blood flow at baseline and during changes in NO availability in (a) the smallest VOI (OVOI minus five voxel layers) i.e., ‘medullary’ blood flow, and (b) in the most superficial voxel layer, i.e., cortical blood flow, cf. fig 3c. Statistically significant differences compared to baseline: *P<0.05.

Fig. 7.

Blood flow in each individual voxel layer. Layer number 0 is the most superficial layer, whereas layer 5 is the smallest VOI (OVOI minus five voxel layers) i.e., ‘medullary’ region. Layers 1 through 4 are those stepwise peeled off the original VOI (OVOI).


