Furosemide increases water content in renal tissue

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Furosemide increases water content in renal tissue. Am J Physiol Renal Physiol 292: F1645–F1651, 2007. First published January 30, 2007; doi:10.1152/ajprenal.00060.2006.—The present study was designed to evaluate the short-term effects of intravenous administration of furosemide on key functions in the kidney cortex and the outer and inner medulla of rats by using magnetic resonance imaging (MRI). Renal tissue water content, renal tissue oxygenation (in relation to the magnetic resonance spin-spin relaxation rate), the apparent diffusion coefficient (ADC) of water, and volume of renal blood flow were measured. Furosemide administration resulted in an increased water content in all regions of the kidney. In parallel with this, we found a significant reduction in ADC in the cortex (2.7 ± 0.1 × 10–3 to 2.3 ± 0.1 × 10–3 mm2/s; P < 0.01) and in the outer medulla (2.3 ± 0.1 × 10–3 to 2.0 ± 0.1 × 10–3 mm2/s; P < 0.01), indicating that the intra- to extracellular volume fraction of water increased in response to furosemide administration. Furosemide also decreased the blood oxygenation in the cortex (49.1 ± 2.9 to 40.9 ± 2.0 s–1; P < 0.01), outer medulla (41.9 ± 2.8 to 33.2 ± 1.6 s–1; P < 0.01) and in the inner medulla (37.1 ± 2.9 to 26.7 ± 1.8 s–1; P < 0.01), indicating an increased amount of oxygenated Hb in the renal tissue. Moreover, renal blood flow decreased in response to furosemide (6.9 ± 0.2 to 4.4 ± 0.2 μl/min; P < 0.001). In conclusion, furosemide administration was associated with increased renal water content, an increase in the intra- to extracellular volume fraction of water, an increased oxygen tension, and a decrease in the renal blood flow. Thus MRI provides an integrated evaluation of changes in renal function, leading to decreased renal water and solute reabsorption in response to furosemide, and, in addition, MRI provides an alternative tool to monitor noninvasively changes at the cellular level.

magnetic resonance imaging; furosemide; loop diuretic; kidney cortex; kidney medulla; renal function; oxygenation

DIURETICS PROMOTE THE RENAL EXCRETION OF both water and solutes from the body. Furosemide is a powerful diuretic acting on the thick ascending limb of the loop of Henle, where ~25% of all sodium is reabsorbed. Furosemide exerts its action by inhibition of the Na+-K+-2Cl− apical membrane cotransporter NKCC2 or BSC-1, which is the most dominating apical NaCl transport protein located to this segment (36). The direct effect of the furosemide-induced volume depletion is an increase in the isoosmotic urine production (by) impairing the concentrating capacity of the kidney. Thus furosemide may secondarily be associated with marked changes in the overall renal water and sodium content and may be associated with changes in water diffusion between different compartments in the kidney.

Magnetic resonance imaging (MRI) allows direct quantification of the renal water content, which is based on measurement of longitudinal relaxation time (T1) of the tissue and a subsequent conversion to percentage of water (13, 41). Previously, this technique has been applied in Long Evans rats, where tissue water content was examined after cerebral ischemia, showing a close correlation between in vivo MR-measured water content in the brain and ex vivo with wet/dry measurements (41). Moreover, the relationship between intra- and extracellular diffusion can be measured by the so-called apparent diffusion coefficient (ADC), a technique that is inherently sensitive to diffusion by the random Brownian motion of protons. Importantly, ADC depends on the intra- and extracellular volume fraction and, to some extent, the cell size and the membrane permeability (39), whereas a decrease in ADC can reflect in cellular swelling and vice versa (24, 25).

Recently, measurement of the oxygen levels in the renal tissue has become possible using a technique referred to as blood oxygen level-dependent (BOLD) MRI (30). The technique has been applied to various conditions in the brain and has also been demonstrated to be promising in quantifying the oxygenation of the human kidney (11, 18, 31). Recently, this technique showed that furosemide is associated with an increase in medullary oxygenation in young women, whereas an unchanged medullary oxygenation was found in older women (11). This discrepancy in the effect of furosemide may reflect age-related changes in renal prostaglandin synthesis or renal hemodynamics. Consequently, to separate these effects, it is important to measure potential changes in renal blood flow (RBF) after furosemide administration.

Noninvasive measurement of blood flow velocity is performed routinely with MRI using a phase subtraction technique in which one-dimensional velocity encoding is generated by a gradient echo phase-contrast sequence (11). The high RBF in conjunction with rapid molecular diffusion of water between intra- and extracellular compartments in the kidney makes changes in the renal intra- and extracellular compartments sensitive to hemodynamics. Additionally, because furosemide acts primarily in the thick ascending limb, medullary oxygen content is affected when the Na+-K+-2Cl− cotransporter is inhibited, increasing the medullary oxygen concentration (3, 30).

Furosemide administration is generally not thought to be associated with marked changes in the vascular system, e.g., no change in the mean arterial blood pressure and a minimal fall in blood volume within 1 h after administration (2). However, several studies have shown that the RBF is slightly reduced after administration of furosemide (3, 7, 14, 15), supporting the view that furosemide acts as a renal vasoconstrictor.

The aim of the present study was to examine whether MRI could detect changes in key renal function parameters in

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response to furosemide. This was achieved by measuring dynamic changes in the tissue water content, ADC, renal oxygen consumption, and RBF before and after administration of furosemide in intact rats. Having established a noninvasive method to investigate such important mechanisms would stimulate MRI to become a more prominent tool in the understanding of basal physiological principles in the kidney.

**MATERIALS AND METHODS**

**Experimental procedure.** Male Wistar rats weighing 350–400 g (120–130 days of age) were anaesthetized with Mekunal (100 mg/kg body wt; Nycomed, Copenhagen, Denmark). The rats were maintained on a dry pellet diet with free access to water before the experiment. For drug administration, a polyurethane catheter (0.015 in. Tygo; Norton Performance Plastics, Taunton, MA) was implanted in the femoral vein. MRI was performed with a Philips NT 1.5 T system (Philips Medical Systems, Best, The Netherlands) using a small surface coil of 4 cm in diameter. The animal was placed supine with the kidneys placed above the imaging coil. The fully anaesthetized rat was then subjected to an imaging protocol as shown in Fig. 1. After the scout images obtaining and the optimal positions were found, a group of baseline scans including BOLD, ADC, RBF, and T1 measurements was initially acquired, followed by intravenous administration of 0.1 mg/kg body wt furosemide (injected in a volume of 1 ml/kg; Furix; Benzon Pharma, Copenhagen, Denmark; n = 6) or a similar volume of saline (n = 5). MRI was subsequently performed, ending with a T1 measurement 57 min after the injection. Rectal temperature was monitored throughout the experimental protocol. All animals were cared for according to the local ethical standards of laboratory animals, and approval was obtained before experimental procedures.

**Renal water content.** Accurate and noninvasive measurements of the kidney water content were performed by determining T1. Even though the kidney demonstrates a large blood volume, a linear increase in T1 vs. percent water content from cortex to inner medulla has been found in rats (23) and rabbits (20). The reciprocal of the total water content (W), defined as grams of water per grams of tissue, is has been found in rats (23) and rabbits (20). The reciprocal of the total water content were performed by determining T1. Even though the kidney demonstrates a large blood volume, a linear increase in T1 vs. percent water content from cortex to inner medulla has been found in rats (23) and rabbits (20). The reciprocal of the total water content (W), defined as grams of water per grams of tissue, is

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$$\text{W} = \frac{1}{\text{C}}$$

where C is the water content of the kidney. The reciprocal of the total water content was used to determine R2*, which is directly proportional to the tissue content of deoxyhemoglobin (9, 30) and is thereby inversely proportional to the tissue concentration of oxygen. BOLD scans were performed in three coronal slices to image both kidneys. Using TE of 4 and 40 ms in combination with a 40° flip angle and a TR = 100 ms resulted in heavily 1/R2* weighted images. As for ADC scans, the position, size, resolution, and thickness are equal to the image parameters chosen for the measurements of the tissue water content. The slope of fitted Log(intensity) vs. TE was performed to derive a R2* map on a pixel-by-pixel frame.

**RBF.** Volume blood flow was measured using a phase-contrast gradient echo sequence equipped with a flow-sensitizing bipolar gradient in the slice direction. The strengths of the flow-encoding gradients were set according to RBF values from the literature (35). Ten slices of 3 mm thickness were prescribed perpendicular to the renal veins. Each slice had an 11 × 11-mm field of view and a resolution of 256 × 256 pixels to ensure appropriate pixels to find and derive the blood flow in the renal veins. Other parameters were TE = 4 ms, TR = 25 ms, 40° flip angle, and four transients. The phase images were subtracted, and quantitative RBF, in units of milliliters per milliliter, was determined by multiplication with the renal vein area.

**Data analysis.** For each time frame, T1 scan with TE = 50 ms, ADC scan with b = 0, and BOLD scan using TE = 4 ms were used as anatomic templates to facilitate placements of regions of interest (ROI). Slices with clearly visualized cortex, inner medulla, and outer medulla were chosen for our analysis. ROIs were manually inserted to encompass those three regions and were then duplicated to the water map, ADC map, and BOLD map, which were made using Osiris Medical Software (Geneva University Hospital, Geneva, Switzerland) and Photoshop (Adobe Systems, San Jose, CA) graphical software. RBF values were summarized for all slices in which the renal vein was clearly seen. The mean values and SEs were calculated for all measurements. The following statistical analysis was performed: ANOVA of data from one observation time to another for the same parameter was performed by a one-way ANOVA model. Where
ANOVA indicated that statistical significance existed, Dunnett’s multiple comparison was used to determine statistical significance among baseline and treated values. Comparisons between furosemide and saline groups were evaluated by Student’s t-test for unpaired data. Differences were considered statistically significant at the 0.05 level. The saline group was considered as the control.

RESULTS

The gradient echo images used as an anatomical template revealed excellent spatial information of the rat kidney. Only minimal artifacts from respiratory movements were seen, and cortex as well as inner and outer medulla were easily recognized in both kidneys (Fig. 2). Pixel-by-pixel color maps of the effect of furosemide and saline on tissue water content, ADC, R2*, and RBF are presented in Fig. 3. Changes are indicated by color shifts, with red shift indicating a relative decrease and blue shift indicating a relative increase. Data are presented and as relative changes (index) compared with data obtained before intravenous administration of furosemide or saline (Figs. 4 –7).

Furosemide increases the renal water content. The renal water content increased significantly in all regions of the kidney following administration of furosemide. In the kidney cortex, the water content increased from 78.5% ± 0.7% at baseline to 81.9% ± 1.0% (P = 0.009) after furosemide. The outer medullary water content increased from 81.8% ± 0.9% at baseline to 85.8% ± 0.8% (P = 0.002) after furosemide, and the inner medullary water content showed a similar increase, from 87.2% ± 0.9% at baseline to 89.4% ± 0.6% (P = 0.007) after furosemide. The renal water content of cortex, outer medulla, and inner medulla did not change significantly in response to saline administration. Thus furosemide induced a marked increase in renal water content. Comparison of the renal water content in the furosemide-treated rats with the saline-treated rats showed that the change was primarily located to the renal outer medulla (P = 0.016) and secondarily to cortex (P = 0.09) and inner medulla (P = 0.22; Fig. 4).

Furosemide decreases the ADC. Indexed ADC values are presented in Fig. 5. Furosemide induced an abrupt decrease in the ADC inside the kidney. ANOVA for repeated measurements comparing ADC values from the furosemide group with the saline group showed no significant differences at 8 or 26 min after administration (8 min: P = 0.316; 27 min: P = 0.086). However, at 46 min after furosemide administration, the relative change in the cortical ADC was significantly larger than the relative change after administration of saline (13 ± 3% vs. 1 ± 5%; P = 0.01), indicating that furosemide induced a shift in the intra- to extracellular volume fraction ratio. Similar reduction in the outer medullary ADC was found in the furosemide group compared with the saline group (11 ± 6% vs. 1 ± 6%; P = 0.036). The relative change in ADC of the inner medulla remained unchanged during the protocol, and no significant differences between groups were found at any time (P > 0.419).

Fig. 2. A coronal magnetic resonance (MR) image of the rat kidneys achieved from a standard gradient-echo sequence. Cortex, outer medulla, and inner medulla are distinguished by gray scale. The images were performed using a conventional clinical MR system equipped with microcoils designed for measurements in small animals.

Fig. 3. Pixel-by-pixel maps of different physiological parameters [tissue water content (A), apparent diffusion coefficient (ADC; B), and blood oxygen level-dependent (BOLD; C)], of the rat kidneys before and after administration of furosemide or saline. The maps are layered on an anatomical image acquired during the functional scan. The color schemes indicate a relative increase or decrease in the tissue water content, ADC, or oxygenation, e.g., pixels that are shifted red indicate a reduced level of oxygenation and pixels that are shifted blue indicate an increased level of oxygenation.
Furosemide decreases renal energy expenditure. Figure 6 shows absolute and relative BOLD MRI (R2*) data. For all regions, the response to furosemide showed a significant difference between baseline and treated R2* values (P < 0.01). R2* values of control kidneys did not change significantly after administration of saline (7.3 ± 0.4; P > 0.2). However, when comparing the responses to furosemide with the responses to saline at 3 min after the administration, we found no significant differences between relative BOLD values in the cortex (−9 ± 7% vs. 0 ± 5%; P = 0.065) nor in the outer medulla (−15 ± 6% vs. −5 ± 4%; P = 0.116). Inner medullary BOLD values differed significantly between the furosemide group and controls (−18 ± 4% vs. −1 ± 7%; P = 0.023). Repeating the scan at 21 min showed a significant difference between relative BOLD values of furosemide and saline groups in the outer medulla (−26 ± 7% vs. −7 ± 10%; P = 0.029) and in the inner medulla (−20 ± 8% vs. 0 ± 4%; P = 0.019). However, relative cortical values remained unchanged at 21 min (−13 ± 8% vs. 0 ± 5%; P = 0.1). At 39 min after administration, a marked reduction in the relative BOLD values of the furosemide group was observed compared with the saline group, and statistics showed a significant difference for cortex (−20 ± 5% vs. −2 ± 3%; P < 0.001), outer medulla (−25 ± 5% vs. −1% ± 3%; P < 0.001), and inner medulla (−32 ± 6% vs. −11 ± 8%; P = 0.007).

Furosemide decreases RBF. Relative changes in RBF in response to furosemide are shown in Fig. 7. RBF decreased significantly after administration of furosemide (−30 ± 14%; P < 0.001), whereas RBF was unchanged in the saline-treated rats (14 ± 9%; P = 0.385). Relative RBF did not differ between furosemide and saline treatment at 16 min (−31 ± 6% vs. −13 ± 13%; P = 0.086) or at 34 min (−32 ± 5% vs. −20 ± 9%; P = 0.095). However, at 52 min, the relative RBF was significantly reduced in the furosemide group (−36 ± 4% vs. −16 ± 5%; P = 0.021).

DISCUSSION

During the present study, changes in renal tissue water content, molecular water diffusion, intrarenal oxygenation, and RBF were measured in response to a furosemide bolus using MRI. The main finding was that furosemide induced a significant increase in the renal water content, predominantly located to the cortex and the outer medulla. In parallel, a significant reduction in the diffusion of the tissue water molecules was demonstrated in the cortex and in the outer medulla, indicating an increase in the intra- to extracellular water compartment ratio. BOLD MRI revealed that blood oxygenation in the outer and inner medulla was increased following administration of furosemide, indicating increased levels of blood oxygenated Hb, i.e., reduced oxygen uptake by the renal tissue. Furthermore, furosemide administration was associated with an immediate reduction in RBF.

Renal water content increases in response to furosemide. Furosemide induced a marked increase in the overall kidney water content. From the MRI approach used in the present study, molecular water diffusion, intrarenal oxygenation, and...
study, tissue water content can be derived by measuring the spin-lattice relaxation rate, a constant that corresponds to the percentage of water in the tissue. This demonstrated that the renal water content increased in all regions of the kidney after furosemide administration. Thus it may be anticipated that handling of water in the different nephron segments changes dramatically immediately after administration of furosemide. The urinary concentrating process depends on the coordinated function of the loop of Henle and the collecting duct. In the thick ascending limb of the loop of Henle, reabsorption of sodium and chloride powers the countercurrent multiplier process responsible for generation of the corticomedullary osmotic gradient, whereas the collecting ducts, under the control of vasopressin, allow variable degrees of osmotic equilibration, resulting in variable water excretion and a reciprocal relationship between urinary flow and urinary osmolality. Furosemide specifically binds to and inhibits the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) apical membrane cotransporter NKCC2 responsible for the active chloride reabsorption in the thick ascending limb of the loop of Henle in the mammalian renal tubule (10). Thus the direct effect of this inhibitor is a reduction in the active reabsorption of chloride in this segment, resulting in a decreased driving force. It is well established that this rapidly leads to an increased diuresis. The increased renal water content after administration of furosemide may therefore primarily represent an increase in the amount of water present in the lumen of the distal tubule and collecting ducts. Characteristically, in a previous study, immunohistochemical localization of rat kidney NKCC2 was restricted to the medullary and cortical thick ascending limb segments (10). Thus the marked increase in renal water content demonstrated in the cortex and outer medulla may reflect that the density of NKCC2 is high in this area. Correspondingly, administration of furosemide leads to an increased amount of water remaining in the renal tubular lumen of the thick ascending limb and collecting duct. Our current MRI system did not allow us to identify which exact cellular segments the increased water content was located to. To perform such examinations, we have to use either a tracer or drug that is characterized by being located specifically to specific segments or by a very high field magnet providing a much higher spatial resolution.

Renal intra-to extracellular volume fraction increases in response to furosemide. Furosemide administration was associated with a significant reduction in the ADC index both in the outer medulla and cortex of the kidney. The origin of the ADC reduction is complex as water diffusion in the renal tissue is affected by a heterogeneous intraRBF distribution, glomerular filtration, tubular secretion and reabsorption, and urine flow (42). Anderson et al. (1) have used a simplified tissue model of osmotically driven cell volume changes on ADC in the rat optic nerve. They found that the changes in membrane polarization and cellular energy were not sufficient to induce significant changes in ADC, whereas changes in the cellular volume fraction induced by cell membrane depolarization are sufficient.

The furosemide-induced reduction in ADC may therefore correspond to an increase in the intra- to extracellular water compartment ratio, indicating that the relative intracellular...

Fig. 7. Relative renal blood flow (RBF) measured by MRI using an estimate of the renal vein flow before and after administration of furosemide or saline. Data are relative to baseline values; *P < 0.05. RBF demonstrated a significant reduction 52 min after administration of furosemide, whereas RBF was not markedly reduced following administration of saline.
volume of water increases compared with the extracellular volume. Thus the ADC reduction could be a result of an acute intact apical water transport in the principal cells of the cortical and outer medullary collecting ducts increasing the cellular volume in these segments of the kidney. The furosemide-induced impairment of the solute gradient would compromise water reabsorption to the interstitial compartment and vasa recta. This finding supports the view that furosemide does not influence short-term regulation of aquaporin 2 in the principal cells. Thus it is anticipated that the trafficking of aquaporin 2 and apical water reabsorption is intact in these segments, whereas the reduced driving force may account for the inhibition of basolateral water transport.

Whether molecules need to cross a membrane or are hindered by different extracellular environments to diffuse in a given direction makes a major difference for ADC measurements (13, 22, 39). In the kidney, the vascular structures supplying the cortex, outer medulla, and inner medulla are different. In the cortex, many glomeruli have long efferent arterioles, which extend to the medulla where they divide, forming vascular bundles, and, in the inner medulla, the cores of these bundles break up into a capillary plexus. Another regional difference concerns the interstitial cells, which become more numerous and larger as one descends from cortex to papilla (40). Changes in ADC should therefore either be a result of hemodynamic changes or be a result of changes in the cellular environment. Because ADC is a scalar obtained from three directions, changes in ADC do not fully reveal anisotropic reorganization of the intracellular compartment. Different experimental approaches have resulted in slightly different ADC values in the rat model, which could be caused by different diffusion-encoding techniques (5, 6, 21, 42). In general, because the structure of the kidney is anisotropic, a full description of the self-diffusion of water inside the kidney should be investigated by more advanced methods, such as application of diffusion tensor imaging (34).

Renal energy expenditure is reduced in response to furosemide. The BOLD technique readily detects the reduction in medullary oxygenation that follows administration of furosemide. We have recently demonstrated that the value of \( R_2^* \) correlates inversely with the tissue content of oxygen (27), meaning that a decreased \( R_2^* \) implies an increase in the oxyhemoglobin concentration, which subsequently results in a proportional increase in blood and tissue \( P_O_2 \). The BOLD MRI technique does not allow distinguishing changes in oxygenation produced by alternations in the supply of oxygen from those produced by changes in oxygen consumption (30). The present study showed that furosemide induces an overall decrease in \( R_2^* \), especially in the medullary regions. These result values are consistent with previous studies in humans (30) and in rats (32, 33). Similar observations were found using invasive oxygen-sensitive microelectrodes (3), from which furosemide was concluded to enhance medullary oxygen availability. The improved level of medullary oxygen may be explained by the decreased consumption of oxygen necessary to maintain the medullary transport of ions, and therefore the equilibrium from oxy- to deoxyhemoglobin is shifted because furosemide acts by reducing regional tubular oxygen consumption for reabsorptive transport of sodium (30). We demonstrated that \( R_2^* \) was mostly decreased in the inner medulla, although one would think that furosemide mainly would act by decreasing the active transport in cells lining the medullary thick ascending limb. This is consistent with previous findings demonstrating that changes induced by furosemide are fully dominated by changes in \( R_2^* \) and that the BOLD effect is not dependent on changes in the tissue water content (30).

**RBF decreases in response to furosemide.** A close correlation between the percentage change of relative RBF and the percentage change of ADC has recently been demonstrated (28, 29), suggesting that a decrease in ADC followed by administration of furosemide was mediated by hemodynamic changes. Our data showed that furosemide induced a significant decrease in RBF, supporting the view that furosemide causes renal vasoconstriction (3). These effects may therefore add to the change in ADC, since renal vasoconstriction may be associated with a decrease in the intravascular volume. Furthermore, volume depletion has effects on renal hemodynamics, including a sustained decrease in glomerular filtration rate and the blood perfusion of the kidney (4, 14, 16, 18, 43), which has been shown to depend linearly on RBF (35). Furosemide has in addition been demonstrated to block the tubuloglomerular feedback response, followed by significant decreases in renal interstitial concentrations of ATP (26). Furthermore, furosemide elicited a marked impairment of RBF and glomerular filtration rate autoregulatory efficiency as well as the autoregulation-associated alterations in RVR, as previously reported by other investigators (8, 17). RBF in the sham-operated rats decreased slightly with time, suggesting that anesthesia may reduce the systemic blood flow. However, the decrease was not statistically significant compared with baseline. Importantly, the present study demonstrated that RBF was reduced at all time points following intravenous administration of furosemide.

In conclusion, in vivo magnetic resonance monitoring of kidney tissue water content following administration of furosemide revealed increased cortical and medullary water content. Diffusion-weighted MRI was able to detect a significant decrease in cortical and medullary ADC after administration of furosemide, indicating an increase in the intra- to extracellular volume fraction ratio. Consistent with previous studies, BOLD imaging revealed increased medullary tissue oxygenation. Thus MRI is a noninvasive method providing direct visualization of the kidney and simultaneous estimation of essential renal physiological parameters in vivo.

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