

Monitoring urea transport in rat kidney *in vivo* using hyperpolarized ^{13}C MRI

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Introduction: Urea plays a vital role in the urinary concentrating mechanism of the kidney by functioning as a key osmolyte¹. While NaCl dominates the outer medulla, urea is important in the inner medulla. In the inner medullary collecting duct (IMCD), facilitated transporters UT-A1 and UT-A3 allow specific reabsorption of urea from the luminal fluid, followed by reabsorption of water down the resulting osmotic gradient via aquaporins. Transport is required since urea is a highly polar molecule with subsequently low diffusivity across lipid bilayers. A knockout mouse lacking UT-A1/A3 has a urinary concentrating defect². Activity of UT-A1 is acutely sensitive to antidiuretic hormone (ADH, or vasopressin), facilitating faster equilibration in the concentration of urea between luminal and interstitial spaces of the inner medulla, resulting in formation of more highly concentrated urine³. This study is the first to describe hyperpolarized [^{13}C]urea as a functional marker in renal imaging. We monitored signal changes localized to the medulla in dynamic imaging of infused urea in rats in acute antidiuretic and diuretic states, testing differential activity of urea transporter UT-A1.

Methods- Hyperpolarization: 99% [^{13}C]urea was dissolved in glycerol to 6.4M, with 23mM trityl radical OX063 and 1.5mM Dotarem. The sample was polarized by DNP in a HyperSense polarizer, and rapidly dissolved in PBS for a 150mM solution. **Animals:** Four male Sprague-Dawley rats were scanned in both antidiuretic and diuretic states on the same day. Standard protocols were used to induce these states⁴. For antidiuresis, rats were deprived of food and water for an overnight period of 16hrs. For the first imaging study in the morning, rats were injected with 1.5mL 150mM urea over 6sec (tail vein). For diuresis, rats were allowed free access to 10% (wt/wt) glucose water for 8hrs, and the experiment was repeated. **Imaging:** Rats were scanned in a GE 3T human scanner. A single axial slice (15 mm) centered on the right kidney (Fig. 1) was imaged every 3sec over 2min. A pulse sequence employing balanced SSFP was used⁵: matrix=32x32, resolution=2.5mmx2.5mm, alpha=±15° (with alpha/2 catalysis), TE/TR=5.75ms/11.5ms, BW=±10kHz. A 6M [^{13}C]urea syringe was strapped anteriorly for transmit gain calibration. Axial/coronal ^1H images were also acquired. **Data analysis:** Images were resampled and overlaid in color on grayscale ^1H images. Data was corrected for T_1 (i.e. divided by e^{-t/T_1} with $T_1 \approx 17$ sec *in vivo*). Images were normalized for polarization,

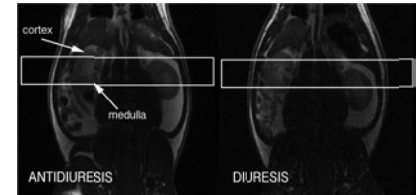


Figure 1. Coronal ^1H images through rat kidneys in antidiuresis (left) and diuresis (right), showing ^{13}C scan slices through rat right kidney.

using the magnitude of a central k-space sample at the 3sec timepoint. Dynamic curves were plotted for ROI's corresponding to the right renal medulla and cortex. We applied the concept of a 'moment of inertia' (MOI) to measure spatial centralization of the urea signal over time, which reflects medullary urea transport since the medulla is located radially inward from cortex. In analogy to a physical moment of inertia, we computed the summation of each image voxel's signal intensity times the square of its distance to the center of the kidney.

Results- Dynamic images from diuretic and antidiuretic states are shown in Fig. 2. Noise is inflated in later time frames by the T_1 correction. Faster medullary enhancement is evident in the case of antidiuresis. This is seen as more rapid centralization of the urea signal. Differentiation between the two states was detected at 30-50sec. The dynamic curves (Fig. 3) showed similar results. In the case of the rat in Fig. 2A, clear separation in medullary signals of 36% occurred by 45 sec (Fig. 3A). Some of the dynamic curves exhibited spurious fluctuations due either to noise, Gibbs ringing, or inability to precisely delineate anatomic margins at this resolution (Fig. 3B). Variable polarization may also affect the curves. As a direct measure of the centralization of urea signal, the MOI (Fig. 4) relates directly to the visual differences apparent in Fig. 2. For each animal, the moment curve takes on a general shape based on anatomy, with consistent variation induced in the two states. The moment is inversely related to the rate of urea transport³. Overall, the antidiuresis curve was statistically lower at any given timepoint ($p=0.0004$, single-sided sign test). The mean maximum difference between curves among the four rats was $6.5 \pm 3.1\%$ ($p=0.012$, paired single-tailed t-test).

Discussion- Through dynamic imaging of hyperpolarized urea, we detected increased activity of urea transporter UT-A1 in antidiuresis *in vivo*. Faster equilibration between IMCD lumen and interstitial space results in a larger space for urea, and thus faster medullary enhancement. Glomerular filtration rate (GFR) should not vary between the two states, if anything potentially reduced under antidiuresis if a 'pre-renal' state develops. Each rat received a tiny dose of glycerol, needed for DNP. High doses of glycerol induce renal failure in rats⁶. However, that dose is 80x our dose. The ability to monitor urea transport *in vivo* is exciting for its role in disease states such as lithium-induced diabetes insipidus⁷ and for novel diuretic drugs that inhibit urea transporters⁸. Hyperpolarized urea may also have an important clinical role as a contrast agent for angiography or perfusion imaging throughout the body, amid growing concern over the risks of iodinated radiographic agents⁹ and paramagnetic Gadolinium chelates¹⁰ in patients with impaired renal function.

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References- 1. Sands and Layton. *Semin Nephrol.* 2009. 2. Fenton et al. *PNAS.* 2004. 3. Klein et al. *JASN.* 2006. 4. Kato and Sands. *Am Journ Physiol.* 1999. 5. von Morze et al. *JMRI.* 2011. 6. Oken et al. *J Clin Invest.* 1966. 7. Klein et al. *Kidney Int.* 2002. 8. Levin et al. *Faseb J.* 2007. 9. McCullough et al. *Rev Cardiovasc Med.* 2006. 10. Kuo et al. *Radiology.* 2007.

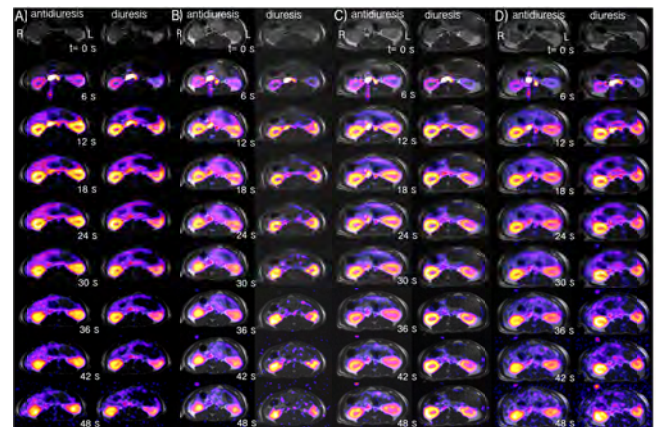


Figure 2. Dynamic renal imaging of hyperpolarized urea (color) in four rats (A-D) in antidiuretic (left) and diuretic (right) states.

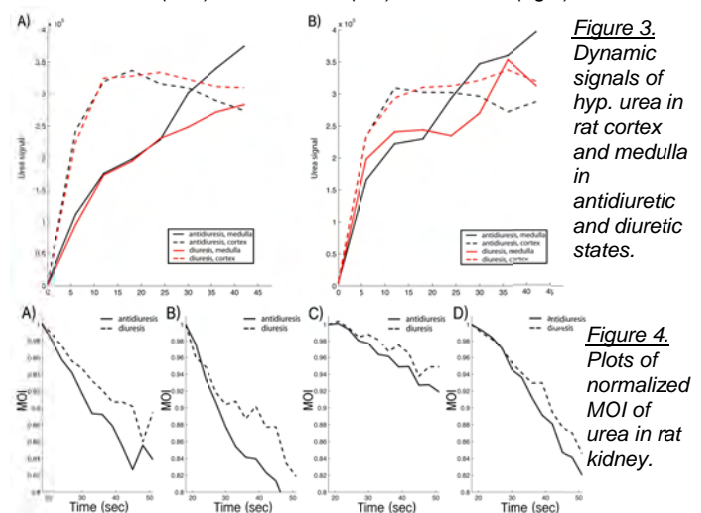


Figure 3. Dynamic signals of hyp. urea in rat cortex and medulla in antidiuretic and diuretic states.

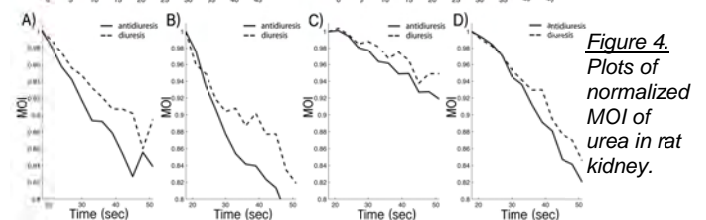


Figure 4. Plots of normalized MOI of urea in rat kidney.