New Insights into the Molecular Regulation of Urine Concentration

Editorial focus on: Metformin, an AMPK activator, Stimulates the Phosphorylation of Aquaporin-2 and Urea Transporter A1 in Inner Medullary Collecting Ducts

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Running title: Metformin, AMPK and urine concentration
Abstract

The ability to concentrate urine was a critical step in evolutionary development, and defects in urine concentration continue to plague humans. In order for kidneys to appropriately regulate urinary water excretion, by varying the concentration and/or dilution of urine, there needs to be integrative interaction of multiple proteins in multiple renal epithelial cells. Two critical proteins in this process are AQP2 and UT-A1. In the manuscript that is the subject of this editorial focus, Dr. Klein and colleagues identify a new and novel mechanism regulating both AQP2 and UT-A1. Specifically, they identify that the adenosine monophosphate (AMP)-stimulated kinase (AMPK) phosphorylates both of these proteins, a critical step necessary for activation of their transport function. In vitro assays, using pharmacologic agonists of AMPK, confirmed increased water and urea transport. Finally, the authors show in a murine model of congenital nephrogenic diabetes insipidus, induced by deletion of the V2 receptor, that the AMPK agonist, metformin, increases the ability to concentrate the urine. Because metformin can stimulate AMPK, and the authors demonstrate that AMPK is present in the IMCD, these results suggest AMPK activation may have an important role in the regulation of urine concentration. These findings offer the potential to open an entirely new line of investigation into the molecular mechanisms regulating urine concentration. Furthermore, because an FDA approved agonist of this system, metformin, is already available for clinical use, these findings may be able to be translated rapidly into clinical use.
Introduction

The ability to excrete a concentrated urine was a critical future in evolutionary development, and continues to be necessary for humans to maintain health. Urine concentration is a complex process, and requires interaction between multiple renal epithelial cell segments and the appropriate function of multiple proteins. Critical aspects of the roles of the thin descending and thick ascending limb of the loop of Henle, the connecting segment and the collecting duct had been recognized and intensively studies over the past several decades. The inability to concentrate the urine appropriately is termed diabetes insipidus, and when it is due to kidney-specific defects it is termed nephrogenic diabetes insipidus (NDI) (1).

The recent study by Klein and colleagues in this issue (5) is an important addition to our understanding of the molecular mechanisms regulating urine concentration and has the potential for rapid clinical translation. Metformin is a medication widely used in clinical medicine for its glucose lowering effects in patients with diabetes, and is believed to work by activating the adenosine monophosphate-activated kinase, AMPK. Klein and colleagues show that AMPK is present in the renal inner medulla and that it phosphorylates two key proteins involved in urine concentration, AQP2 and UT-A1. In vitro microperfusion studies show that the AMPK agonist, metformin, increases IMCD water and urea permeability, and that an alternative AMPK agonist, AICAR, has similar effects on urea permeability. Finally, they show that in a model of nephrogenic diabetes insipidus, genetic deletion of the V2 receptor, that metformin increases urine concentration.

The molecular mechanisms through which metformin is having these effects is likely to involve activation of the cytosolic energy sensor, AMPK. AMPK is a heterotrimeric protein,
composed of one alpha subunit, one beta subunit and one gamma subunit (2), and Klein and
colleagues show that both known alpha subunits, alpha-1 and alpha-2, are expressed in the inner
medulla (5). AMPK is activated by AMP and ADP binding to the beta-subunit, and inhibited by
ATP, thereby enabling it to function as a “cytosolic energy sensor.” However, AMPK is also
regulated by a variety of other mechanisms, including intracellular Ca\(^{2+}\), liver kinase B (LKB),
calcium-calmodulin-dependent kinase kinase (CaMKK\(\beta\)) and transforming growth factor \(\beta\-
activated kinase (TAK1) (2). Although metformin is widely recognized to activate AMPK, its
effects also include AMPK-independent mechanisms, including interaction with mTOR,
PRAS40 and RAPTOR (7). Klein and colleagues do show the alternative AMPK activator,
AICAR, has similar effects as metformin on IMCD urea permeability, supporting the likelihood
that metformin is active through AMPK. However, it is important to recognize that metformin
and AICAR can have similar biological effects, but through distinct, but differing, mechanisms
that do not involve AMPK (7).

The studies add to a growing list of evidence that AMPK has important biological roles
other than serving solely serving as a cytosolic “energy sensor.” AMPK is been shown to
regulate multiple and transport processes in the kidney, including H-ATPase, NaPi2a, ROMK,
ENaC and KCNQ1 (8). It may be involved in the progression of chronic kidney disease, in renal
cystogenesis in autosomal dominant polycystic kidney disease and in acute kidney injury (3, 6,
9). Finally, it may be involved in renal cytokine responses (4). Of important concern,
particularly as clinical implications of AMPK activation are examined, is the report that AMPK
activation increases renal medullary cell apoptosis in both normally hydrated and dehydrated
mice with type II diabetes mellitus (10).
Where do we go from here? First, Klein and colleagues are to be congratulated for identifying a novel, and potentially rapidly translatable, mechanism regulating AQP2, UT-A1 and urine concentration. Second, several important questions remain to be examined. For example, does metformin act through AMPK activation or through AMPK-independent effects. If AMPK is involved, which of the alpha subunits is necessary? Is metformin’s ability to stimulate urine concentration specific for V2 receptor deletion-specific models of nephrogenic DI or does it have the same effect in other causes of nephrogenic DI? Why does metformin not routinely cause excessive urine concentration and lead to hyponatremia in patients who do not have diabetes insipidus? Finally, given that the most common form of NDI is due to lithium therapy, is not reversible with lithium discontinuation, and places patients at increased risk of hypernatremia, it is hoped that carefully performed preclinical and clinical trials of metformin therapy in first animals and then humans with lithium-induced NDI will be forthcoming.
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5. **Klein JD, Y Wang, MA Blount, PA Molina, LM LaRocque, JA Ruiz and JM Sands.** Metformin, an AMPK activator, stimulates the phosphorylation of Aquaporin 2 and Urea Transporter A1 in Inner Medullary Collecting Ducts. *Am J Physiol Renal Physiol*


7. **Liu X, RR Chhipa, S Pooya, M Wortman, S Yachyshin, LML Chow, A Kumar, X Zhou, Y Sun, B Quinn, C McPherson, RE Warnick, A Kendler, S Giri, J Poels, K Norga, B Viollet, GA Grabowski and B Dasgupta.** Discrete mechanisms of mTOR and

