A timely characterization of vasopressin-sensitive adenylyl cyclase isoforms in the mouse inner medullary collecting duct

Mitsi A. Blount

Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia

SECOND MESSENGER SIGNALING in the kidney mediates several of the organ’s basic physiological functions including the regulation of blood pressure, control of fluid volume, and maintenance of the body’s water homeostasis in times of duress. Tight regulation of the second messenger cAMP is critical since a two- to threefold increase of this cyclic nucleotide produces a maximum physiological response in most tissues (8). Most cAMP-sensitive cellular processes that occur by PKA activity must fit within a narrow range of cAMP concentration for optimal modulation of cellular function (8). This is especially true in the inner medulla, where vasopressin (AVP)-stimulated cAMP signaling mediates the body’s ability to conserve water (17). There are potentially many ways that the inner medullary collecting duct (IMCD) could regulate intracellular cAMP concentration; however, without a better understanding of how AVP stimulates cAMP production in these cells such methods remain elusive.

In their article in the American Journal of Physiology-Renal Physiology, Strait et al. (21) provide an informative report that identifies the adenylyl cyclase (AC) isoforms in mouse IMCD that are activated by AVP. To date, there are nine mammalian transmembrane ACs as well as a tenth soluble form that has distinct catalytic and regulatory properties (12). The nine membrane-bound ACs are classified into four categories based on regulatory properties: group I includes Ca2+-stimulated AC1, AC3, and AC8; group II contains Gβγ-stimulated AC2, AC4, and AC7; group III encompasses Gαi/Ca2+-inhibited AC5 and AC6; and group IV is the forskolin-insensitive AC9. Because of the low abundance of AC expression and the unavailability of satisfactory antibodies for most of the isoforms, most of the data for tissue distribution rely on detection of mRNA levels. In some cases, isoform expression can be confirmed by functional assays based on the different regulatory properties, but interpretation of these results is often complicated by the fact that most cells express two or more AC isoforms (12). The difficulty in identifying AC isoforms may explain why there is little investigation of ACs in the IMCD. The few studies that have been performed not only focus exclusively on rat IMCD but also report conflicting expression patterns (1, 4, 9, 18). In this article, Strait et al. (21) were able to screen acutely isolated mouse IMCDs for AC isoform mRNA and protein expression. The authors found that the only AC isoforms in the mouse IMCD are AC3, AC4, and AC6.

Although it is not completely clear why multiple AC isoforms are expressed in a particular cell, the distinct properties, cellular location, and overall expression of individual isoforms can regulate specific signaling pathways. Currently, there are no potent AC isoform-specific inhibitors. This has made the biochemical characterization of AC isoform function extremely difficult. The authors undertook the challenging study of determining how AVP mediates cAMP synthesis with two innovative approaches: 1) altering signaling pathways that uniquely regulate specific AC isoforms by pharmacological intervention and 2) small interfering RNA (siRNA) knockdown of AC3, AC4, or AC6.

In addition to increasing cAMP, AVP has been shown to cause an increase in Ca2+ concentration in the IMCD (20). Other investigators have suggested that calmodulin regulates AC activity in the rat IMCD (5, 9). Of the three AC isoforms identified in this highlighted study, AC3 and AC6 are regulated by calcium. The authors found that AVP-mediated cAMP production was in part dependent on the calcium-stimulated AC3. Knockdown of this isoform with siRNA confirmed the importance of this enzyme in AVP-mediated signaling. Interestingly, thapsigargin and CaMKII inhibition reduced AVP-induced cAMP synthesis, suggesting that AC6 is also involved in AVP action. The authors confirmed this finding by showing that siRNA knockdown of AC6 reduced AVP-stimulated cAMP synthesis. While most of the AVP-mediated cAMP accumulation was found to be regulated by calcium, the authors found that a small fraction was unaffected, suggesting that calcium-insensitive AC4 could also mediate some of the AVP response. However, siRNA knockdown of AC4 failed to show any effect.

This article by Strait et al. is perhaps long overdue. In recent years, several investigators have begun to focus on AVP-mediated effects in the IMCD. The AVP-stimulated increase in cAMP often triggers several cellular responses through PKA. PKA phosphorylation of aquaporin (AQP)-2 is important for AVP-stimulated trafficking of this transporter to the apical plasma membrane of the IMCD (10, 11), leading to increased levels of water reabsorption. Similarly, movement of the urea transporter UT-A1 to the membrane is also the result of PKA-mediated phosphorylation of the transporter (3). AVP stimulates movement of UT-A3 to both the basolateral and apical membranes of the IMCD (2). The AVP-mediated increase in cAMP also stimulates the guanine nucleotide exchange factor Epac. Perfused rat IMCD treated with an Epac-selective cAMP agonist mimics AVP-stimulated movement of AQP2 to the apical membrane (24). Our laboratory has recently found (23) that activation of Epac increases urea transport, UT-A1 phosphorylation, and UT-A1 plasma membrane accumulation. It is possible that each of these processes is regulated by individual AC isoforms or through compartmentalization of cAMP. Specific AC isoforms can be tethered to lipid rafts, caveolae, or A-kinase anchoring proteins (AKAPs) (6, 7, 13), which create small pools of highly concentrated cAMP at the plasma membrane. This could be important in AVP-stimulated trafficking of AQP2 or the urea transporters in the IMCD. Along those same lines, AC isoforms can also form protein-protein interactions with other proteins to regulate trafficking. Recently,
AC6 has been shown to bind to snapin (22). We have observed (14, 15) that snapin mediates trafficking of both AQP2 and UT-A1 to the apical plasma membrane of the IMCD. This initial characterization of AC isoforms performed by Strait and colleagues (21) provides the groundwork to answer these and many other questions. Teasing out the signaling mechanisms behind AVP-mediated urine concentration will have a great physiological impact, particularly for those afflicted with nephrogenic diabetes insipidus. Creation of transgenic mouse models that knock down AC3, AC4, or AC6 expression would be ideal to study the importance of AVP signaling in the kidney. Reportedly, AC6-knockout mice have been generated and do appear to have a urine-concentrating defect (16). Given the findings reported here, it will be exciting to see what data the AC6-null mice yield.

These experiments performed by Strait et al. (20) have shed new light onto understanding of the downstream effects of AVP in the IMCD. By characterizing the AVP-sensitive AC isoforms we can now begin to investigate the many cellular process and regulatory mechanisms mediated by cAMP. These studies will no doubt provide the framework for the acquisition of new information on the underlying mechanisms behind the dysregulation of water homeostasis that occurs in common clinical disorders such as congestive heart failure, cirrhosis, and nephrotic syndrome, as well as nephrogenic diabetes insipidus.

GRANTS
The author is supported by National Institute of Diabetes and Kidney Diseases Grant K01-DK-082733.

DISCLOSURES
No conflicts of interest are declared by the author.

REFERENCES