Collecting duct expression of N-methyl-d-aspartate receptor subtype NR3a regulates urinary concentrating capacity

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**N-METHYL-D-ASPARTATE RECEPTORS (NMDARs)** are known to be essential for proper development and function of the central nervous system (CNS) (5). NMDARs are activated by glutamate, the most common excitatory neurotransmitter in the CNS, and NMDARs constitute a subfamily of ion channels identified by specific molecular composition and unique pharmacological and functional properties. NMDARs are molecularly organized as heteromeric complexes incorporating different subunits within a repertoire of three subtypes: NR1, NR2, and NR3, the latter of which has two subunits (NR3a and NR3b) (3). In addition to their expression in the brain, NR1 and NR2 have been demonstrated in both the renal cortex and medulla (2), and recently increased NR1 expression has been implicated in renal ischemia-reperfusion injury (7), suggesting that this family plays important roles outside the brain.

In an issue of the *American Journal of Physiology: Renal Physiology*, Sproul and colleagues (6) add another exciting dimension to NMDARs by demonstrating that NR3a and NR3b are highly expressed in the developing kidney and show persistent high levels of NR3a protein abundance in the renal medulla and papilla of the adult mouse. In assessing the human genome of NR3a and NR3b, the authors identified a potential transcription factor binding site upstream of NR3a, which matched the known consensus binding sequence for the tone-inducible transcription factor TonEBP. Based on this observation they hypothesized that there may be toxicity-driven expression of NR3a in the medullary region of the adult kidney, which is chronically exposed to a hypertonic environment. To examine this, they used an elegant design combining a targeted proteomics approach with knockdown of NR3a both in vivo and in vitro carefully dissecting the potential importance of NR3a in kidney function. By confocal imaging and colocalization of NR3a with *Dolichos biflorus* agglutinin, the authors demonstrate that NR3a is localized to the basolateral membrane of the collecting duct. This was confirmed in inner medullary collecting duct (IMCD) cells, and by incubation of IMCDs in 0.1% oxygen for 24 h there was a fourfold increase in NR3a protein expression, demonstrating that hypoxia regulates NR3a. Exposure of IMCDs to graded osmolality further suggests that media toxicity may additionally increase NR3a expression, which was confirmed in vivo by increased NR3a expression in the papilla of the kidney in mice subjected to 18-h water restriction, suggesting that NR3a is important for urine concentration.

The final concentration of the urine depends on the medullary osmotic gradient built up by the loop of Henle and the water permeability of the collecting ducts carrying the urine through the cortex and medulla (4). Collecting duct water permeability is regulated by vasopressin binding to the vasopressin V2 receptor regulating aquaporin-2 (AQP2) trafficking to the apical membrane. Regulation of intracellular calcium is involved in AQP2 trafficking and appears to involve a calcium-sensing receptor and the Ca\(^{2+}\)/calmodulin-dependent enzyme myosin light chain kinase, hypothesized to mediate local AQP2 shuttling (1). The results of the study by Sproul and colleagues (6) using partial knockdown achieved with short hairpin (sh) RNA in IMCD cells suggest that NR3a may also be directly/indirectly involved in AQP2-regulated trafficking, since impairment of the Ca\(^{2+}\)-dependent trafficking pathway resulting from elevated intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) may explain the decrease in surface expression of glycosylated AQP2 observed in IMCD cells with reduced NR3a. In NR3a knockout cells, stimulation of vasopressin also led to an unexpected transepithelial osmotic gradient despite the activation of vasopressin receptors. This may be due to increased salt transport caused by amiloride-sensitive epithelial Na channel (ENaC) activity and reduced water movement across the epithelium. The latter is supported by reduced AQP2 expression in both shRNA-transfected IMCDs and in NR3a knockout mice. Interestingly, in IMCDs only decreased expression of the glycosylated form of AQP2 was observed. This could be a direct effect of elevated [Ca\(^{2+}\)], but this needs to be addressed further together with a thorough dissection of AQP2 regulation by NMDARs.

One of the interesting observations of the present paper is the finding that NR3a regulates Ca\(^{2+}\) permeability but fails to respond to changes in [Ca\(^{2+}\)]\(_i\) to classic NMDAR agonists and antagonists, despite the presence of other collecting duct NMDAR subunits. This may suggest that NR3a has other effects on cell function and regulation of [Ca\(^{2+}\)]\(_i\), that may be independent of the classic role of NMDAR subunits (6). The significance of NR3a being abundantly expressed in the neonatal kidney and declining with age is not answered in the present paper, but potentially NR3a could play a role for nephron development.

The novel finding that NR3a is located to collecting duct principal cells in the adult kidney is exciting and may be the first step to unraveling yet another important element in the complex regulation of urinary concentrating capacity. Since one major function of NR3a in the kidney collecting duct appears to be preservation of low cytosolic calcium to maintain functional calcium-dependent signaling including vasopressin-induced trafficking of AQP2. Thus NR3a may be an important target for protection of collecting duct cells and, under conditions that are associated with high vasopressin levels, may be one mechanism that protects the function of the principal cells to reabsorb water, thereby helping to maintain the countercurrent multiplication system. The present paper therefore potentially opens the door for exploring new pathways which are...
important for understanding kidney physiology and pathophysiology.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

REFERENCES


