Primary cultures of inner medullary collecting duct cells: an isolated system to study the mechanisms of renin regulation.

Alexis A Gonzalez*, Lucienne S Lara**, Minolfa Prieto-Carrasquero* and L. Gabriel Navar*. *Physiology Department and Hypertension Renal Center of Excellence, Tulane University School of Medicine New Orleans, LA, **Instituto de Ciencias Biomedicas, Universidade Federal do Rio de Janeiro, Brazil.

All of the components of the renin-angiotensin-system (RAS) are expressed in kidneys and regulated independently from the "classic" systemic RAS. The final effector, Angiotensin II (Ang II), is present at high levels in the kidneys of hypertensive animals. An important fraction of this Ang II is generated in proximal tubule cells. However, emerging evidence demonstrates the expression of renin in principal cells of collecting ducts (CD) and its up-regulation by Ang II. The presence of angiotensinogen (AGT) in the urine and the expression of angiotensin converting enzyme (ACE) in the distal nephron support the concept that renin in the CD might contribute to additional intrarenal Ang II generation. Although it has been shown that Ang II stimulates CD renin via Ang II type 1 receptor (AT1R); little is known about the possible mechanisms. During chronic activation of RAS, many other factors may contribute to renin regulation in principal CD cells, including activation of the mineralocorticoid receptor (MR) by aldosterone, the increase in Na+ reabsorption through the epithelial sodium channels (ENaC); activation of vasopressin type 2 receptor (V2R); and the activation of E prostanoid receptors by prostaglandin E2 (PGE2) in response to Ang II. Accordingly, the objective of this study is to characterize the presence RAS components and E Prostanoid receptors (EP1-4) in an isolated system composed by primary cultures of inner medullary collecting duct (IMCD) cells. Expression of renin, AT1R, MR, ENaC, Aquaporin-2, and EP receptors was evaluated by immunoblotting, immunofluorescence and qRT-PCR techniques. Functional AT1R activity was evaluated by changes in intracellular calcium (Ca++2) in response to Ang II treatment. Our results demonstrate the presence of mRNA and protein for renin, aquaporin-2, aENaC, MR and AT1R. Incubation with Ang II (10^-8 M) showed a robust increment in intracellular Ca++2, demonstrating the functional expression of AT1R in the IMCD cell plasma membrane. EP1 receptor was highly expressed whereas EP3 and EP2 were detected at low levels, EP4 was not detect. These results provide support for a prospective study on CD renin regulation by interaction among signaling pathways of RAS components and EP receptors.

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