MOLECULAR AND FUNCTIONAL STUDIES OF MEMBRANE ION TRANSPORTERS

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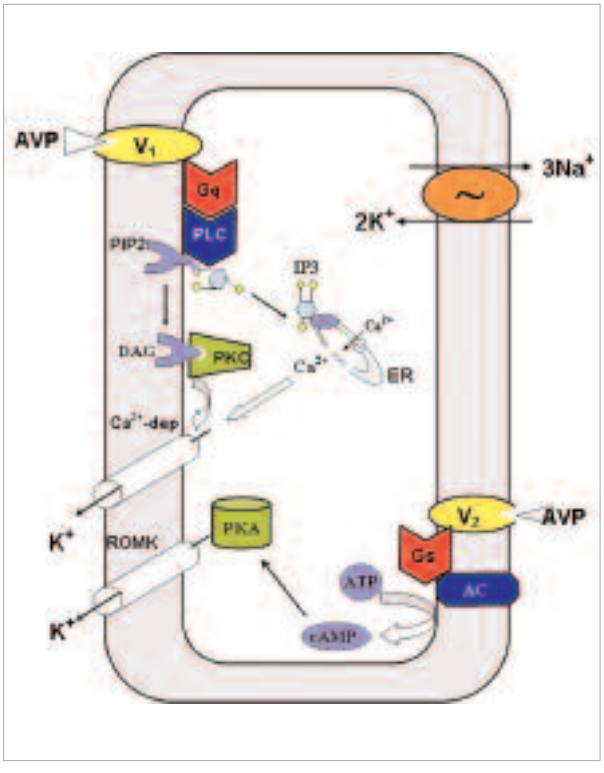

Schematic drawing of the action of vasopressin on a principal cell of the collecting duct or of the connecting segment. V1 and V2, vasopressin receptors; G, G-proteins; PKA, protein kinase A; PKC, protein kinase C; AC, adenylate cyclase; ER, endoplasmic reticulum; AVP, arginine-vasopressin

The general objective of this project is the investigation of the molecular and functional mechanisms of ion transport in cells, particularly of the epithelial type, originating from renal and other tissues. Among the methods that will be used for this purpose are renal micropuncture and microperfusion, molecular biology including transfection of wild type and mutant transporters into cultured cells, electrophysiology ("patch-clamp") for the analysis of individual ion channels, determination of cell volume regulation and the role of ion transporters in this regulation, measurement of cell ion activities by fluorescence microscopy allowing for the determination of cell pH and calcium levels. These studies will be performed in mammalian kidney, intestine, colon crypts, cells in primary and permanent cultures such as MDCK, T84, IRPTC and others. Transporters of H^+, HCO3- and K^+ will be investigated, including Na^+/H^+ exchangers, H^+ and H^+/K^+ ATPases, K^+ channels, $Cl^-/HCO3^-$ exchangers, and $Na^+/HCO3^-$ cotransporters, passive mechanisms, and the role of hormones (aldosterone, angiotensin, vasopressin, atrial natriuretic factor, parathyroid hormone) in the regulation of these mechanisms will be studied. Techniques for the determination of transepithelial and transmembrane (apical and basolateral) ion fluxes using microelectrodes or cell fluorescence will be used. Molecular properties of transporters such as the isoforms of the Na^+/H^+ exchanger and of protein kinase C, their genetic modulation and the role of protein regulators (NHERF) in the regulation of the transfer of H^+ will be investigated. Cell signaling of the regulation of H^+ and K^+ ion transport will be studied in different experimental conditions.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Fluorescence and confocal microscopy: Cell pH and calcium level were determined by fluorescence techniques (BCECF for pH and Fluo 4 for Ca) in cultured renal and intestinal cells in order to study the signaling mechanisms of H^+ and Ca^{2+} transport, as well as other regulation mechanisms by angiotensin and vasopressin.

The effects of aldosterone on the intracellular pH recovery rate (pHirr) via Na^+/H^+ exchanger and on the cytosolic free calcium ($[Ca^{2+}]i$) were investigated in rat S3 segment *in vitro*. Aldosterone [10^{-12}, 10^{-10} or 10^{-8} M with 1 h, 15 or 2 min preincubation (pi)] caused a dose dependent increase in the pHirr, but aldosterone (10^{-6} M with 1 h, 15 or 2 min pi) decreased it.

Microperfusion of renal tubules *in vivo*: The direct action of aldosterone (10^{-12} M) on net bicarbonate reabsorption ($JHCO_3^-$) was evaluated by stationary microperfusion of *in vivo* middle proximal tubule (S2) of rat kidney, by using H^+ ion-sensitive microelectrodes. Aldosterone in luminally perfused tubules caused a significant increase in $JHCO_3^-$. Aldosterone perfused into peritubular capillaries also increased $JHCO_3^-$ when compared with basal levels during intact capillary perfusion with blood.

Studies in potassium channel (ROMK) knockout mice were performed, showing that the loss of these channels was largely compensated by maxi-K channels, which are calcium and PD dependent. In rat studies it was shown that vasopressin acts on K^+ transport by V1 (luminal) and V2 (basolateral) receptors, the former mediated by maxi-K channels.

Studies are focused on the Na^+/H^+ exchanger NHE3: We have investigated the mechanisms of chronic regulation of NHE3 by analyzing its promoter activity under influence of changes in pH, and at the parathyroid (PTH) and angiotensin II (AII) levels.

Role of dipeptidyl peptidase IV (DPPIV) in the function of NHE3: Rats were fed for seven days a specific inhibitor of DPPIV. NHE3 activity was depressed due to lower expression of this transporter in the microvilosities of the proximal tubule brush-border, causing natriuresis, diuresis and a more alkaline urine compatible with NHE3 inhibition.

Phenylpropenes are a class of substances produced by angiosperm plants, among which eugenol is included, with a variety of biological functions. In electrophysiological experiments we have demonstrated that eugenol and a series of analog compounds block the action potential firing in mammalian nerves through a reversible, fast inhibitory action on voltage-gated sodium channels.

Purinergic receptors: Fluctuations in the intracellular calcium concentration of satellite cells kept in fresh cultures of cells isolated from the dorsal root ganglion of rats, as measured by fluorescence microscopy, indicate the presence of purinergic receptors in the satellite cells. In addition, our data are consistent with such receptors being of the P2Y, metabotropic type.

MAIN PUBLICATIONS

Oliveira-Souza M, Musa-Aziz R, Malnic G, Mello-Aires M. 2004. Arginine Vasopressin stimulates H^+-ATPase in MDCK cells via V1 (cell Ca^+) and V2 (cAMP) receptors. *Am. J. Physiol. Renal Physiol.* **286**: F402-F408

Amorim JBO, Musa-Aziz R, Mello-Aires M, Malnic G. 2004. Signaling path of the action of AVP on distal K^+ secretion. *Kidney Int*. **66**:696-704.

Aziz Raif Musa, Souza MO, Mello-Aires M. 2005. Signalling pathways in the biphasic effect of ANG II on Na^+/H^+ exchanger in T84 cells. *Journal of Membrane Biology, USA*. **205**:1-12.

Bailey M, Cantone A, Yan Q, MacGregor G, Leng Q, Amorim JBO, Wang T, Hebert SC, Giebisch G, Malnic G. 2006. Mechanism of urinary potassium excretion in the ROMK-deficient mouse model of type II Bartter's syndrome. *Kidney Int.* **70(1)**:51-59.

Carraro-Lacroix LR, Ramirez MA, Zorn TMT, Rebouças NA, Malnic G. 2006. Increased NHE1 expression is associated with serum deprivation-induced differentiation in immortalized rat proximal tubule cells. *Am. J. Physiol. Renal Physiol*. **291(1)**:F129-F139.

Caruso-Neves C, Kwon SH, Guggino WB. 2006. Albumin endocytosis in proximal tubule cells is modulated by angiotensin II through an AT2 receptor-mediated protein kinase B activation. *Proc. Natl. Acad. Sci, USA*. **103**:18810-18815.

Amorim JBO, Musa-Aziz R, Lessa LMA, Malnic G. 2006. Effect of uroguanylin on potassium and bicarbonate transport in rat renal tubules. *Canadian Journal of Physiol. Pharmacol.* **84**:1003-1010.

Beltrán AR, Ramírez MA, Carraro-Lacroix LR, Hiraki Y, Rebouças NA, Malnic G. 2008. NHE1, NHE2, and NHE4 contribute to regulation of cell pH in T84 colon cancer cells. *Pflugers Arch*. **455**:799-810.

Girardi A, Fukuda L, Rossoni L, Malnic G, Rebouças N. 2008. Dipeptidyl Peptidase IV Inhibition Downregulates Na^+-H^+ Exchanger NHE3 in Rat Renal Proximal Tubule. *Am. J. Physiol. Renal Physiol*. **294**:F414-F422.

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