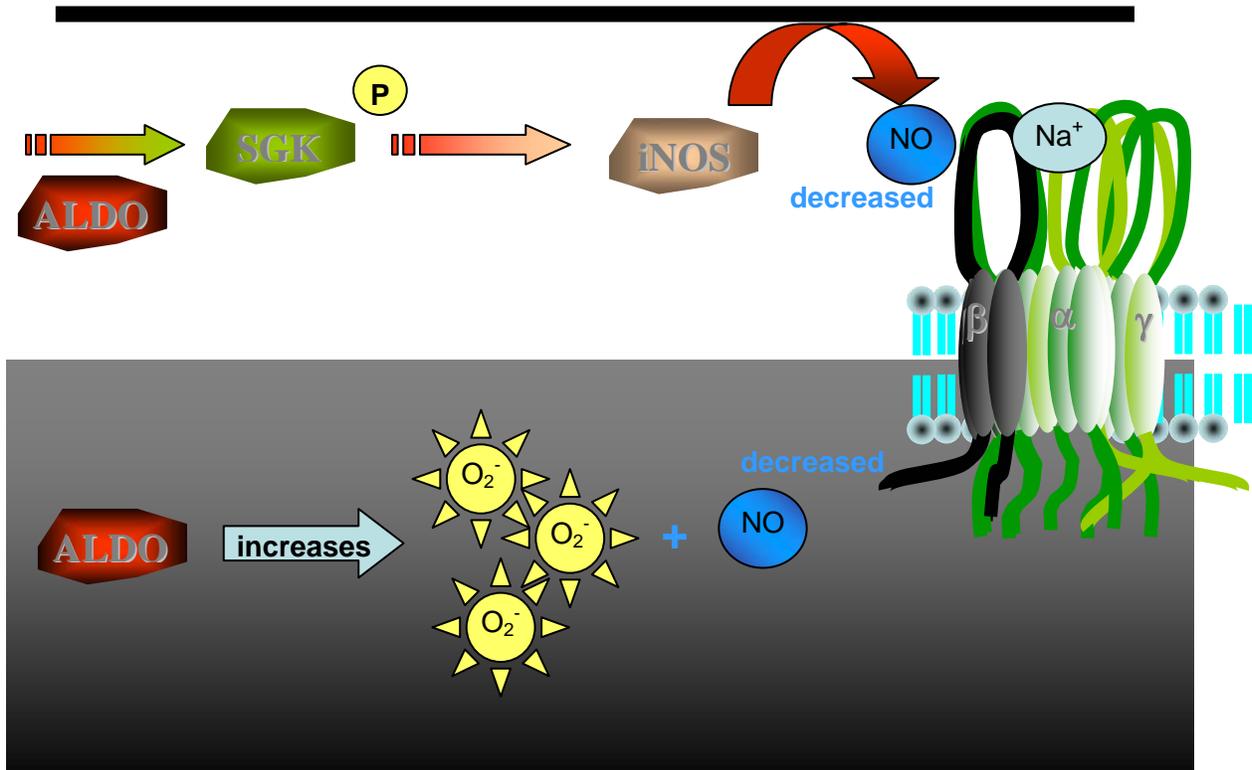


## Current Kidney Research Interests.....

key words: distal kidney cells, aldosterone, ENaC, SGK, nitric oxide, reactive oxygen species

### MODEL FOR ALDOSTERONE REGULATION OF NO AVAILABILITY IN Na TRANSPORTING CELLS



Aldosterone regulates nitric oxide inhibition of epithelial Na channel (ENaC) activity in A6 distal kidney cells. My N. Helms and Douglas C. Eaton. Emory University; Dept. of Physiology and Center for Cell and Molecular Signaling; Atlanta, GA 30322

*Introduction.* Nitric oxide (NO) is a highly diffusible, short lived free radical that is synthesized in cells from L-arginine in a reaction catalyzed by NO synthase (NOS). We, and others, have recently shown that nitric oxide is involved in the regulation of sodium balance. Recently, using single channel patch clamp techniques, we showed that apical application of 1.5 $\mu$ M nitric oxide releasing compound, PAPA-NONOate, to A6 distal kidney cells significantly decreased ENaC open probability ( $P_o$ ) from  $0.186 \pm 0.043$  to  $0.045 \pm 0.009$  ( $n=7$ ,  $P < 0.05$ ) without changing the unitary current. In addition, NO donor compounds also significantly decreased transepithelial current in A6 and M-1 mouse cortical collecting duct (CCD) cells grown on permeable supports,

compared to an inactive control compound. The inhibitory effects of nitric oxide on sodium transport were observed within 1 minute, and persisted for at least 10 minutes without significant change. Based on these findings, our aim was to better understand the role of nitric oxide in the regulation of epithelial sodium channels. Since aldosterone and glucocorticoids, the principle physiological regulators of ENaC, decrease NO by inhibiting iNOS activity, without effecting iNOS mRNA expression, we hypothesized that one mechanism by which aldosterone produces an increase in Na reabsorption is by reducing NO's inhibitory effect on ENaC activity.

*Aldosterone decreases nitric oxide release in sodium transporting epithelia.* Our data suggests that NO release is indeed regulated by aldosterone. Na transporting alveolar epithelial cells treated with 1.5 $\mu$ M aldosterone (for 4 hours) released 50% less NO molecules compared to control cells.  $^{32}$ P labeling of M-1 CCD cells grown with/without 1.5 $\mu$ M aldosterone also suggests that aldosterone regulates NO production, possibly via phosphorylation of inducible NOS isoforms. (Several NOS isoforms have been shown to be inhibited by phosphorylation). We immuno-precipitated iNOS protein from M-1 CCD cells labeled with  $^{32}$ P-Pi [using both anti-iNOS and anti-SGK1 (serum and glucocorticoid kinase) antibodies] cultured in the presence or absence of 1.5 $\mu$ M aldosterone and found that 1) aldosterone increased  $^{32}$ P phosphate incorporation into iNOS and 2) immunoprecipitation of SGK co-immunoprecipitated phosphorylated iNOS. Further, our studies show that SGK1 and iNOS are closely associated in epithelial cells (as determined by dual labeling immunohistochemistry), and that SGK enzyme phosphorylates *mi*NOS S733 and S903 residues in *in vitro* kinase assays. SGK is a known regulator of ENaC activity, and our current model for SGK1 regulation of ENaC presumes that aldosterone-activated SGK1 decreases iNOS activity via direct phosphorylation of iNOS, consequently lowering production of nitric oxide.

*Aldosterone increases superoxide anion production, which regulates NO availability and limits NO inhibition of ENaC function.* *In vivo*, nitric oxide quickly interacts with superoxide anions ( $O_2^-$ ) to form the potent oxidant peroxynitrite ( $ONOO^-$ ). Since several ion channels are modulated by redox mechanisms, we wanted to determine the role of  $O_2^-$  and  $ONOO^-$  formation in ENaC function. We increased endogenous levels of  $O_2^-$  in A6 cells by directly applying cell permeable superoxide dismutase inhibitor (SODi), or increased exogenous  $O_2^-$  production by combining xanthine oxidase and hypoxanthine. In contrast, we reduced  $O_2^-$  in A6 cells by applying the cell-permeable,  $O_2^-$  scavenger, TEMPO. Using single channel patch clamp techniques, we found that decreasing  $O_2^-$  levels, using 250 $\mu$ M TEMPO, significantly decreased ENaC NPo in A6 cells (from  $.96 \pm .32$  to  $.51 \pm .22$ ,  $n=10$ ,  $P<.05$ ), whereas agents that increased superoxide levels (SODi and xanthine oxidase) did not significantly alter ENaC NPo. Interestingly, pre-treating A6 cells with SODi, prevented the nitric oxide induced decreases in ENaC activity described above. Moreover, application of xanthine oxidase with hypoxanthine (which releases  $\sim 10\mu$ moles  $O_2^-$ ) reversed nitric oxide inhibition of ENaC in A6 cells. Seemingly,  $O_2^-$  prevents NO inhibition of ENaC activity. Furthermore, application of 10 $\mu$ M  $ONOO^-$  did not significantly alter ENaC NPo; thus peroxynitrite may contribute to the pathology of oxidative stress, but has no immediate inhibitory effect on ENaC. Importantly, we found that aldosterone increased  $O_2^-$  production in A6 cells. Dihydroethidium (DHE) labeling of superoxides was 50% higher in A6 cells treated with aldosterone, compared to untreated control cells. Our data suggests that aldosterone induces  $O_2^-$  production, which quickly binds and traps NO molecules, limiting nitric oxide inhibition of ENaC. Peroxynitrite formation from  $O_2^-$  and NO reactivity does not inhibit sodium channel activity.

*In summary*, our studies show that the regulation and synthesis of NO, which inhibits ENaC activity, in sodium transporting epithelia is under the control of aldosterone. It has been suggested that high levels of  $O_2^-$  release during oxidative stress can cause NO depletion, leading to vasoconstriction and blood pressure elevation. Our data supports this, as we have shown that  $O_2^-$  suppresses NO inhibition of Na reabsorption using single channel patch clamp electrophysiology. Together, our studies have identified key regulatory proteins that may be targeted in drug therapies for hypertensive disease and provide important rational for further developing antioxidant therapy.