Effect of Angiotensin II and Nitric Oxide on H^+-ATPase Activity in the Mammalian Collecting Duct

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Intercalated cells play an important role in acid-base transport in the mammalian collecting duct. Type A intercalated cells secrete protons and reabsorb bicarbonate, whereas type B intercalated cells secrete bicarbonate. However, little is known about the regulation of these transport processes. Angiotensin II (Ang II) is known to be involved in the regulation of solute transport in the kidney and its effect on sodium and bicarbonate reabsorption in the proximal tubule is well established. Although Ang II binding sites and Ang II AT_1 receptor mRNA have been demonstrated in the collecting duct, knowledge of the effect of Ang II on collecting duct function is limited. However, recent physiologic studies have suggested an effect of Ang II on bicarbonate transport in the cortical collecting duct (CCD). Moreover, in preliminary studies, we have observed ultrastructural changes in intercalated cells after infusion of Ang II, suggesting that Ang II might affect intercalated cell function.

Proton transport in intercalated cells is mediated by a vacuolar-type H^+-ATPase that is located in the apical plasma membrane and apical vesicles in type A intercalated cells and in the basolateral plasma membrane and intracellular vesicles in type B intercalated cells. In addition, there is evidence that the collecting duct secretes protons in exchange for potassium by means of an H^+-K^+-ATPase that is also located in intercalated cells.

To explore the role of Ang II in the regulation of intercalated cell function, we have examined its effect on H^+-ATPase and H^+-K^+-ATPase activity in microdissected segments of the rat CCD. H^+-ATPase activity was measured as bafilomycin-sensitive ATPase activity and H^+-K^+-ATPase activity was measured as Sch-28080-sensitive ATPase activity, using a fluorometric microassay. Incubation of CCD segments with Ang II for 60 min caused a dose-dependent inhibition of H^+-ATPase activity with maximum inhibition at 10^{-8}M Ang II. The inhibitory effect of Ang II was blocked by simultaneous incubation with 10^{-6}M Losartan, indicating that inhibition was mediated via AT_1 receptors. Incubation with PD-123319, an AT_2 receptor antagonist, had no effect on Ang II-mediated inhibition of H^+-ATPase activity. Incubation of CCD segments with Ang II had no effect on Sch-28080-sensitive H^+-K^+-ATPase activity. These results demonstrate that Ang II inhibits the vacuolar H^+-ATPase in the rat CCD via activation of AT_1 receptors and support a role for Ang II in the regulation of proton and bicarbonate transport in the CCD.

Because of evidence that NO inhibits H^+-ATPase mediated pH regulation in macrophages, we next examined the effect of NO donors on H^+-ATPase activity in microdissected CCD segments. Incubation of CCD segments

with the NO donors, sodium nitroprusside or SIN-1, caused a dose-dependent decrease in H^+-ATPase activity. Incubation of CCD segments with lipopolysaccharide (LPS) and interferon γ, potent inducers of the inducible form of nitric oxide synthase (NOS) decreased H^+-ATPase activity by 85%. This effect was prevented by simultaneous incubation with N-nitroarginine, a competitive inhibitor of NOS, indicating that the inhibition of H^+-ATPase activity was caused by NO production. These results demonstrate that NO inhibits H^+-ATPase activity in the rat CCD and suggest that an inducible form of NOS may play a role in regulating the vacuolar H^+-ATPase in the intercalated cells. We conclude that Ang II as well as NO may be involved in the regulation of proton and bicarbonate transport in the CCD.