Role of Intercalated Cells in Regulation of Acid-Base Balance in the Mammalian Kidney

C. Craig Tisher, M.D. and Kirsten M. Madsen, M.D., Ph.D.

Division of Nephrology, University of Florida, Gainsville, FL, USA

In the mammalian kidney the collecting duct plays an important role in the final regulation of urine pH. Under normal conditions tubular fluid pH decreases along the collecting duct due to net secretion of protons. It is well established from studies in both the rat and rabbit that the initial portion of the collecting duct located in the kidney cortex secretes both protons and bicarbonate leading to either acidification or alkalinization of the tubular fluid.

The collecting duct can be subdivided into three segments, the cortical (CCD), outer medullary (OMCD), and inner medullary collecting duct (IMCD). Between the distal convoluted tubule (DCT) and the CCD is a transition region called the connecting segment or connecting tubule (CNT). The CCD and OMCD consist of two major types of cells, principal cells and intercalated cells. The latter constitute approximately one-third of the cells in both the CCD and the OMCD in the outer stripe of the outer medulla ($OMCD_o$). In the rabbit intercalated cells gradually disappear in the OMCD in the inner stripe of the outer medulla ($OMCD_i$), whereas in the rat intercalated cells still constitute one-third of the cells in the $OMCD_i$, but gradually disappear in the initial portion of the inner medullary collecting duct ($IMCD_i$). In spite of these differences in cellular composition, the OMCDi is the major site of acid secretion in the collecting duct of both the rat and rabbit.

Principal cells and CNT cells are involved in the reabsorption of sodium and secretion of potassium, and they are characterized by the presence of high levels of Na^+-K^+-ATPase activity. Intercalated cells were for several years considered likely candidates for acid and base transport because of their high content of carbonic anhydrase. Further support for the involvement of intercalated cells in acid secretion was provided by morphologic studies demonstrating significant ultrastructural changes in the intercalated cells of the OMCD of rats with acute respiratory acidosis and chronic metabolic acidosis.

Intercalated cells in the OMCD are characterized by the presence of apical tubular and vesicular membrane structures, the so-called tubulovesicles, which are coated with short rods or studs on their cytoplasmic side, and prominent microplicae on the luminal surface. Following both respiratory and metabolic acidosis there is an increase in the surface area of the apical plasma membrane concomitant with a decrease in the number of tubulovesicles in the apical region suggesting a transfer of membrane from the tubulovesicles to the apical membrane. Based on these observations we proposed that a proton pump is located in the apical tubulovesicles. Following stimulation of hydrogen ion secretion, membrane containing the proton pump is transferred from the tubulovesicles and inserted into the apical membrane to secrete protons. Support for this hypothesis was subsequently provided by immunocytochemical studies demonstrating that antibodies against H^+-ATPase label both apical tubulovesicles and

the apical plasma membrane of intercalated cells. Further support for the involvement of intercalated cells in acid secretion and bicarbonate reabsorption derived from immunocytochemical studies demonstrating that antibodies against erythrocyte band 3 protein, a Cl^--HCO_3^- exchanger, label the basolateral membrane of intercalated cells indicating that these cells can reabsorb bicarbonate.

Subsequent morphologic studies have provided evidence for the existence of two distinct populations of intercalated cells, type A and type B, in the CCD of the rat. Type A cells contain prominent apical tubulovesicular structures similar to those described in intercalated cells in the OMCD. They possess an apical proton pump and a basolateral Cl^--HCO_3^- exchanger and have higher levels of carbonic anhydrase II immunoreactivity than type B cells. The luminal surface of type A intercalated cells is covered by prominent microplicae or a mixture of microplicae and microvilli, whereas type B intercalated cells possess short stubby microvilli. Stimulation of acid secretion causes morphologic changes in type A cells similar to those demonstrated in intercalated cells in the OMCD, indicating their involvement in proton secretion. In contrast, morphologic changes were not observed in type B cells during acute respiratory acidosis. Chronic metabolic acidosis is associated with an increase in the size of type A intercalated cells and intercalated cells in the $OMCD_i$ whereas type B intercalated cells become small and difficult to identify.

Type B intercalated cells are characterized by the presence of numerous cytoplasmic vesicles throughout the cytoplasm, many mitochondria, and lower levels of carbonic anhydrase II immunoreactivity than type A cells. Immunocytochemical studies have demonstrated H^+-ATPase immunoreactivity distributed diffusely in the cytoplasm or located in the basal region of type B cells. In addition, functional studies using fluorescent pH-sensitive probes have provided evidence for the presence of a Cl^--HCO_3^- exchange process across the luminal membrane of peanut lectin-positive intercalated cells in the rabbit that correspond to type B cells in the rat. Based on these observations it was suggested that peanut lectin-positive cells in the rabbit (type B cells) are involved in bicarbonate secretion in the CCD. Studies from our laboratory suggested that stimulation of bicarbonate secretion causes an increase in the size of B cells in the rat CCD, thus providing additional support for a role of type B intercalated cells in bicarbonate secretion. In contrast, B cells are decreased in size following chronic metabolic acidosis indicating that they may be inactive during acidotic conditions. In the rat, type B cells constitute approximately 40% of the intercalated cells in the CCD, whereas in the rabbit 70% of the intercalated cells are peanut lectin-positive.

It has been suggested that type A and type B intercalated cells might represent two different manifestations of the same cell type that can reverse its polarity based on the acid-base status of the animal, or the intracellular pH of the intercalated cells in the CCD. However, the absence of band 3 immunoreactivity in B cells and the differences in ultrastructure and in carbonic anhydrase immunoreactivity between type A and type B cells speak against this hypothesis, and favor the existence of two distinct cell types.

Studies have also provided evidence for another mechanism of acid secretion in the collecting duct. In the isolated OMCD from potassium-depleted rabbits, potassium was reabsorbed in exchange for protons by a process similar to that responsible for acid secretion in the parietal cells of the gastric mucosa. In agreement with these observations biochemical studies have demonstrated ouabain-insensitive K^+-ATPase activity in both the rat and rabbit collecting

duct. Using a monoclonal antibody against gastric H^+-K^+-ATPase, we demonstrated immunoreactivity in intercalated cells in both the rat and rabbit collecting duct, thus providing additional support for a proton potassium exchange mechanism in the collecting duct. These observations are also supported by morphologic studies demonstrating that potassium depletion is associated with ultrastructural changes in intercalated cells similar to those described in acidotic conditions, thus supporting their involvement in both acid secretion and potassium reabsorption.

Intercalated cells gradually disappear in the rabbit $OMCD_i$, and they are rarely observed in the inner half of this segment, which consists of only one cell type. However, in spite of the scarcity of intercalated cells, the $OMCD_i$ of the rabbit is considered to be a major site of proton secretion in the collecting duct. The cells in this segment and in the initial part of the IMCD exhibit histochemical staining for carbonic anhydrase along the plasma membrane, and rod-shaped intramembranous particles have been described in the apical membrane, a finding characteristic of acid-secreting cells. However, these cells do not possess H^+-ATPase activity or band 3 protein, the transporters characteristic of acid-secreting intercalated cells, and they do not contain cytoplasmic carbonic anhydrase.

Acid secretion has been reported also in the terminal portion of the IMCD which consists mainly of one cell type, the IMCD cell, and does not contain intercalated cells. The mechanism of acid secretion in this segment of the collecting duct is not known, and histochemical as well as immunocytochemical studies have failed to demonstrate carbonic anhydrase in IMCD cells. There is no evidence for immunoreactivity for either H^+-ATPase or band 3 protein in the IMCD cells, although studies of cultured cells from the papillary collecting duct have reported evidence for both H^+ pumps, Cl^--HCO_3^- exchange and carbonic anhydrase activity in these cells. Studies of morphologic adaptation to acid-base disturbances have not been reported in the IMCD.