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Topical Review

Cell pH and Transepithelial H/HCO_3 Transport in the Renal Proximal Tubule

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Introduction

H/HCO_3 transporters have been identified on the cell membranes of both polar and nonpolar cells. In nonpolar cells, these transporters subserve functions such as cell pH and cell volume defense. In addition, these transporters have been demonstrated to be activated by signals which elicit cell growth. As a group, these functions have been referred to as "housekeeping" functions and the transporters as housekeeping transporters. In that polarized epithelial cells also must defend cell pH, defend cell volume, and grow, they also require the transporters capable of contributing to these housekeeping functions. In addition, certain epithelia effect transepithelial H/HCO_3 transport and thus require these transporters to subserve an additional function. By proper placement in a polarized epithelium, a single transport mechanism can contribute to transepithelial transport as well as to housekeeping functions.

Proximal Tubule Cells Possess H/HCO_3 Transporters Similar to, Yet Distinct from, Those Present in Nonpolar Cells

The proximal tubule is responsible for the reabsorption of 70–80% of HCO_3 filtered by the glomerulus. This is accomplished by apical membrane H secretion and basolateral membrane HCO_3 efflux. Here, we will briefly describe the important H/HCO_3 transport mechanisms which have been found in the proximal tubule. The interested reader is referred to a recent review for a more extensive discussion of these (Alpern, 1990). In the present review, emphasis will be placed on how these transporters serve to effect transepithelial H/HCO_3 transport in a regulated fashion, while at the same time maintaining a constant cell composition.

The major mechanism for apical membrane H secretion is an Na/H antiporter (Murer, Hopfer & Kinne, 1976). This antiporter is similar to that present in nonpolar cells in that it is inhibited by amiloride and allosterically activated by decreases in cell pH, but is different than the housekeeping Na/H antiporter in two ways. First, the apical membrane Na/H antiporter is less sensitive to amiloride and its analogues than is the housekeeping Na/H antiporter (Haggerty et al., 1988). Second, and most importantly, Na/H antiporters in nonpolar cells tend to be inactive at resting cell pH unless the cell is stimulated (Clark & Limbird, 1991). While such a pattern may be acceptable for housekeeping functions, it would not be acceptable if the antiporter is to mediate transepithelial H secretion. Thus, the apical membrane Na/H antiporter, while similar to housekeeping Na/H antiporters in that it is allosterically activated by decreases in cell pH, is different in that it functions at a significant rate at resting cell pH. Indeed, this may be responsible for the fact that resting cell pH in the proximal tubule is 0.15–0.3 pH units higher than in most nonpolar cells. The apical membrane Na/H antiporter has been shown to mediate approximately 2/3 of transepithelial H secretion (Preisig et al., 1987), and by functioning in parallel with Cl/base exchangers to mediate all of transcellular NaCl absorption in the proximal tubule (Preisig & Rector, 1988). In addition to the apical membrane Na/H antiporter, a basolateral membrane Na/H antiporter is present in the most distal parts of the proximal tubule (S3 segment) and in some of the most juxtamedullary nephrons (Kurtz, 1989; Geibel, Giebisch & Boron, 1989). This basolateral membrane Na/H antiporter is presumably of the housekeeping variety, and does not contribute to transepithelial H/HCO_3 transport.

Key Words Na/H antiporter · $Na/HCO_3/CO_3$ symporter memory · c-fos · c-jun · regulation

A cDNA encoding an amiloride-sensitive Na/H antiporter has been cloned (Sardet et al., 1989). This cDNA encodes a protein of 815 amino acids, with an aminoterminal hydrophobic domain including 10–12 transmembrane spanning regions and a carboxyterminal hydrophilic domain which represents the major cytoplasmic portion of the protein. Based on the tissue distribution of gene expression, as well as the amiloride sensitivity of the expressed protein, it is believed that the cloned Na/H antiporter gene encodes the housekeeping Na/H antiporter. This gene is now referred to as NHE-1. By reverse transcriptase PCR, its mRNA has been localized to those proximal tubular segments found to possess basolateral membrane Na/H antiporter (Krapf & Solioz, 1991). Additional isoforms of Na/H antiporter genes have recently been cloned by low stringency screening of intestinal and renal cDNA libraries (Sardet et al., 1991). These studies should yield the sequence of the apical membrane Na/H antiporter.

In parallel with the apical membrane Na/H antiporter, proximal tubule cells exhibit a Na-independent, amiloride-insensitive H transporting mechanism (Chan & Giebisch, 1981; Preisig et al., 1987). This mechanism is able to defend cell pH against an acid load and is responsible for approximately 1/3 of transepithelial H secretion (Kurtz, 1987; Preisig et al., 1987; Preisig, 1992). Based on immunocytochemical studies, this transport mechanism is most likely an H ATPase of the vacuolar type (Brown et al., 1988).

The major mechanism for base efflux across the basolateral membrane is a Na-coupled electrogenic transporter which carries one Na, one HCO_3 and one CO_3 (Boron & Boulpaep, 1983; Alpern, 1985; Yoshitomi, Burckhardt & Fromter, 1985; Soleimani & Aronson, 1989). Although first described in the proximal tubule, this transporter has now been found in numerous different polar epithelia and in some nonpolar cells. Its function in nonpolar cells has not been delineated, but it is able to defend cell pH against acid or alkali loads (*see below*).

Lastly, the proximal tubule possesses basolateral membrane Na-coupled and Na-independent Cl/HCO_3 exchangers (Alpern & Chambers, 1987). A $Na(HCO_3)_2$/Cl exchanger would be expected to function in the HCO_3 influx-Cl efflux mode, and thus could contribute to transepithelial NaCl absorption. While this transporter is present in many nonpolar cells where it participates in cell pH defense to an acid load, no such role has been demonstrated in the proximal tubule (Krapf et al., 1988). Lastly, a Na-independent Cl/HCO_3 exchanger on the basolateral membrane can contribute to base efflux. In nonpolar cells such Cl/HCO_3 exchangers have been shown to defend against an alkaline pH challenge. In order to accomplish this function, the transporter has been demonstrated in numerous cells to be allosterically activated by increases in cell pH. On the basolateral membrane, the transporter has the potential to contribute to base efflux mediating transcellular H transport, as well as to cell pH defense. In most of the proximal tubule, however, most of $NaHCO_3$ absorption occurs independently of Cl, and base efflux is likely mediated by the Na/HCO_3/CO_3 cotransporter (Alpern, 1990). However, it has been suggested that the most distal parts of the proximal tubule as well as the most juxtamedullary located nephrons may rely on a different mechanism for base efflux such as a Cl/HCO_3 exchanger (Geibel et al., 1989, Kurtz, 1989; Nakhoul, Chen & Boron, 1990). Figure 1 summarizes the H/HCO_3 transporters present in the proximal tubule. Other H/HCO_3 transporters such as an apical Cl/base exchanger and apical and basolateral H leaks have not been discussed because their activities are very low (for review, *see* Alpern, 1990). The area within the dashed line in Fig. 1 includes those transporters believed to be the most important in cell pH defense and transepithelial H/HCO_3 transport.

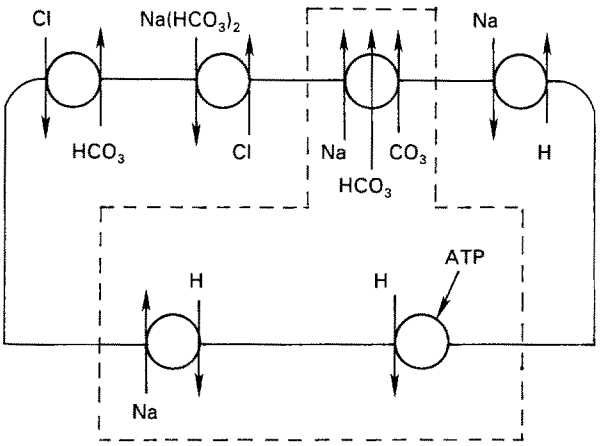

Fig. 1. Simplified model of proximal tubule H/HCO_3 transport mechanisms. The apical membrane is at the bottom of the figure. The transporters enclosed within the dashed line are the ones found to be most significant for cell pH defense and transepithelial H/HCO_3 transport.

Proximal Tubule Cells Regulate Intracellular and Extracellular pH

As the function of most proteins is affected by ambient pH, the regulation of cytoplasmic pH within narrow limits is key to cellular function. Maintenance of normal cell pH is accomplished in part through maintenance of extracellular pH in the nor-

mal range. However, even in the presence of a normal extracellular fluid pH, an electrochemical gradient exists that would passively drive H into most cells leading to unacceptable levels of cell acidity (pH 6.4 in a cell with a voltage of −60 mV). In addition, cell pH faces a second challenge related to metabolic acid production.

In order to address the mechanisms responsible for maintenance of normal cell pH, investigators have typically studied the cell's response to sudden addition of acid or alkali (for review, *see* Roos & Boron, 1981). In nonpolar cells, these studies have shown that the Na/H antiporter, the $Na(HCO_3)_2/Cl$ exchanger, and the H ATPase contribute to defense against an acid load. A Na-independent Cl/HCO_3 exchanger effects defense against an alkaline load.

Similar studies have been performed in the rabbit proximal convoluted tubule. In this segment, the apical membrane Na/H antiporter and the basolateral membrane $Na/HCO_3/CO_3$ cotransporter contribute to defense against an acid load; the $Na/HCO_3/CO_3$ cotransporter defends against an alkaline load (Krapf et al., 1988). This segment was not found to possess a basolateral membrane Na/H antiporter, but in other segments of the nephron which do possess this transporter, it would contribute to cell pH defense against an acid load. In addition, in the rat proximal convoluted tubule and in the more distal portion of the rabbit proximal tubule (S3), an apical membrane H ATPase contributes to defense against an acid load (Kurtz, 1987; Preisig, 1992).

While these acid/alkali load experiments are easy to perceive, the integrated system by which the cell regulates cell pH is more complex. As a starting point, it is useful to envision a cell in a "basal" state where there is no metabolic acid generation, no H leak into the cell, and transporters responsible for cell pH defense are inactive. If one then adds a small rate of metabolic acid production and a slow H leak, the defense of cell pH would require an acid extruding mechanism to run at a slow rate. Steady state cell pH will be defined by that cell pH at which acid addition to the cell through metabolism and H leak, is equal in rate to acid extrusion from the cell. The lower cell pH becomes, the slower will be the rates of metabolic acid generation (Halperin et al., 1969) and acid leak into the cell, and the faster will be the rate of acid extrusion. Eventually, a cell pH is achieved where these rates are equal.

In the renal proximal tubule cell, rates of metabolic acid production and nonspecific H leak into the cell are relatively slow compared to rates of transepithelial H secretion. Nevertheless, the principles of cell pH regulation are the same. The major acid loading mechanism is basolateral membrane HCO_3 efflux across the $Na/HCO_3/CO_3$ cotransporter. The major acid efflux mechanisms are apical membrane Na/H antiport and apical membrane H ATPase. Because of the polarized distribution of these transporters they effect transepithelial H/HCO_3 transport while at the same time regulating cell pH. Steady-state cell pH will be determined as the cell pH at which the rate of apical membrane acid extrusion is equal to the rate of basolateral membrane base extrusion.

If one of these processes changes in rate, the cell will no longer be in a steady state and cell pH will move to a value which brings the cell back to a steady state. For instance, if apical membrane Na/H antiporter activity suddenly increases, the rate of apical membrane H transport will increase. Because the rate of apical membrane H extrusion will exceed the rate of basolateral membrane base efflux, cell pH will rise. The increase in cell pH secondarily slows the Na/H antiporter and the H ATPase, while at the same time accelerating the $Na/HCO_3/CO_3$ cotransporter, until a new steady state is achieved where rates of apical H extrusion and basolateral membrane base efflux are again equal.

The magnitude of the cell pH change required before a new steady state is achieved depends on the sensitivity of the H/HCO_3 transport mechanisms to cell pH. If they are exquisitely sensitive to cell pH changes, minimal changes in cell pH will be required. This would allow the proximal tubule to fulfill its two important roles with regard to pH regulation: varying the rate of transepithelial H secretion to regulate extracellular fluid pH; and maintaining cell pH within a narrow range so that proximal tubular cell function remains normal. As will be discussed below, H/HCO_3 transporters are indeed exquisitely sensitive to cell pH.

Changes in cell [Na] may also participate in establishment of the steady state with minimal changes in cell pH. Thus, an increase in Na/H antiporter activity, in addition to alkalinizing the cell, may also increase cell [Na]. This increase in cell [Na] could then increase the rate of the $Na/HCO_3/CO_3$ cotransporter and slow the Na/H antiporter, minimizing the required change in cell pH to achieve steady state. At present, because cell [Na] is not as routinely measured as cell pH, this mechanism has not been rigorously tested. It is possible that the Na/K ATPase will modify its rate in order to minimize any changes in cell [Na].

Regulation of Transepithelial H Secretion Involves Regulation of Apical and Basolateral Membrane Transport Mechanisms

The reabsorption of large amounts of filtered HCO_3 requires secretion of H ions at a rapid rate. In addi-

Table. Mechanisms of transporter regulation

Substrate concentration
Allosteric interactions
Phosphorylation/dephosphorylation
Membrane trafficking
Synthesis/degradation—transcription and translation

tion, the regulation of renal HCO_3 reabsorption requires large changes in rates of proximal tubular H secretion in different conditions. In the steady state, any change in the transport of H or its equivalent must be of an equal magnitude on each membrane. Thus, if transepithelial H transport is increased by 50%, there must be a 50% increase in the cumulative rates of the Na/H antiporter and H ATPase, and a 50% increase in the rate of the Na/HCO_3/CO_3 cotransporter. The Table lists the general mechanisms by which transporter rate can be regulated. There is accumulating evidence that most of these mechanisms of transporter regulation are relevant to proximal tubular H secretion.

Substrate Concentrations and Allosteric Interactions Acutely Regulate H/HCO_3 Transporters

As with most transport mechanisms, H/HCO_3 transporters are regulated by substrate concentrations. This has been best studied for the Na/H antiporter. When external substrate dependence was examined, it was found that Na and H compete for a similar site, each with saturable kinetics (Aronson, Suhm & Nee, 1983). Regulation of the Na/H antiporter by internal substrate concentrations is more complex. In addition to substrate effects, cell H concentration has been found to be a powerful allosteric regulator of Na/H antiporter rate (Aronson, Nee & Suhm, 1982). Thus, decreases in cell pH, or equivalently, increases in cell H concentration have a profound effect on Na/H antiporter rate that is greater than can be explained by substrate binding. As noted above, this large sensitivity to cell pH allows the Na/H antiporter to significantly change its rate in response to small changes in cell pH, and thus helps to maintain cell pH within narrow limits.

The Na/HCO_3/CO_3 cotransporter is regulated by changes in [Na], [HCO_3], and [CO_3] (Soleimani & Aronson, 1989). At a given CO_2 tension, [HCO_3] is proportional to [OH], and [CO_3] is proportional to $[OH]^2$. Thus kinetically the rate of this transporter would be expected to be proportional to the $[OH]^3$. This should lead to marked sensitivity to internal and external pH. In addition, the transporter carries two negative charges and thus is extremely sensitive to changes in cell voltage (Alpern, 1985). Lastly, the possibility of allosteric regulation by cell pH has been raised, but is not settled. There is presently little information on the regulation of the H ATPase by changes in cell and luminal pH in the proximal tubule. Nevertheless, studies on a similar transporter in the turtle urinary bladder suggest that the transporter is regulated by both of these determinants, being stimulated by decreases in cell pH and increases in luminal pH (Steinmetz, 1974). Thus, all three of the key proximal tubule H/HCO_3 transporters are exquisitely sensitive to cell pH, allowing large changes in transcellular H/HCO_3 transport with minimal changes in cell pH.

H/HCO_3 Transport Is Regulated by Protein Kinases

The above discussion has concentrated on regulatory mechanisms which can be considered instantaneous in that substrate and allosteric regulation are present only as long as the concentrations of substrates or regulators are altered. In addition, a number of mechanisms exist which can be considered forms of memory in that the regulation can persist after the inducing mechanism is no longer present, and may even persist for significant times in membrane preparations which have been removed from the intact cell. These forms of memory can be divided into short-term (minutes to hours) and long-term (hours to days) memory, and in many respects their mechanisms may be analogous to those present in the central nervous system. One mechanism of short-term memory is phosphorylation/dephosphorylation of proteins. The apical membrane Na/H antiporter has been studied in brush-border membranes and found to be inhibited by cyclic AMP-dependent protein kinase and by Ca calmodulin-dependent protein kinase 2, and to be stimulated by protein kinase C (Weinman & Shenolikar, 1986; Weinman, Shenolikar & Khan, 1987; Weinman et al., 1988). Interestingly, in studies examining regulation of amiloride-sensitive and amiloride-resistant Na/H antiporters in culture, results have been somewhat different (Casavola, Helmle-Kolb & Murer, 1989). Whereas cAMP-dependent protein kinase inhibits both types of antiporters, protein kinase C stimulated the amiloride-sensitive (housekeeping) form, but inhibited the amiloride-resistant (apical) form.

These protein kinase effects most likely mediate hormonal regulation of Na/H antiporter activity. Parathyroid hormone and dopamine are believed to inhibit Na/H antiporter activity by activation of cyclic AMP-dependent protein kinase (Kahn et al.,

1985; Pollock, Warnock & Strewler 1986; Felder et al., 1990). Stimulation of angiotensin II and alpha 2 catecholamine receptors increases Na/H antiporter activity, possibly by inhibition of adenylyl cyclase and secondarily of cyclic AMP-dependent protein kinase (Nord et al., 1987; Liu & Cogan, 1989). However, the possibility that other signaling systems may contribute to these forms of hormonal regulation has not been eliminated.

Once again, changes in apical membrane H secretion must be accompanied by changes in basolateral membrane base efflux. If these protein kinases directly regulated only the apical membrane Na/H antiporter, changes in cell pH would be required in order to secondarily regulate the rate of the $Na/HCO_3/CO_3$ cotransporter. However, if these kinases also directly regulated basolateral membrane base efflux, changes in cell pH would not be required or could be minimal. Indeed, direct regulation of the $Na/HCO_3/CO_3$ cotransporter by these protein kinases has been demonstrated. Cyclic AMP-dependent protein kinase and Ca-calmodulin-dependent protein kinase 2 inhibit, and protein kinase C stimulates, the $Na/HCO_3/CO_3$ cotransporter (Ruiz & Arruda, 1992). Angiotensin II has been demonstrated to increase Na/H antiporter and $Na/HCO_3/CO_3$ cotransporter activities (Geibel, Giebisch & Boron, 1990). PTH, on the other hand, has been found only to regulate apical membrane transporter rate (Sasaki & Marumo, 1991).

Exocytotic Membrane Insertion May Regulate Apical Membrane Transport

The proximal tubule is rich in subapical vesicles, and is very active in endocytosis/exocytosis. There has been extensive work in the distal nephron and in the turtle urinary bladder demonstrating that decreases in cell pH lead to exocytotic insertion of apical membrane containing H ATPases (Gluck, Cannon & Al-Awqati, 1982). Studies have also demonstrated that decreases in cell pH lead to exocytosis in the proximal tubule (Schwartz & Al-Awqati, 1985), but the functional significance of this effect has not been established. Proximal tubular endosomes have been demonstrated to contain H ATPases and an Na/H antiporter, making them a possible repository for transporters (Sabolic, Haase & Burckhardt, 1985; Gurich & Warnock, 1986). The endosomal Na/H antiporter has been noted to be insensitive to amiloride, but this may be due to a sidedness effect, in that amiloride is being applied to the cytoplasmic surface (Gurich & Warnock, 1986). Endocytosis and exocytosis may serve to regulate apical membrane H secretion rate in response to hormones or other perturbations. Angiotensin II has been proposed to increase Na/H antiporter activity in part by inducing exocytosis (Bloch et al., 1992). Endo/exocytosis would also fit under the category of memory, and could be short term or long term, depending on the time course.

Long-Term Memory Regulates H/HCO_3 Transporters

Chronic regulation of H/HCO_3 transporters in the proximal tubule can be effected by various mechanisms that could act solely or in combination. First, persistence of the initial stimulus could lead directly to a sustained alteration in driving forces, allosteric effects, intracellular signaling events, protein phosphorylation and/or in the balance between membrane insertion and retrieval of H/HCO_3 transporters, thus maintaining altered transporter activities. Second, the initial stimulus could affect the rate of synthesis of new transporter proteins (i.e., regulate transcription or translation) or their rate of degradation.

Information about chronic adaptations of H/HCO_3 transporters has concentrated on the regulation of the apical Na/H antiporter and the basolateral $Na/HCO_3/CO_3$ cotransporter. Various in vivo conditions, chronic acidosis, chronic K depletion, chronic hyperfiltration (chronic increases in glomerular filtration rate) and several hormones have been shown to chronically increase the activities of the apical Na/H antiporter and the basolateral $Na/HCO_3/CO_3$ cotransporter. Discussion of each of these conditions is beyond the scope of this review (for review, *see* Alpern, 1990). We concentrate therefore on recent experimental insights into the mechanisms of chronic adaptation of H/HCO_3 transporters in response to chronic acidemia.

Chronic acidemia (induced either by a primary decrease in extracellular HCO_3 concentration or by a primary increase in ambient CO_2 tension) has been studied most intensively. The activities of the apical Na/H antiporter and the basolateral $Na/HCO_3/CO_3$ cotransporter are stimulated quantitatively in parallel, thus allowing an increase in proximal tubule HCO_3 reabsorptive capacity at a cell pH that is not different from control (Cohn, Klahr & Hammerman, 1983; Kinsella, Cujkid & Sacktor, 1984; Tsai et al., 1984; Akiba, Rocco & Warnock, 1987; Preisig & Alpern, 1988; Krapf, 1989). Similar parallel stimulation of the apical Na/H antiporter and basolateral $Na/HCO_3/CO_3$ cotransporter has been found in chronic K deficiency and hyperfiltration (Soleimani et al., 1990; Preisig & Alpern, 1991). Once again, such a response allows large changes in transcellular

flux with minimal changes in cell pH (Preisig & Alpern, 1988, 1991; Krapf, 1989).

The increase in activity of these transporters is detectable even when isolated proximal tubules or membrane vesicles containing these transporters are removed from the chronically acidemic animal and studied in nonacidemic control conditions. This observation is consistent with an intrinsic adaptation of these transporters that has become independent of the initial change in electrochemical driving forces (memory). Kinetically, the increase in apical Na/H antiporter activity is characterized by an increase in the maximal transport capacity (Vmax), while the apparent affinities for Na and H/HCO_3 ions are unchanged (Km) (Tsai et al., 1984; Akiba et al., 1987). The nature of this kinetic change and the associated memory effect suggest that the increase in transport activity may be due to recruitment of silent membrane transporters by covalent modification or exocytotic insertion, or to changes in the synthesis and/or degradation of the transporter proteins.

Further studies have utilized tissue culture to examine the mechanism by which chronic acidosis increases Na/H antiporter activity. Studies in cultured rabbit proximal tubule cells have demonstrated that incubation in acid media for 48 hr causes an increase in Na/H antiporter activity which persists after the cells are removed from the acid media (Horie et al., 1990). This adaptation occurs if cells are incubated in low [HCO_3] acid media or in high pCO_2 acid media.

As discussed above, Na/H antiporters have been divided into two groups based on sensitivity to amiloride and its analogues (Haggerty et al., 1988). Housekeeping Na/H antiporters are sensitive to nanomolar concentrations of ethylisopropylamiloride (EIPA), and are found on nonpolar cells and on the basolateral membrane of a number of epithelia. A second Na/H antiporter type requires micromolar concentrations of EIPA for inhibition and is localized on the apical membrane of epithelia. To examine which type of Na/H antiporter is regulated by acid incubation, studies have utilized renal epithelial cell lines with proximal tubule-like characteristics. In MCT cells (SV40-transformed mouse cortical tubules) grown on solid support, the Na/H antiporter is EIPA sensitive, and is increased by approximately 60% by acid incubation for 24 hr (Moe et al., 1991). Similarly, in OKP cells (opossum kidney, clone P) which possess an EIPA-resistant antiporter, acid incubation × 24 hr causes a 90% increase in Na/H antiporter activity (Moe et al., 1991). Lastly, in LLC-PK1-Cl4 cells (a cloned pig kidney cell line), which possess an apical EIPA-resistant antiporter and a basolateral EIPA-sensitive Na/H antiporter, both antiporters are activated by acid incubation (Igarashi, Ganz & Reilly, 1990). Thus, it appears that in renal epithelial cells, both types of Na/H antiporter are activated by chronic acidemia. It should be noted that in all of the above studies there are no hormones present, implying a direct effect of acid on the cells.

Chronic Activation of the Na/H Antiporter by Acid Requires Protein Synthesis and is Associated with Increases in Na/H Antiporter mRNA Abundance

In these tissue culture studies, the effect of acid preincubation on Na/H antiporter activity was inhibited by cycloheximide, suggesting a role for protein synthesis (Horie et al., 1990). It was not clear from these studies whether the synthesized protein was the Na/H antiporter or a regulatory protein. The recent cloning and sequencing of the human growth factor-activated Na/H antiporter (NHE-1) has allowed further investigation of the molecular mechanism of the acid-induced Na/H antiporter activation. Using a rat renal Na/H antiporter probe with high homology to the human Na/H antiporter cDNA, it was found that the increase in proximal tubule Na/H antiporter activity is associated with an increase in renal cortical Na/H antiporter mRNA expression if the acidemia is induced by a decrease in extracellular HCO_3 concentration (metabolic acidemia), but not if the acidemia is induced by high ambient CO_2 tension (respiratory acidemia) (Fig. 2*A*) (Krapf et al., 1991). Studies in cultured MCT cells and LLC-PK1-Cl4 cells have found that incubation in acid media, either low HCO_3 or high pCO_2 increases Na/H antiporter mRNA two- to threefold (Fig. 2*B* and *C*) (Igarashi et al., 1990; Moe et al., 1991). This effect requires 24 hr of acid incubation. The reason why high pCO_2 increases Na/H antiporter mRNA in tissue culture but not in vivo is not clear, but it may be quantitative in that the degree of acidosis achieved chronically is greater in culture. It is presently not known whether this increase in Na/H antiporter mRNA expression is due to transcriptional and/or post-transcriptional mechanisms. Thus, activation of the EIPA-sensitive Na/H antiporter (NHE-1) by chronic acid incubation is associated with increases in the abundance of NHE-1 mRNA.

It could be argued that the increase in Na/H antiporter activity occurring in cultured cells exposed to acid media represents an adaptation to defend cell pH, and has little to do with the response of the proximal tubule to acidosis. According to such a theory, one might expect any cell in culture to increase Na/H antiporter activity in response to chronic incubation in acid media. However, primary

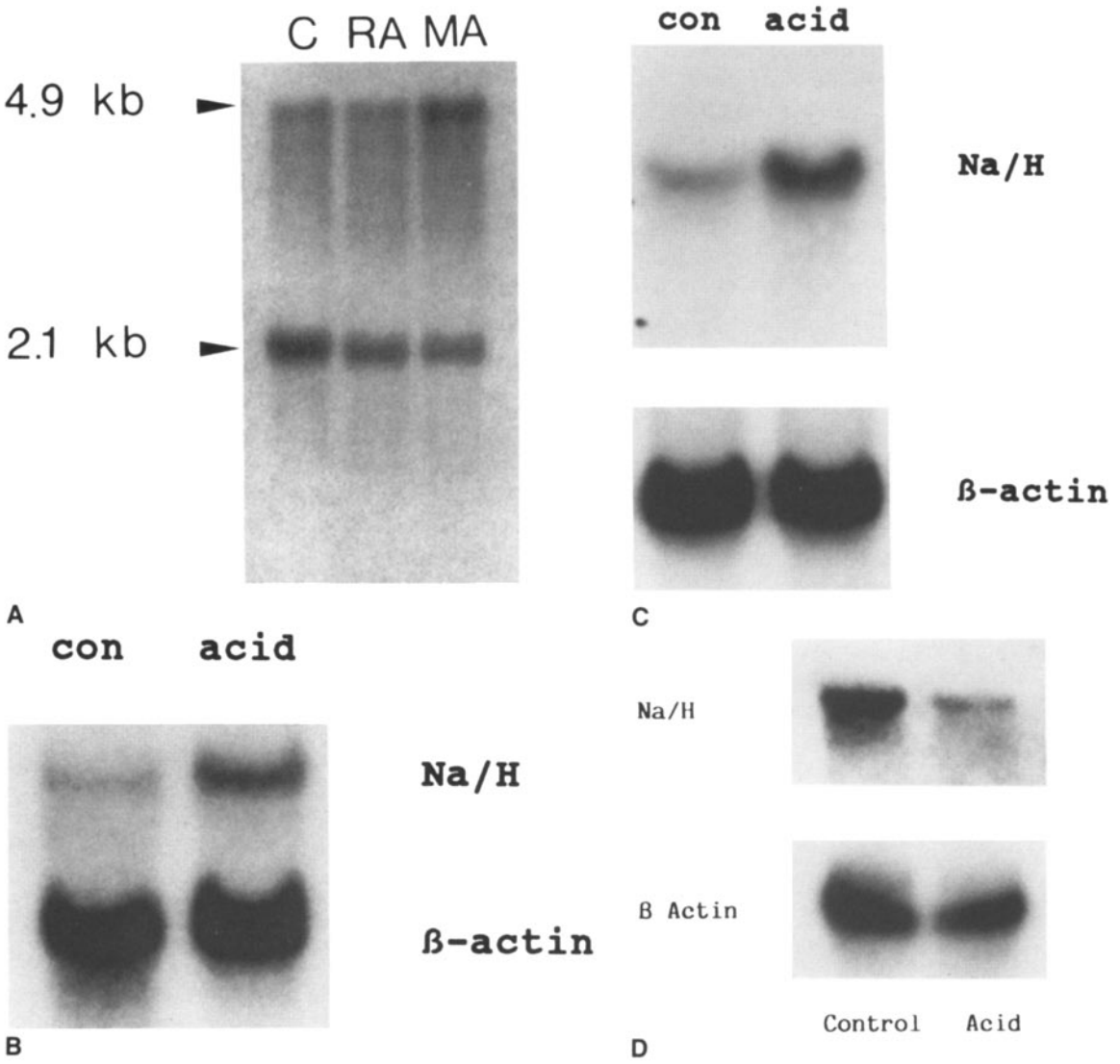

Fig. 2. Effect of acidosis on the abundance of NHE-1 mRNA. (*A*) NHE-1 mRNA (4.9 kb) and beta actin mRNA (2.1 kb) are compared in poly A^+ selected RNA from control (*C*), respiratory acidosis (*RA*), and metabolic acidosis (*MA*) rats. Rats were made acidotic for five days. (*B*) Effect of low [HCO_3] acid incubation × 24 hours on NHE-1 mRNA in cultured MCT cells. (*C*) Effect of high pCO_2 acid incubation × 24 hr on NHE-1 mRNA abundance in cultured MCT cells. (*D*) Effect of low [HCO_3] acid incubation × 24 hr on NHE-1 mRNA abundance in cultured NIH 3T3 cells. Abundance of NHE-1 mRNA was normalized by abundance of beta actin mRNA. The acid-induced decrease in β-actin mRNA in panel *D* is not due to unequal loading, but represents suppression of this mRNA by acidosis. (*A*) from Krapf et al., 1991; (*B, C,* and *D*) from Moe et al., 1991.

cultures of human foreskin fibroblasts incubated in acid media for 48 hr demonstrated a decrease rather than an increase in Na/H antiporter activity (Horie et al., 1990). Similar results were found in NIH 3T3 cells (a fibroblast cell line) incubated in acid media for 24 hr (Moe et al., 1991). At this point, the molecular basis of the difference between renal proximal tubule cells and fibroblasts is not clear. However, this difference is not due to different isoforms of the Na/H antiporter. As noted above, incubation of MCT cells or LLC-PK1-C14 cells in acid media leads to an increased abundance of NHE-1 mRNA. When similar studies were performed in NIH 3T3 cells, acid incubation caused an 80% decrease in NHE-1 mRNA abundance (Fig. 2*D*) (Moe et al., 1991). Thus, mRNA abundance for this particular isoform is increased in renal proximal tubule cells and decreased in fibroblasts in response to acid incubation.

Recent studies have begun to address how intracellular signaling pathways mediate increased Na/H antiporter mRNA expression in response to metabolic acidemia. In chronic acidosis, chronic hyperfiltration, and chronic K deficiency, increases in Na/H antiporter and $Na/HCO_3/CO_3$ cotransporter

activities are associated with proximal tubular hypertrophy. This raises the suggestion that protein kinase C, an important component of cell growth, may play a role in the response to these conditions. Three lines of evidence support this thesis. First, chronic activation of protein kinase C in cultured proximal tubule cells causes a persistent increase in Na/H antiporter activity (Horie et al., 1992*a*). Addition of phorbor esters × 2 hr to rabbit proximal tubule cells in primary culture caused an increase in Na/H antiporter activity which persisted 24 hr after removal of the phorbol ester. This effect was dependent on protein kinase C in that it did not occur with an inactive phorbol ester analogue which cannot activate protein kinase C, and it was blocked by sphingosine. This effect was inhibited by actinomycin D and cycloheximide, suggesting that it was dependent on transcription and translation. Lastly, two hours of phorbol ester addition caused a twofold increase in Na/H antiporter mRNA four hours later.

The second line of evidence is that in MCT cells, inhibition of protein kinase C prevents the effect of acid incubation on Na/H antiporter activity (Horie et al., 1992*b*). Protein kinase C was inhibited by sphingosine or by protein kinase C downregulation (pretreatment with high concentrations of phorbol esters for 48 hr). In that MCT cells possess an EIPA-sensitive Na/H antiporter, these results are only relevant to NHE-1.

The last line of evidence is that acid incubation causes activation of protein kinase C-responsive genes. To demonstrate this, the effect of acid incubation on AP-1 activity was measured in MCT cells. Many genes whose expression is regulated by protein kinase C, possess in their 5′ flanking region a consensus nucleotide sequence, TGA(C/G)TCA, referred to as an AP-1 binding site or a TRE (TPA response element). When protein kinase C is activated, AP-1 activity increases, leading to increased binding to the AP-1 binding site and secondarily causing increased transcription of the protein kinase C-responsive genes. AP-1 activity is most potently expressed by a heterodimer of c-fos and c-jun.

To test if acid incubation causes an increase in AP-1 activity, MCT cells were stably transfected with a reporter gene construct containing six AP-1 binding sites upstream to a minimal interferon promoter and the reporter gene, chloramphenicol acetyltransferase (CAT). Incubation of these cells in acid media caused an increase in CAT activity and CAT mRNA (Fig. 3) (Horie et al., 1992*b*). In addition, acid incubation caused an increase in c-fos and c-jun mRNA at 15–30 min and 1–2 hr, respectively (Fig. 4). These studies demonstrate in MCT cells that acid incubation causes a response in c-fos, c-jun, and AP-1 activity typical of protein kinase C activation.

Based on the above results, it appears likely that protein kinase C plays a key role in the acid-induced activation of the NHE-1 isoform of the Na/H antiporter, and possibly in the hypertrophy seen in this condition, as well as in chronic hyperfiltration and chronic K deficiency. Increased levels of diacylglycerol, the endogenous activator of protein kinase C, have been found in the renal cortex following uninephrectomy (a cause of hyperfiltration) (Salihagic et al., 1988). It is not presently clear whether fos, jun, and AP-1 play a key role. Studies examining the effect of uninephrectomy on c-fos mRNA have been conflicting (Beer et al., 1987; Norman et al., 1988; Ouellette et al., 1990; Sawczuk et al., 1990; Nakamura et al., 1992), but recently increases in c-fos and c-jun have been demonstrated at 1 hr following surgery (Nakamura et al., 1992). Interestingly, the promoter regions from the human and rabbit NHE-1 genes have now been cloned and contain AP-1 binding sites (Miller et al., 1991; Reboucas, Blaurock & Igarashi, 1991). It remains to be examined whether protein kinase C plays a key role in the activation of the apical EIPA-resistant Na/H antiporter by acid. In addition, the roles of other signaling pathways and the roles of transcriptional *vs.* post-transcriptional mechanisms in the chronic regulation of the Na/H antiporter requires further study.

Summary

The proximal tubule contains a number of H/HCO_3 transporters. Three of these, the apical membrane Na/H antiporter and H ATPase, and the basolateral membrane $Na/HCO_3/CO_3$ cotransporter, play a major role in transcellular H/HCO_3 transport and cell pH regulation. Regulation of transcellular transport must involve regulation of these transport mechanisms. Studies have now demonstrated regulation of transporter activities by substrate concentrations, allosteric mechanisms, kinases, exocytosis/endocytosis, and synthesis. Because of the large H/HCO_3 fluxes maintained by these transporters, they possess the ability to significantly alter proximal tubule cell pH. This effect on cell pH is minimized by two mechanisms. First, the H/HCO_3 transporters are exquisitely sensitive to changes in cell pH, allowing them to change their rate significantly in response to small changes in cell pH. Secondly, regulation in a number of key physiologic settings, such as angiotensin II, chronic acidosis, chronic K deficiency, and chronic hyperfiltration occurs directly at the level of both apical and basolateral membrane transporters. Parallel regulation of these transporters

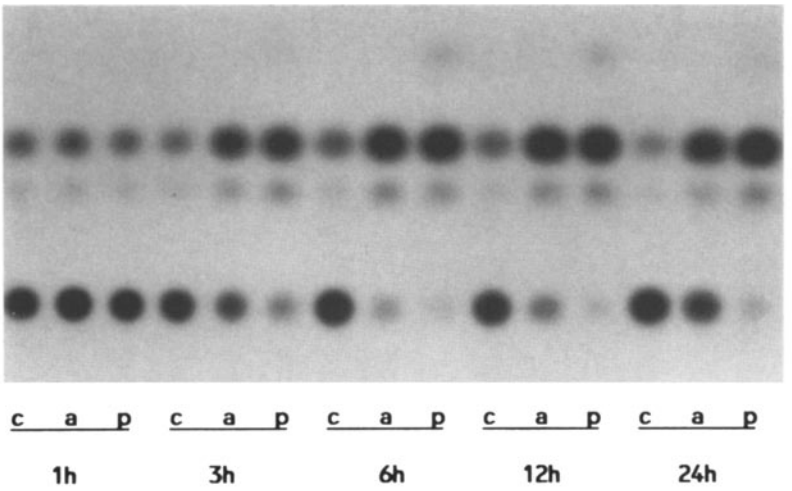

Fig. 3. Chloramphenicol acetyltransferase activity in control (*c*), acid incubated (*a*), and phorbor ester treated (*p*) cultured MCT cells stably transfected with 6AP-1/CAT. Label at the bottom of the figure indicates unacetylated chloramphenicol, while label above indicates acetylation. Both acid and phorbor ester treatment lead to increased chloramphenicol acetyltransferase activity at 3–24 hr. From Horie et al., 1992*b*.

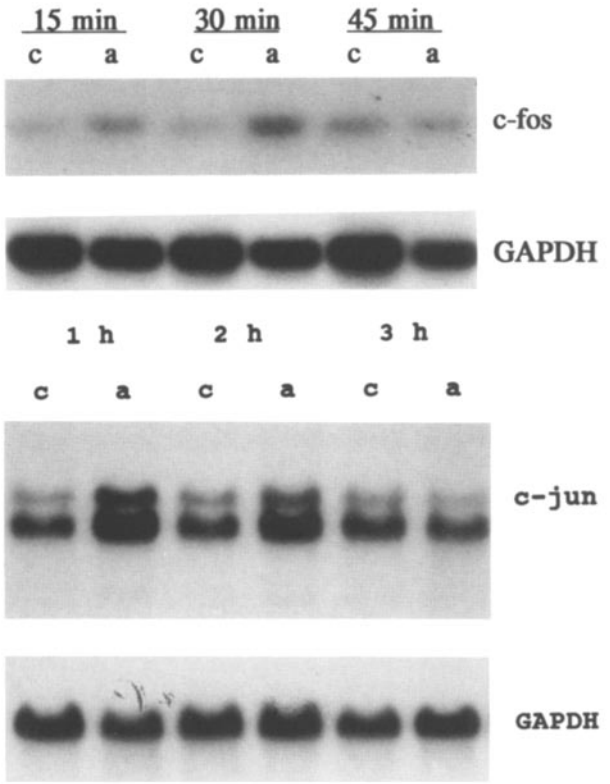

Fig. 4. c-fos and c-jun mRNA abundance in control (*c*) and acid incubated (*a*) MCT cells. Results are normalized to GAPDH mRNA. From Horie et al., 1992*b*.

allows large changes in flux in the absence of any changes in cell pH.

The cloning of the gene encoding the Na/H antiporter isoform, NHE-1, has allowed the beginning of the application of molecular biology to the study of the regulation of proximal tubule transport. The generation of useful antibodies, as well as the cloning of other isoforms and genes encoding other H/HCO_3 transporters, should allow this field to progress at an increasingly rapid rate.

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