BRIEF REVIEWS

The Plasma Membrane Sodium-Hydrogen Exchanger and Its Role in Physiological and Pathophysiological Processes

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SUMMARY. The plasma membranes of most if not all vertebrate cells contain a transport system that mediates the transmembrane exchange of sodium for hydrogen. The kinetic properties of this transport system include a 1:1 stoichiometry, affinity for lithium and ammonium ion in addition to sodium and hydrogen, the ability to function in multiple 1:1 exchange modes involving these four cations, sensitivity to inhibition by amiloride and its analogues, and allosteric regulation by intracellular protons. The plasma membrane sodium-hydrogen exchanger plays a physiological role in the regulation of intracellular pH, the control of cell growth and proliferation, stimulus-response coupling in white cells and platelets, the metabolic response to hormones such as insulin and glucocorticoids, the regulation of cell volume, and the transepithelial absorption and secretion of sodium, hydrogen, bicarbonate and chloride ions, and organic anions. Preliminary evidence raises the possibility that the sodium-hydrogen exchanger may play a pathophysiological role in such diverse conditions as renal acid-base disorders, essential hypertension, cancer, and tissue or organ hypertrophy. Thus, future research on cellular acid-base homeostasis in general, and on plasma membrane sodium-hydrogen exchange in particular, will enhance our understanding of a great variety of physiological and pathophysiological processes. (Circ Res 57: 773-788, 1985)

FOR many years it was held that acid-base equivalents merely distributed passively across the plasma membrane of cells according to a Donnan equilibrium. Neutralization of acid produced from intermediary metabolism was presumed to rely first upon cytoplasmic and membrane buffer action, and, second, upon the diffusive movement of protons (H+) out of the cell down an electrochemical gradient. With the advent of refined and reliable electrode techniques for actually measuring transmembrane voltage differences and intracellular H+ activity, however, it has become clear that intracellular pH (pHi) is maintained at a much higher value than predicted if protons were in Donnan equilibrium across the plasma membrane (Roos and Boron, 1981). Measurements from numerous vertebrate and invertebrate cell types have given pHi values ranging from 6.8-7.3, whereas if H+ were in electrochemical equilibrium across the membrane, pHi would average 6.3-6.5, because the typical cell has a large interior-negative membrane potential. Thus, there is continual passive influx of H+ into the cell, and/or passive efflux of hydroxyl (OH−) or bicarbonate (HCO3−) ions out of the cell (Fig. 1). It is clear that maintenance of pHi must depend upon an energy-requiring active H+ extrusion (or OH− accumulation) system.

One such mechanism for active H+ extrusion that has been documented in the plasma membrane of a wide variety of cell types is a carrier-mediated Na+-H+ exchange (antiport, countertransport) system that catalyzes the coupled transmembrane exchange of sodium (Na+) for H+. In the intact cell, the plasma membrane Na+-H+ exchanger mediates the uphill extrusion of protons directly coupled to the downhill flux of Na+ into the cell (Fig. 1). This antiport process is not directly dependent upon any exergonic chemical reaction, such as ATP hydrolysis; rather, energy for the uphill extrusion of H+ is obtained exclusively from the steep inwardly directed Na+ gradient, which is maintained by the primary active extrusion of Na+ via the separate plasma membrane Na+,K+-ATPase system. Thus, Na+-H+ antiport is an example of secondary active transport.
This transport system has been identified in virtually every type of cell that has been examined to determine its presence, including ova, sperm, erythrocytes, lymphocytes, skeletal muscle, cardiac muscle and Purkinje fibers, neurons, capillary endothelia, fibroblasts, renal tubular cells, intestinal and gall bladder epithelia, and a variety of cells in culture (Aranson, 1985). The principal physiological role of the Na\(^+\)-H\(^+\) exchanger is to regulate intracellular pH. However, as a corollary of this role, the Na\(^+\)-H\(^+\) exchanger has an important additional role as a signal-transducer for various stimuli that influence cell function by altering intracellular pH. Examples include sperm activation of oocytes, the initiation of cell growth and proliferation in response to serum growth factors, and certain actions of insulin and thrombin. Moreover, as a transporter of Na\(^+\), this system plays a role in the regulation of cell volume. Finally, the Na\(^+\)-H\(^+\) exchanger has an important role in mediating the net transport of acid-base equivalents and Na\(^+\) across various epithelia, such as the renal proximal tubule and small intestine.

This review presents a current overview of the physiological roles subserved by the plasma membrane Na\(^+\)-H\(^+\) exchanger in different animal cells. The intrinsic properties of the antiporter will be presented first, as these have bearing on the interpretation of the many investigations that pertain to its various roles in diverse cells and tissues. Of burgeoning interest is how the transport system might be regulated and how its state of activity might be important in certain pathophysiological states.

Properties of the Na\(^+\)-H\(^+\) Exchanger

The distinctive nature of this transport system, like any other transport system, resides in its specific properties with regard to stoichiometry, selectivity for substrates, sensitivity to inhibitors, and capacity for regulation. The first clear-cut demonstration of directly coupled Na\(^+\)-H\(^+\) exchange was made in microvillus membrane vesicles derived from the luminal membranes of rat intestinal mucosa and renal proximal tubular cells (Murer et al., 1976). In this experimental preparation, uphill \(^{22}\)Na influx can be driven by an outward proton gradient, and outward extrusion of H\(^+\) can be driven by an inward Na\(^+\) gradient. In intact cellular preparations, the technique is essentially the same: Na\(^+\)-dependent H\(^+\) extrusion can be assayed with intracellular H\(^+\)-sensitive microelectrodes, with pH-sensitive fluorescent dyes, or with nuclear magnetic resonance (NMR); and H\(^+\)-coupled Na\(^+\) movements can be assayed isotopically or with intracellular Na\(^+\)-sensitive electrodes (see Nuccitelli and Deamer, 1982). The transport properties derived from these various approaches show remarkably close agreement.

Stoichiometry

Several observations indicate that the stoichiometry of the Na\(^+\)-H\(^+\) antiport process is one-for-one. In a number of studies with intact cell systems, the net flux of Na\(^+\) in one direction and the net flux of H\(^+\) in the opposite direction have been directly quantified (Cala, 1980; Moolenaar et al., 1981b; Boron and Boulpaep, 1983; Grinstein et al., 1984). In each of these studies, the directly measured ratio of these fluxes did not differ significantly from 1.0. Second, in vesicle studies, maneuvers that alter the membrane electrical potential difference have no effect on Na\(^+\) influx via this transport pathway (Murer et al., 1976; Kinsella and Aronson, 1980; Cohn et al., 1982; Knickelbein et al., 1983), and in intact cell systems, stimulating or inhibiting Na\(^+\)-H\(^+\) antiport does not cause any measureable change in the transmembrane electrical potential difference (Aickin and Thomas, 1977; Cala, 1980; Deitmer and Ellis, 1980; Vigne et al., 1982; Grinstein et al., 1983), indicating that the operation of the Na\(^+\)-H\(^+\) exchanger is electrically silent and must have a Na\(^+\):H\(^+\) coupling ratio close to 1.0. Third, when the transmembrane Na\(^+\) gradient is thermodynamically balanced by an H\(^+\) gradient of the same magnitude (i.e., when Na\(^+\)/H\(^+\) = H\(^+\)/H\(^+\)), the Na\(^+\)-H\(^+\) exchanger is at equilibrium and mediates no net flux of Na\(^+\) and H\(^+\) (Moody, 1981; Kinsella and Aronson, 1982), which also implies that the exchange is electroneutral and that the coupling ratio is 1.0. A Na\(^+\):H\(^+\) coupling ratio of 1.0 is consistent with stoichiometries of 1:1, 2:2, 3:3, etc., but the interaction of external Na\(^+\) and external H\(^+\) with the Na\(^+\)-H\(^+\) exchanger conforms to simple saturating Michaelis-Menten kinetics, with a Hill coefficient of 1.0 (Burnham et al., 1982; Aranson et al., 1983; Ives et al., 1983a; Gunther and Wright, 1983; Frelin et al., 1983; Paris and Pouyssegur, 1983; Grinstein et al., 1984). This is most consistent with the presence of only a single binding site for external Na\(^+\) or H\(^+\), and indicates that the stoichiometry of exchange is actually one Na\(^+\) for one H\(^+\).

Alternative Substrates and Modes

The Na\(^+\)-H\(^+\) exchanger can operate in more than one mode with a narrow selection of monovalent cations. In addition to mediating Na\(^+\)-H\(^+\) exchange...
interacts competitively with the Na\(^+\)-H\(^+\) exchanger. Furthermore, external H\(^+\) interacts competitively with both systems can transport Li\(^+\) and actually have a higher affinity for Li\(^+\) than for Na\(^+\), and both systems are ATP-independent and ouabain-insensitive. Coupled Na\(^+\)-H\(^+\) exchange has been demonstrated in mammalian erythrocytes (Dissing and Hoffman, 1982; Parker, 1983; Parker and Glosson, 1984), but whether the same antiport system that mediates Na\(^+\)-Li\(^+\) exchange also mediates the Na\(^+\)-H\(^+\) exchange in these erythrocytes is not yet clear.

Inhibitors

Sensitivity of the plasma membrane Na\(^+\)-H\(^+\) exchanger to a variety of transport inhibitors has been evaluated. Ouabain, furosemide, disulfonic acid stilbene derivatives, and acetazolamide have no direct effects on this pathway (Kinsella and Aronson, 1980). The best inhibitors identified to date are the diuretic drug amiloride and its analogues. With the exception of one study (Ives et al., 1983a), amiloride has generally been found to be a purely competitive inhibitor with respect to external Na\(^+\) (K\(_i\) ~ 10\(^{-4}\) to 10\(^{-3}\) M) (Kinsella and Aronson, 1981b; Vigne et al., 1982; Paris and Pouyssegur, 1983), implying that amiloride interacts at or near the Na\(^+\) transport site. Inhibition by this agent is immediate in onset and is rapidly reversible. Note that, compared to its effect on Na\(^+\)-H\(^+\) exchangers, amiloride is 10–100 times more potent as an inhibitor of the Na\(^+\) channels found in the apical membranes of such epithelia as the colon and the cortical collecting tubule of the kidney (Benos, 1982). The effects of amiloride as a diuretic employed clinically probably arise solely from inhibition of apical Na\(^+\) channels in the distal nephron, rather than from inhibition of Na\(^+\)-H\(^+\) exchange.

Nevertheless, amiloride has been employed extensively as an experimental probe of Na\(^+\)-H\(^+\) exchange activity in various tissues. Inhibition of H\(^+\) efflux or Na\(^+\) influx along with inhibition of a particular cell function is often taken as evidence for a specific role of Na\(^+\)-H\(^+\) exchange in that cell function. Although amiloride is useful and informative in this regard, it is not an ideal agent when employed in this manner, and such experiments need to be interpreted carefully. Because amiloride and Na\(^+\) compete for access to the external transport site of the Na\(^+\)-H\(^+\) exchanger, and because the K\(_m\) for Na\(^+\) is quite low (K\(_m\) ~ 3–50 mm), amiloride is not an effective inhibitor of Na\(^+\)-H\(^+\) exchange in the presence of physiological Na\(^+\) concentrations (140–150 mm) unless relatively high concentrations of the drug (>10\(^{-4}\) to 10\(^{-3}\) M) are used. At these concentrations, amiloride may have additional effects, such as inhibiting paracellular cation permeability in epithelia (Balaban et al., 1979), and inhibiting Na\(^+\),K\(^+\)-ATPase (Soltoff and Mandel, 1983). In addition, at high concentrations, amiloride can act as a weak base and nonspecifically alter transmembrane pH gradients (Dubinsky and Frizzell, 1983). Finally, high concentrations of amiloride can inhibit protein synthesis in cells and in cell-free systems, implying that, under certain circumstances, this drug has a direct toxic action (Leffert et al., 1982). In spite of
these constraints, amiloride has been useful as an experimental probe of Na⁺-H⁺ exchange activity, particularly when used in the presence of lower than physiological Na⁺ concentrations. Recently, amiloride analogues with substitution of the 5-amino group by alkyl or alkenyl groups have been found to be up to 100 times more potent than amiloride in inhibiting Na⁺-H⁺ exchangers (Vigne et al., 1984). These analogues should be more useful than amiloride for studying the functions of Na⁺-H⁺ exchangers under physiological conditions.

Other inhibitors of the Na⁺-H⁺ exchanger include the alkaloids, harmaline and quinidine, which are less specific than amiloride (Kinsella and Aronson, 1980; Parker, 1983), the carboxyl-activating reagent N-ethoxy carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (Burnham et al., 1982), and the histidine-specific reagent diethylpyrocarbonate, an irreversible inhibitor (Grillo and Aronson, 1983). Whether any of these agents are useful for probing the function and roles of the Na⁺-H⁺ exchanger under physiological conditions is yet to be determined.

**Regulation by Intracellular H⁺**

A general observation in most studies reviewed is that raising the internal H⁺ concentration dramatically stimulates the rate of Na⁺-H⁺ exchange. Since this system is thermodynamically energized by the Na⁺o/Na⁺i and H⁺o/H⁺i gradients, it is predicted that as the outward H⁺ gradient steepens in the face of an unchanging inward Na⁺ gradient, the rate of antiport should increase proportionally. However, in renal microvillus membrane vesicles, the rate of Na⁺ influx by Na⁺-H⁺ exchange has a greater than linear dependence on internal H⁺ concentration (Aronson et al., 1982), and in whole renal cells and in human lymphocytes, efflux of H⁺ via Na⁺-H⁺ exchange shows a steeper dependence on internal pH than expected for a simple Michaelis-Menten process (Boron and Boulpaep, 1983; Grinstein et al., 1984). In view of the considerable data indicating that the stoichiometry of Na⁺-H⁺ exchange is one-for-one, these observations have led to the proposal that internal H⁺ can allosterically activate the exchanger through an interaction that is independent of its role as a transportable ion (Aronson et al., 1982). The evidence in support of this concept derives from two observations: first, that raising the internal H⁺ concentration actually stimulates Na⁺-Na⁺ exchange in vesicles, an effect opposite to that expected if internal H⁺ were to interact (and compete) only at the internal transport site, and second, that raising internal H⁺ stimulates net Na⁺ efflux in exchange for external H⁺, also an effect opposite to that expected if internal H⁺ were interacting only at the transport site (Aronson et al., 1982). Conceptually, one explanation for the striking stimulatory effect of internal H⁺ is that the transport system possesses one or more functional groups on its inner, cytoplasmic surface, the protonation of which causes activation of the exchanger.

This kinetic regulation of Na⁺-H⁺ exchange activity by internal protons has important consequences for the cell. Activation of Na⁺-H⁺ exchange by a falling cytoplasmic pH would greatly enhance the ability of plasma membrane Na⁺-H⁺ exchangers to extrude protons promptly, thereby protecting against intracellular acid loads. Conversely, this property is reflected by the existence of an apparent "activity threshold," because, as pH rises to or above a certain value, the rate of H⁺ extrusion is sharply curtailed in most tissues. This kinetically protects against the generation of an intracellular alkalosis inasmuch as the average Na⁺o/Na⁺i gradient of approximately 10 could theoretically drive pH 1.0 unit higher than extracellular pH if there were not some mechanism to prevent the system from reaching thermodynamic equilibrium. It appears that the kinetic dependence on internal H⁺ activity is just such a "brake" for this system. Of interest in this regard is recent evidence that suggests that stimulation of the plasma membrane Na⁺-H⁺ exchanger by purified growth factors results from a shift in its sensitivity to intracellular pH (Moolenaar et al., 1983). Hence, altering the apparent affinity of the transporters for internal H⁺ ions may be an important mechanism for physiological regulation of this transport system.

**Possible Regulation by Intracellular Ca**²⁺

In addition to the regulation of Na⁺-H⁺ exchange by internal pH, it has been proposed that antipot activity might also be importantly influenced by intracellular calcium (Ca²⁺) activity, perhaps through the mediation of calmodulin (Benos, 1982; Owen and Villereal, 1982a, 1982b). This hypothesis is founded on the observation in fibroblasts that elevations in free intracellular Ca²⁺ induced by the calcium ionophore A23187 markedly stimulate amiloride-sensitive Na⁺ influx, and that treatment of these cells with intracellular Ca²⁺ antagonists or with calmodulin antagonists blocks activation of Na⁺-H⁺ exchange in a dose-dependent manner (Villereal, 1981; Owen and Villereal, 1982a; 1982b; Mix et al., 1984). Furthermore, in sea urchin eggs, fertilization is associated with an immediate rise in free intracellular Ca²⁺ and a subsequent activation of Na⁺-H⁺ exchange (see next section). Since treatment of the unfertilized egg with A23187 also induces egg activation and Na⁺-dependent H⁺ extrusion, it has been proposed that intracellular Ca²⁺ might be a modulator of Na⁺-H⁺ exchange in these cells (Whitaker and Steinhardt, 1982; Busa and Nuccitelli, 1984). However, some doubt has been cast upon the hypothesis that Ca²⁺ is a regulator of Na⁺-H⁺ exchange activity. Treatment of either mouse neuroblastoma cells (Moolenaar et al., 1981a) or mouse fibroblast cells (Frelin et al., 1983) with a range of A23187 concentrations in the presence of
external Ca++ does not modify the rate of amiloride-sensitive Na+ uptake, nor does it trigger mitogenesis. Also, Grinstein et al. (1984) have reported that the Ca++-ionophore ionomycin does not activate Na+-H+ exchange in human lymphocytes, and preloading renal microvillus membrane vesicles with 10^-7 to 10^-3 M calcium has no significant effect on renal Na+-H+ exchange activity (Aronson et al., 1982). Thus, the exact interplay between intracellular Ca++ activity, calmodulin, and the Na+-H+ exchanger is uncertain.

An alternative mechanism for Ca++-dependent regulation of the plasma membrane Na+-H+ exchanger is via the action of the calcium and phospholipid-dependent protein kinase (i.e., protein kinase C). In several different cell types, phorbol esters, which are relatively specific activators of protein kinase C, stimulate the rate of plasma membrane Na+-H+ exchange (Burns and Rozengurt, 1983; Mooienaar et al., 1984). If Na+-H+ exchange activity is regulated by protein kinase C-induced phosphorylation under physiological conditions, then it may be sensitive not only to changes in intracellular Ca++ activity, but also to changes in availability of diacylglycerol, a potent endogenous activator of protein kinase C (Berridge, 1984). Diacylglycerol is a product of phosphoinositide hydrolysis. Another such product is inositol triphosphate, which is known to stimulate the release of Ca++ from intracellular storage pools. Thus, receptor-mediated hydrolysis of phosphoinositides to diacylglycerol and inositol triphosphate may be an important mechanism for regulating activity of the Na+-H+ exchanger through the action of protein kinase C (Berridge, 1984).

Physiological Roles

In almost all animals cells, a steep inwardly directed Na+ gradient is maintained across the plasma membrane due to the primary active extrusion of Na+ from the cell via the Na+-K+-ATPase (sodium pump). Because Na+_/Na+exceeds H+_/H+ under most physiological conditions, the net thermodynamic driving force acting on plasma membrane Na+-H+ exchangers generally favors the net exchange of external Na+ for internal H+. Thus, it is easy to conceptualize how the transport system could function in the protection of intracellular pH against intracellular acid loads, especially in light of the demonstrated ability of internal protons to activate proton extrusion. Of great interest are the recent observations that the plasma membrane Na+-H+ exchange system serves a variety of functions in addition to merely defending pH against acid loads. These roles can be broadly distinguished into three categories: (1) intracellular pH change is caused by activation of Na+-H+ exchange, and the ensuing pH rise is permissive or serves as a signal for generating a biological response; (2) changes in activity of plasma membrane Na+-H+ exchange alter the intracellular content of Na+, as in cell volume regulation; and (3) plasma membrane Na+-H+ exchange participates in net transepithelial movement of Na+ and H+, as in the processes of acid secretion and Na+ reabsorption by gallbladder, intestine, and kidney.

Regulation of Intracellular pH

Since the development of accurate means for intracellular pH and voltage measurements, there has been general agreement that H+ activity is lower inside cells than would be predicted if H+ were simply passively distributed in equilibrium with the membrane potential. In most cell systems studied to date, active H+ extrusion is accomplished by a Na+-coupled ion exchange mechanism, whereby downhill Na+ influx is coupled either singly to H+ efflux or Na+ influx is coupled to Cl- efflux and HCO3- influx (Roos and Boron, 1981). Which system is present or predominates in any given tissue appears to depend at first approximation on animal class and perhaps tissue type: with one exception, invertebrate cells seem to rely on the Na+-dependent Cl--HCO3- exchange system, whereas vertebrate cells appear to rely more on Na+-H+ exchange with or without an independently operating Cl--HCO3- exchange. The systems are distinguished by external and internal ionic requirements and by their sensitivity to inhibitors: Na+-H+ exchange is inhibited by amiloride, and Cl--HCO3- exchange but not Na+-H+ exchange is inhibited by disulfonic stilbene derivatives (DIDS, SITS).

For example, in the snail neuron (Thomas, 1977), the squid giant axon (Boron and Russell, 1983), and the giant barnacle muscle fiber (Boron et al., 1981), effective recovery of pH, after the cell interior is acidified depends on the presence of external Na+ and HCO3- and internal Cl-. Based on experiments with either ion-sensitive electrodes or radioisotope tracers, the actual stoichiometry of ion fluxes appears to be 1 Na+ in, 1 Cl- out, and 2 equivalents of HCO3- in (Boron and Russell, 1983). The entire process is electroneutral and can be inhibited by SITS, a selective anion inhibitor, but not by amiloride. Although a Na+-H+ exchange may occur along with the Cl--HCO3- exchange in these tissues, an independent Na+-H+ exchange has not been discerned. Unlike other tissues to be discussed, there is an absolute dependence on Cl- and HCO3-, and deletion of any one ion prevents pH recovery. In another invertebrate preparation, the crayfish neuron, Moody (1981) found that regulation of pH occurs somewhat differently. In this case, two operations were needed to effect pH recovery: Na+-H+ exchange, and a Na+-dependent Cl--HCO3- exchange.

A more prominent role for Na+-H+ exchange in pH regulation has been documented in a variety of vertebrate cells. Aickin and Thomas (1977) recorded an intracellular pH of 7.07 in mouse skeletal muscle situated in buffered Ringers at pH, 7.40. After intra-
cellular acid loading, pH, recovered spontaneously over about 30 minutes, and was associated with an influx of Na+. Recovery of pH could be blocked more than 90% by removal of outside Na+ or application of 10^-4 M amiloride. A less active Cl--HCO3--exchange process was also noted in this tissue, and it made a minor contribution to pH recovery. Other similar types of investigations in vertebrate skeletal muscle have documented that Na+-H+ exchange and Cl--HCO3--exchange co-exist independently in the plasma membranes of this tissue (Roos and Boren, 1978; Abercrombie et al., 1983). Although both systems appear to function importantly toward pH recovery when the cell is acid loaded, activity of the amiloride-sensitive Na+-H+ exchanger appears to be more significant, particularly when the muscle cell is depolarized (Roos and Boren, 1978; Abercrombie et al., 1983).

Regulation of internal pH in mammalian cardiac tissue also depends importantly on Na+-H+ exchange. Ellis and Thomas (1976) were the first to document that both mammalian Purkinje tissue and ventricular muscle cells possess active mechanisms for pH maintenance. Resting intracellular pH was 7.27, and after cytoplasmic acidification, pH recovery was prompt. This recovery process was dependent upon external Na+, was accompanied by Na+ influx, was amiloride-sensitive, and was unaffected by Cl- or HCO3- deletion or SITS addition (Deitmer and Ellis, 1980). This strongly suggests that Na+-H+ exchange is the chief if not sole pH regulator in this tissue. A similar conclusion was reached by Piwnica-Worms and Lieberman (1983), who investigated regulation of intracellular pH in spontaneously beating cardiac muscle cells in culture by using the pH-sensitive chromophore, 6-carboxyfluorescein. Significantly, either lowering external Na+ from 144 mM to 0.8 mM or application of 1 mM amiloride caused a rapid cytosolic acidification. These data strongly imply that the Na+-H+ exchanger is continuously active in the contracting cardiac cell, where it compensates for the generation of metabolic acid or for the passive entry of H+ or exit of HCO3- across the cell membrane. A Cl--HCO3--exchange system that is independent of Na+-H+ antiport also exists in the Purkinje fiber (Vaughan-Jones, 1979). However, this anion transport system does not appear to play a major part in acid extrusion in the heart, but, rather, it may serve to facilitate Cl- entry into the cell and thereby regulate intracellular Cl- activity (Vaughan-Jones, 1979).

The Na+-H+ exchange system has been identified as the principal mechanism for pH regulation in a number of other tissues and cells. In such epithelia as the gallbladder (Weinman and Reuss, 1982) and the renal proximal tubule (Boron and Boulpaep, 1983), Na+-H+ exchange is the principal mechanism for controlling intracellular pH. Grinstein et al. (1984) have found that lymphocytes activate an amiloride-sensitive electroneutral Na+-H+ exchange following cytoplasmic acidification, and Betz (1983) has identified Na+-H+ exchange in isolated brain capillary endothelium from the rat. Finally, a variety of cultured animal cells possess a relatively quiescent Na+-H+ antipporter that is dramatically stimulated when the cell interior is acidified slightly, indicating a direct link between activity of the Na+-H+ exchanger and intracellular pH (Moolenaar et al., 1981b; 1983; Rindler and Saier, 1981; Vigne et al., 1982; Schuldiner and Rozengurt, 1982; Rothenberg et al., 1983; Paris and Pouyssegur, 1983; Frelin et al., 1983).

In general, acid-loaded cells recover only to the baseline pH, and then seem to curtail H+ transport sharply, even though the driving force for Na+-H+ exchange, the inwardly directed Na+ gradient, still remains favorable for additional H+ extrusion. This phenomenon probably reflects the kinetic sensitivity of the transport system to internal H+ as discussed earlier (Aronson et al., 1982; Boron and Boulpaep, 1983). Thus, H+ extrusion rates are dramatically increased as internal H+ rises, and are dramatically slowed as internal H+ falls in spite of a persistently steep thermodynamic driving force for continued H+ extrusion. The physiological importance of this phenomenon resides in the fact that the cell must maintain its pH within narrowly defined limits for optimal enzyme and organelle functioning. If there were not a kinetic modulator for this system, and if activity depended solely on thermodynamic forces, the mechanism would be sluggish in defense of acid loads, and would tend to drive steady state pH to excessively alkaline values.

**Intracellular pH as Mediator of Cell Response to Activating Stimuli**

**Cell Proliferation**

Within the first few minutes after fertilization of an oocyte, there is a dramatic increase in DNA and protein synthesis, an increase in metabolic rate, and initiation of cell division (Whitaker and Steinhardt, 1982; Busa and Nuccitelli, 1984). One of the earliest events triggered by sperm-egg fusion is an increase in intracellular pH. In studies with sea urchin eggs, this cytoplasmic alkalization appears to be due to an active proton extrusion process that is quiescent in the unfertilized egg but is activated 5–10 minutes after the fertilization event. Both the proton efflux and the increase in pH depend upon the presence of external Na+ and have been linked to Na+ influx; as well, these Na+ and H+ fluxes are amiloride-sensitive. Thus, it is postulated that activation of sea urchin eggs by sperm-egg fusion involves activation of Na+-H+ exchange, which is critical to further development. The subsequent alkalization of the cytoplasm may act as a regulator of the biosynthetic events (Whitaker and Steinhardt, 1982; Busa and Nuccitelli, 1984). Of additional interest in this regard is that initiation of motility in sea urchin and rat
sperm also appears to rely upon a Na+-dependent H+-efflux system and an associated rise in pH, (Wong et al., 1981; Lee et al., 1982).

An even more convincing role for Na+-H+ antiport and alterations of cytoplasmic pH in the regulation of cell proliferation and growth has been documented with a variety of mammalian cells in culture. When quiescent cells in culture are stimulated to grow and divide by the application of serum, a dramatic and persistent increase in Na+ influx occurs (Smith and Rozengurt, 1978). The cell growth response can be suppressed by either a reduction in external Na+ (Smith and Rozengurt, 1978) or by the presence of amiloride (Koch and Leffert, 1979; Rozengurt and Mendoza, 1980; Moolenaar et al., 1982). Similarly, in a number of cultured cell preparations it has been observed that (1) serum-activated Na+ influx is simultaneous with a rise in pH due to H+ extrusion, (2) serum and purified growth factors stimulate a rise in pH, and this is strictly dependent on external Na++; (3) the pH increase, like Na+ influx, is inhibited by amiloride, and (4) the fluxes of Na+ and H+ are electroneutral (Moolenaar et al., 1981a, 1981b, 1982, 1983; Pouyssegur et al., 1982; Schuldiner and Rozengurt, 1982; Cæsset al., 1983; Paris and Pouyssegur, 1983; Frelin et al., 1983). Moreover, when mutant fibroblasts which specifically lack Na+-H+ exchange activity are exposed to mitogens, cytoplasmic pH does not rise and DNA synthesis is not activated to the same magnitude as in the wild type fibroblast (L’Allemain et al., 1984).

The biochemical steps by which the binding of growth factors to their membrane receptors is linked to activation of Na+-H+ exchange are unknown at present. In this regard, Villereal (1981) has suggested that the serum activation of Na+-H+ exchange seen in human fibroblasts might be secondary to an elevation in intracellular free Ca++ activity. When quiescent fibroblasts are activated by growth factors, there is a prompt mobilization of Ca++ from intracellular Ca++ storage pools, causing a rise in free intracellular Ca++ activity, as measured by quin-2 fluorescence (Mix et al., 1984); this occurs at the same time that Na+-H+ exchange activity is stimulated by serum. In the absence of serum growth factors, the calcium ionophore A23187 induces a dose-dependent increase in amiloride-sensitive Na+ influx and cell proliferation (Villereal, 1981). Amiloride blocks serum-stimulated Na+ influx but does not block Ca++ mobilization, suggesting that the serum-induced rise in Ca++ is an event that is independent of Na+ influx; treatment with agents that block intracellular Ca++ mobilization or with agents that inactivate Ca++-calmodulin complexes inhibits serum-stimulated Na+ influx and blocks cell activation (Owen and Villereal, 1982a, 1982b). Therefore, it has been proposed that serum growth factors act primarily by mobilizing Ca++ from intracellular pools, and the consequent formation of Ca++-calmodulin complexes then activates Na+-H+ exchange, causing a rise in pH. However, as discussed earlier, activation of Na+-H+ exchange by calcium ionophores is an inconsistent finding. Also as discussed earlier, the critical event may instead be a receptor-mediated hydrolysis of phosphoinositides to yield diacylglycerol, a potent stimulator of protein kinase C. A rise in Ca++ may not be necessary or sufficient for stimulation of Na+-H+ exchange via the mediation of protein kinase C (Moolenaar et al., 1984). It appears that, perhaps, as the result of protein kinase C-mediated phosphorylation, growth factors alter the proton modifier site of the Na+-H+ exchanger, resulting in an increased affinity for cytoplasmic H+, and, thus, a shift in the curve for activation by internal H+ (Moolenaar et al., 1983).

Whatever the precise biochemical steps that result in the activation of Na+-H+ exchange by serum growth factors, it is the resulting cytoplasmic alkalinization that is responsible, at least in part, for the subsequent biosynthetic and mitotic events. For example, the early phosphorylation of certain proteins that occurs with mitogen activation of quiescent fibroblasts is strongly influenced by pH, and seems to be a limiting prerequisite for further activation of biosynthetic events (Pouyssegur et al., 1982). Likewise, a small rise in pH, accelerates other critical operations within the cell, such as glycolysis (Fideman et al., 1982; Moolenaar et al., 1983), DNA synthesis (Zetterberg and Engstrom, 1981; Gerson et al., 1982), and protein synthesis (Winkler et al., 1980). Mitosis can be induced in serum-starved fibroblasts simply by alkalinizing the cells, while serum-induced mitosis can be halted by a small acidification (Busa and Nuccitelli, 1984). Furthermore, mutant fibroblasts that lack Na+-H+ exchangers do not alkalinize when exposed to serum mitogens, and their rate of DNA synthesis and cell growth rises very slowly compared with wild type cells that possess Na+-H+ exchangers (L’Allemain et al., 1984). Thus, it is clear that an internal pH shift brought about by activation of Na+-H+ exchange is probably a critical feature of cell activation, but other regulatory signals are probably equally important and may not be related to pH shifts at all. This point has been emphasized by those workers who have demonstrated the parallel importance of internal Ca++ activity (Winkler et al., 1980; Mix et al., 1984) and by those investigators who have noted that the protein phosphorylation events induced by mitogens were only partially suppressible by inhibiting Na+-H+ exchange activity (Pouyssegur et al., 1982).

Moreover, new DNA synthetic activity can occur upon cell exposure to growth factors in the absence of a pH rise, albeit at a much slower rate (L’Allemain et al., 1984). An interesting observation in this context is that the calcium-calmodulin interaction appears to be pH sensitive, such that a rise in pH from 7.0 to 7.5 might increase calcium binding to calmodulin by more than 60% (Busa and Nuccitelli, 1984).
This implies that calmodulin-mediated reactions might be pH sensitive and that activity of the Na⁺-H⁺ exchanger might have a modulating function for calmodulin-mediated responses. An alkaline pH shift might serve to amplify Ca²⁺-calmodulin signals involved in cell activation, and some calmodulin responses may depend upon this alkaline amplification. Furthermore, if Na⁺-H⁺ exchange itself is indeed modulated by a calmodulin-dependent mechanism, then an enhanced Ca²⁺-calmodulin interaction might serve to sustain Na⁺-H⁺ exchange activity in the face of a higher than normal pH.

Stimulus-Response Coupling in Neutrophils, Lymphocytes, and Platelets

Stimulus-response coupling in certain differentiated cells also appears to bear a relationship to activation of Na⁺-H⁺ exchange and subsequent cytoplasmic alkalization. When mature lymphocytes are activated by exposure to bacterial lipopolysaccharide or concanavalin A, there is an abrupt rise in intracellular pH that is associated with a burst of RNA and protein synthesis, followed by a second higher and more protracted rise in pH that strongly correlates with the rate of RNA synthesis (Gerson et al., 1982, 1983). An early surge of amiloride-inhibitable Na⁺ influx occurs during lymphocyte activation (Rosoff and Cantley, 1983), and lymphocyte activation and subsequent DNA synthesis can be inhibited by micromolar doses of amiloride, an effect due to inhibition of cation flux and not nonspecific toxicity (Heikkila et al., 1983; Rosoff and Cantley, 1983).

Similarly, activation of mammalian neutrophils by complement component C5a and by the synthetic chemotactic agent formyl-methionyl-leucyl-phenylalanine causes a significant increase in Na⁺ influx and cytoplasmic pH (Naccache et al., 1977a; Molski et al., 1981). These events have been correlated with the functional ability of the neutrophil to migrate, degranulate, and generate antibacterial granular supernatants (Naccache et al., 1977b). Both the Na⁺ influx and the rise in pH induced by f-met-leu-phe are amiloride-sensitive but DIDS-insensitive, suggesting that this response is mediated by Na⁺-H⁺ exchange (Molski et al., 1981).

Platelet activation by thrombin also appears to involve activation of plasma membrane Na⁺-H⁺ antiport (Horne et al., 1981; Greenberg-Sepersky and Simons, 1984). With the use of internalized pH-sensitive fluorescent indicators, it has been observed that thrombin stimulation of washed human platelets leads to a dose-dependent increase in Na⁺ influx and a concomitant rise in cytoplasmic pH. The rise in pH produced by a saturating dose of thrombin is reduced 50% by 10⁻⁴ M amiloride and is prevented completely by 10⁻³ M amiloride. The secretion of serotonin by the platelet, which serves to attract and aggregate other platelets, is also thrombin-dependent and is partially inhibited by amiloride. The dose-response curve for thrombin-induced serotonin secretion is nearly identical to that of the thrombin-induced pH rise. Taken together, these data imply a primary role for Na⁺-H⁺ exchange in platelet activation by thrombin but do not exclude other parallel events. For example, activation of platelets and neutrophils also involves a rise in intracellular free Ca²⁺, and protein phosphorylations are prerequisite steps for the full physiological cellular response (Kaibuchi et al., 1983; Korchak et al., 1984). The relationships among Na⁺ influx, elevation of pH, and these events are presently unclear.

Effects of Hormones

Rather convincing evidence has been presented which suggests that cytoplasmic alkalization secondary to activation of Na⁺-H⁺ exchange is one of the early events mediating the action of insulin to stimulate glycolysis in frog skeletal muscle. The addition of insulin to muscle strips increases pH by 0.1 to 0.15 pH unit (Moore, 1981; Fidelman et al., 1982). The possibility that activation of plasma membrane Na⁺-H⁺ exchange is responsible for this rise in pH is suggested by the observations that the elevation in pH is associated with an increased influx of Na⁺, that lowering extracellular Na⁺ prevents the insulin-induced pH rise, and that both the insulin-induced Na⁺ influx and the pH rise can be inhibited by 0.5 mM amiloride (Moore, 1981). That insulin stimulation of glycolysis results from the rise in pH per se is indicated by the findings that changes in glycolytic activity closely parallel the changes in pH produced by insulin, and that glycolysis is significantly reduced when the insulin-induced rise in pH is inhibited by amiloride or by lowering extracellular Na⁺ (Fidelman et al., 1982). It was known earlier that glycolysis in cell-free extracts from rat diaphragm and in guinea pig leukocytes is increased by small increases in medium pH (Ui, 1966; Halperin et al., 1969). The likely explanation for this effect is that the activity of the rate-limiting enzyme of glycolysis, phosphofructokinase, is very sensitive to small changes in ambient pH and can be maximally stimulated by a pH elevation as small as 0.1–0.2 pH unit (Trivedi and Danforth, 1966).

Other hormones also appear to influence Na⁺-H⁺ exchange activity. For example, when rats are adrenalectomized, Na⁺-H⁺ exchange activity as measured in isolated renal microvillus membrane vesicles is diminished compared to control rats (Freiberg et al., 1982). In these adrenalectomized rats, Na⁺-H⁺ exchange activity can be restored by the administration of dexamethasone but not aldosterone. Hence, it appears that glucocorticoids but not mineralocorticoids stimulate renal Na⁺-H⁺ exchange activity. That this effect can be demonstrated in isolated membrane vesicles studied in vitro indicates that there has been an intrinsic change in the number or activity of the Na⁺-H⁺ exchangers in the membrane.
(Freiberg et al., 1982). Stimulation of renal Na\(^{+}\)-H\(^{+}\) exchange by glucocorticoids may explain the effect of these hormones to augment renal acid excretion (Wilcox et al., 1982). Whether glucocorticoids stimulate Na\(^{+}\)-H\(^{+}\) exchange in other target tissues or whether changes in pH, are important for mediating the metabolic effects of glucocorticoids is not known.

Preliminary investigations also indicate an interaction between thyroid hormone and Na\(^{+}\)-H\(^{+}\) exchange activity. Induction of a hypothyroid state with thiouracil in rats causes a decrease in renal brush border Na\(^{+}\)-H\(^{+}\) exchange activity (Kinsella and Sacktor, 1984). As thyroid extract is given back to hypothyroid animals, a dose-related increase in renal Na\(^{+}\)-H\(^{+}\) exchange is observed. A physiological correlation is suggested by the fact that hypothyroidism in the intact rat is associated with urinary sodium and bicarbonate wasting (Michael et al., 1979). Again, it is not known to what extent changes in Na\(^{+}\)-H\(^{+}\) exchange and pH, may be important for mediating the effects of thyroid hormone on other tissues.

It is interesting to note that the three hormones just discussed—insulin, glucocorticoids and thyroid hormone—all are known to enhance the specific activity of Na\(^{+}\),K\(^{+}\)-ATPase in a variety of target tissues (Edelman, 1974; Asano et al., 1976; Resh et al., 1980; Kaji et al., 1981). The increased specific activity of Na\(^{+}\),K\(^{+}\)-ATPase induced by mineralocorticoids in certain target tissues appears to be secondary to stimulation of Na\(^{+}\) entry into the cell via an increased number of Na\(^{+}\) channels (Marver, 1984). Analogously, it is possible that insulin, glucocorticoids, and thyroid hormone stimulate Na\(^{+}\),K\(^{+}\)-ATPase activity secondary to increased Na\(^{+}\) entry via Na\(^{+}\)-H\(^{+}\) exchange.

In addition, at least one hormone has been described that inhibits Na\(^{+}\)-H\(^{+}\) exchange activity. Renal membrane vesicles isolated from parathyroidectomized dogs have enhanced Na\(^{+}\)-H\(^{+}\) exchange (Cohn et al., 1983), and parathyroid hormone inhibits Na\(^{+}\)-H\(^{+}\) exchange and reduces pH\(_i\) in cultured renal cells (Pollock et al., 1984). Inhibition of Na\(^{+}\)-H\(^{+}\) exchange may explain the ability of parathyroid hormone to reduce the rate of proximal tubular acidification (Lino and Burg, 1979). It is not known whether alterations in Na\(^{+}\)-H\(^{+}\) exchange and pH\(_i\) result from the action of parathyroid hormone in other target tissues such as bone.

Finally, it has been reported that angiotensin peptides stimulate amiloride-sensitive Na\(^{+}\) influx in vascular smooth muscle cells (Smith and Brock, 1983). The possibility that this may represent an activation of plasma membrane Na\(^{+}\)-H\(^{+}\) exchange has not been examined.

**Cell Volume Regulation**

In the steady state, most cells appear to maintain their cell volume through primary active extrusion of Na\(^{+}\) via the Na\(^{+}\),K\(^{+}\)-ATPase system (MacKnight and Leaf, 1977). However, when cells are exposed to anisotonic media, ouabain-insensitive ion flux pathways are activated, which restore cell volume to original size within minutes (Krengenow, 1981; Spring and Ericson, 1982). Based on specific ionic requirements and inhibitor sensitivity, two types of electroneutral transport mechanisms have been proposed to mediate the regulatory volume increase that occurs after cell shrinkage in hyperosmotic media: (1) a Na\(^{+}\),K\(^{+}\)-2Cl\(^{-}\} cotransport system that is inhibited by loop diuretics such as furosemide (Krengenow, 1981); (2) an amiloride-sensitive Na\(^{+}\)-H\(^{+}\) antiport system, which when activated in parallel with Cl\(^{-}\}-HCO\(_3\)\(^{-}\} antiport, results in net cellular gain of Na\(^{+}\), Cl\(^{-}\}, and H\(_2\)O. Activation of Na\(^{+}\)-H\(^{+}\) exchange by cell shrinkage has been described in salamander and dog erythrocytes, and *Necturus* gallbladder (Cala, 1980; Ericson and Spring, 1982; Parker, 1983). For example, after *Amphiuma* erythrocytes shrink in hypertonic media, cell re-expansion occurs within 3 hours with a dramatic increase in Na\(^{+}\) influx that is amiloride-sensitive, and accompanied by an increase in intracellular pH and a decrease in medium pH (Cala, 1980). Similar volume-responsive sodium and proton movements are observed in dog erythrocytes (Parker, 1983) and in human lymphocytes (Grinstein et al., 1983). The process of cell volume regulation by gallbladder epithelial cells in hypertonic media appears to be much the same, although actual H\(^{+}\) fluxes have not been measured (Ericson and Spring, 1982).

What actually activates Na\(^{+}\)-H\(^{+}\) exchange at the initiation of regulatory volume increase is an open question. Evidence has been presented that cell shrinkage per se is not the trigger (Cala, 1980; Spring and Ericson, 1982). Alternative proposals have included a shrinkage-associated fall in intracellular pH (Cala, 1980) and/or a change in intracellular Ca\(^{++}\} (Cala, 1983). However, affirmative data for either one of these mechanisms are lacking, and measurements in hypertonically shrunken lymphocytes have failed to show any significant shift in pH or in free cytoplasmic Ca\(^{++}\} (Grinstein et al., 1983).

**Transepithelial Ion Transport**

Epithelial cells mediate the net transport of solutes and water from one extracellular compartment to another, and thereby play a determining role in the ionic composition and volume of extracellular fluids. Although the involvement of Na\(^{+}\)-H\(^{+}\) exchange in transepithelial fluxes of Na\(^{+}\} and H\(^{+}\} would seem most obvious, this transport system also indirectly but importantly contributes to the transport of Cl\(^{-}\} and organic anions in several epithelia.

Studies both in isolated membranes vesicles (Murer et al., 1976; Kinsella and Aronson, 1980) and in isolated perfused tubules (Boron and Boulpaep, 1983) have clearly demonstrated the presence of Na\(^{+}\)-H\(^{+}\) exchange across the luminal membrane of...
the renal proximal tubular cell. In the amphibian (Boron and Boumier, 1983), but not the mammalian proximal tubule (Ives et al., 1983b; Sabolic and Burckhardt, 1983), Na"+-H"+ exchange also occurs in the basolateral membrane. Along the mammalian proximal tubule, over 90% of the filtered bicarbonate load is reabsorbed and a lumen-acid transtubular pH gradient is generated. The role of luminal membrane Na"+-H"+ exchange in facilitating the process of H"+ secretion and HCO"3~ absorption along the mammalian proximal tubule is illustrated in Figure 3A. The "primary active" transport of Na"+ across the basolateral membrane via the Na,K-ATPase generates an inward electrochemical gradient for Na"+ across the luminal membrane. This Na"+ gradient then serves as the driving force for secondary active H"+ secretion via the Na"+-H"+ exchanger. Normally, luminal HCO"3~ acts as the principal acceptor for secreted H"+, with its eventual disappearance after conversion of H2CO3 to CO2 and H2O, a reaction catalyzed by carbonic anhydrase. The intracellular OH"- generated by H"+ secretion combines with CO2 to form intracellular HCO"3~, another reaction catalyzed by carbonic anhydrase. Thus, the net effect of active H"+ secretion by the Na"+-H"+ exchanger is to cause the intracellular accumulation of HCO"3~ against its electrochemical gradient. Hence, there is a large passive driving force for the exit of HCO"3~ across the basolateral membrane of the cell. As recently reviewed elsewhere, current evidence suggests that at least 80% of active H"+ secretion and HCO"3~ reabsorption in the proximal tubule takes place via the luminal membrane Na"+-H"+ exchanger (Aronson, 1983).

The presence of luminal membrane Na"+-H"+ exchange has also been clearly demonstrated in the small intestine (Murer et al., 1976; Gunther and Wright, 1983; Knichelbein et al., 1983) and gall bladder (Weinman and Reuss, 1982). There is acidification of the luminal fluid in both the jejunum (Turnberg et al., 1970) and gall bladder (Whitlock and Wheeler, 1969). Thus, the model shown in Figure 3A is probably applicable to the process of H"+ secretion in these epithelia as well.

In other epithelia, such as pancreas (Swanson and Solomon, 1975) and choroid plexus (Wright, 1977), there is net secretion of HCO"3~. In these cases, there is probably Na"+-H"+ exchange across the basolateral membrane of the cell and downhill exit of HCO"3~ across the luminal membrane.

When a Cl"-OH"- (or HCO"3~) exchange system is present, Na"+-H"+ exchange can promote active transepithelial transport of Cl"-. For example, Figure 3B illustrates the case in which both Na"+-H"+ and Cl"-HCO"3~ exchangers are present on the luminal membrane of an epithelial cell. In this scheme, secondary active H"+ secretion via Na"+-H"+ exchange is driven by the luminal membrane Na"+ gradient and serves to maintain intracellular OH"- above the concentration at which it would be in electrochemical equilibrium across the luminal membrane. In the presence of CO2, intracellular HCO"3~ is generated from OH"-, a reaction accelerated by carbonic anhydrase. The resulting electrochemical HCO"3~ gradient from cell to lumen then drives Cl"- uptake across the luminal membrane by Cl"-HCO"3~ exchange. Such tertiary active Cl"- accumulation elevates the intracellular Cl"- concentration above equilibrium so that there is a favorable driving force for passive Cl"- exit across the basolateral membrane.

The simultaneous presence of Na"+-H"+ and Cl"-OH"- exchangers on the same membrane was first shown by Liedtke and Hopfer (1982) using luminal membrane vesicles from rat small intestine. Na"+-H"+ and Cl"-OH"- (or HCO"3~) exchangers have subsequently been demonstrated in luminal membrane vesicles isolated from Necturus proximal tubule (Seifter and Aronson, 1984) and from rabbit ileum (Knichelbein et al., 1983), but not from rabbit or rat jejunum (Gunther and Wright, 1983; Cassano et al., 1984). Stimulation of Cl"- uptake by H"+ gradients has been observed in luminal membrane vesicles isolated from the rabbit proximal tubule, a finding interpreted to indicate the presence of Cl"-OH"- exchange in this preparation (Warnock and Yee, 1981). However, when adequate precautions are taken to avoid electrical coupling of Cl"- and H"+ fluxes through independent conductive pathways, actual Cl"-OH"- exchange cannot be documented in rabbit or rat renal microvillus membranes (Seifter et al., 1984; Sabolic and Burckhardt, 1983). Similarly, studies on the intact proximal tubule of the rabbit have failed to demonstrate measurable Cl"-OH"- exchange across the luminal membrane (Schwartz, 1983; Sasaki et al., 1984). Experiments employing ion-spe-
cific microelectrodes have documented the simultaneous presence of Na\(^+\)-H\(^+\) and Cl\(^-\)-OH\(^-\) (or HCO\(_3^-\)) exchangers in the apical membrane of the *Necturus* gall bladder (Reuss 1984), but there is dispute as to whether these exchangers contribute the major fraction of NaCl entry under basal conditions (Reuss 1984) or only during cell volume regulation (Ericson and Spring, 1982). Simultaneous Na\(^+\)-H\(^+\) and Cl\(^-\)-HCO\(_3^-\) exchange probably also occurs across the apical membrane of the mouse renal cortical thick ascending limb of Henle, although this has not yet been directly demonstrated (Friedman and Andreoli, 1982).

Clearly, double ion exchange is a commonly found but not ubiquitous mechanism for mediating Na\(^+\)-coupled Cl\(^-\) transport in diverse epithelia. It is interesting to note that in many of the epithelia in which this mechanism is found, such as the gall bladder, ileum, and renal cortical thick ascending limb, Na\(^+\)-coupled Cl\(^-\) transport is stimulated by CO\(_2\) and is sensitive to inhibitors of carbonic anhydrase (Fisher et al., 1981; Heintze et al., 1981; Nelson et al., 1975; Friedman and Andreoli, 1982). These characteristics probably arise from the fact that at physiological pH values, Cl\(^-\) exchanges with HCO\(_3^-\) much more rapidly than with OH\(^-\) (Seifter and Aronson, 1984; Knucklebein et al., 1984). Thus, the generation of HCO\(_3^-\) from intracellular OH\(^-\) (Fig. 3B) may, under certain conditions, be rate-limiting for Na\(^+\)-coupled Cl\(^-\) transport occurring by the parallel operation of Na\(^+\)-H\(^+\) and Cl\(^-\)-HCO\(_3^-\) exchangers.

There are at least two mechanisms whereby Na\(^+\)-H\(^+\) exchange can promote the transepithelial transport of organic anions. First, the processes of active H\(^+\) secretion and HCO\(_3^-\) absorption resulting from luminal membrane Na\(^+\)-H\(^+\) exchange can drive the net absorption of lipid-soluble weak acids that cross cell membranes predominantly by nonionic diffusion (Fig. 3C). This mechanism probably accounts for the absorption of organic anions such as glycocain, butyrate, propionate, and acetate in the mammalian proximal tubule (Ullrich et al., 1971). Similar mechanisms probably account for absorption of lipid-soluble weak acids in the jejunum (Jackson and Morgan, 1975) and for their secretion in the choroid plexus (Wright, 1977). A second mechanism for organic anion transport indirectly coupled to Na\(^+\)-H\(^+\) exchange is shown in Figure 3D. In this case, the transport of organic anion across the luminal membrane occurs by a carrier-mediated process of OH\(^-\)-anion exchange or H\(^+\)-anion cotransport. Such a mechanism probably contributes at least in part to urate reabsorption in the mammalian proximal tubule (Blomstedt and Aronson, 1980).

Pathophysiological Roles

To be sure, a clear-cut role for Na\(^+\)-H\(^+\) exchange in any specific pathophysiological state is yet to be proved with certainty. Nevertheless, in view of the several important physiological roles of the plasma membrane Na\(^+\)-H\(^+\) exchanger just discussed, it requires only a small leap of imagination to invoke a role for this transport system in various pathological states. A few examples will be described briefly.

**Renal Acid-Base Disorders**

As mentioned earlier, Na\(^+\)-H\(^+\) exchange appears to be the principal mechanism for mediating H\(^+\) secretion and HCO\(_3^-\) reabsorption in the proximal tubule. Factors affecting the number or activity of Na\(^+\)-H\(^+\) exchangers in the proximal tubule may therefore influence the efficiency of reclamation of filtered bicarbonate, and may thereby contribute to determining the steady state concentration of plasma bicarbonate. For example, the elevated renal threshold for bicarbonate excretion during hypercapnia may result from the fact that elevating Pco\(_2\) lowers intracellular pH (Struyvenberg et al., 1968), an effect leading to allosteric stimulation of the luminal membrane Na\(^+\)-H\(^+\) exchanger by intracellular H\(^+\) (Aronson et al., 1982). Another example is the increased number or activity of Na\(^+\)-H\(^+\) exchangers in the luminal membranes of proximal tubular cells during potassium depletion (Seifter and Harris, 1984), an effect that may contribute to the maintenance of metabolic alkalosis in this disorder. Additional examples include the mild metabolic acidosis of hyperparathyroidism and the metabolic alkalosis of hyperglucocorticoid states. These two disorders may at least in part reflect the effects of parathyroid hormone and glucocorticoids to inhibit and stimulate, respectively, the number or activity of Na\(^+\)-H\(^+\) exchangers in the luminal membrane of proximal tubular cells (Freiberg et al., 1982; Cohn et al., 1983).

**Essential Hypertension**

As recently reviewed elsewhere (Haddy, 1983; Parker and Berkowitz, 1983; Blaustein, 1984), several different alterations of transmembrane Na\(^+\) transport in patients with hypertension have been described. One of the more consistent and reproducible findings has been an increased activity of the red cell transport system mediating Na\(^+\)-Li\(^+\) exchange in certain subgroups of patients with essential hypertension and their first degree relatives (see Smith et al., 1984, for a comprehensive compilation of these data). Under physiological conditions in the absence of Li\(^+\), the red cell Na\(^+\)-Li\(^+\) exchanger mediates 1:1 Na\(^+\)-Na\(^+\) exchange and, thus, has no impact on intracellular electrolyte content (Duhm and Becker, 1979). The role of abnormal Na\(^+\)-Li\(^+\) countertransport in the pathogenesis of hypertension has therefore been obscure.

One possibility is that the transport system assayed for its Na\(^+\)-Li\(^+\) exchange activity in red cells is really a Na\(^+\)-H\(^+\) exchanger. As discussed above, the plasma membrane Na\(^+\)-H\(^+\) exchanger has several features in common with the pathway mediating Na\(^+\)-Li\(^+\) exchange in red cells, and Na\(^+\)-H\(^+\) exchange has been demonstrated in human red cells.
Several mechanisms have been suggested by which increased activity of the plasma membrane Na\(^+\)-H\(^+\) exchanger in cells other than erythrocytes could play a pathophysiological role in essential hypertension (Aronson, 1982; Funder et al., 1984). First, increased Na\(^+\)-H\(^+\) exchange across the luminal membrane of cells in the proximal tubule or cortical thick ascending limb of Henle would tend to enhance Na\(^+\) reabsorption and thereby cause a defect in renal salt excretion. By analogy with other abnormalities that tend to impair renal Na\(^+\) excretion, such as chronic renal insufficiency or hyperaldosteronism, a defect of this type would require that the kidney be perfused at greater than normal arterial pressure in order to maintain salt balance (Guyton et al., 1974). In addition, the secondary release of natriuretic, digitalis-like, humoral factors could lead to an increase in peripheral vascular resistance (Hamlyn et al., 1982; Poston et al., 1982). In the steady state condition with sustained hypertension, "escape" from the primary defect in salt excretion would prevent a detectable increase in extracellular fluid volume. In addition, the expected tendency of augmented Na\(^+\)-H\(^+\) exchange to generate metabolic alkalosis would be offset by inhibition of distal nephron H\(^+\) secretion, which occurs by an ATP-driven H\(^+\) pump, rather than by Na\(^+\)-H\(^+\) exchange (Koeppen and Steinmetz, 1983). There is considerable evidence that a defect in renal salt excretion is a cause of essential hypertension in at least some patients (Haddy, 1980; de Wardener and MacGregor, 1982).

Increased activity of the plasma membrane Na\(^+\)-H\(^+\) exchanger in vascular smooth muscle cells could alternatively play a pathophysiological role in essential hypertension. Blaustein (1977, 1984) has suggested that Na\(^+\)-Ca\(^++\) exchange across the plasma membrane is an important determinant of the steady state cytosolic concentration of Ca\(^++\) in vascular smooth muscle cells, and thus of steady state vessel wall tension. According to this view, any factor that elevates intracellular Na\(^+\) will tend to elevate intracellular Ca\(^++\) and increase vascular tone. Thus, increased activity of the Na\(^+\)-H\(^+\) exchanger in vascular smooth muscle could cause an elevated peripheral resistance by virtue of a rise in intracellular Na\(^+\). In addition, it is likely that vascular tone is sensitive to changes in intracellular pH. For example, in a variety of vascular beds, hypercapnia induces vasodilation, probably secondary to a fall in intracellular pH (Shepherd, 1983). Thus, increased activity of the Na\(^+\)-H\(^+\) exchanger in vascular smooth muscle might also lead to an elevated peripheral resistance by virtue of a rise in intracellular pH.

Cancer

As outlined previously, activation of plasma membrane Na\(^+\)-H\(^+\) exchange is an early event in the response of cells to mitogenic growth factors. One such mitogenic substance known to activate Na\(^+\)-H\(^+\) exchange is platelet-derived growth factor (PDGF) (Burns and Rozengurt, 1983; Cassel et al., 1983). PDGF-like molecules are produced by a number of transformed cell lines, including naturally occurring human tumors (Bowen-Pope et al., 1984). Moreover, the oncogene product of the simian sarcoma virus is extensively homologous with PDGF (Waterfield et al., 1983). Tumor promoters such as phorbol esters have been recently found to stimulate plasma membrane Na\(^+\)-H\(^+\) exchange (Burns and Rozengurt, 1983). Thus, it appears likely that activation of plasma membrane Na\(^+\)-H\(^+\) exchange plays an important part in oncogenic transformation.

Activation of plasma membrane Na\(^+\)-H\(^+\) exchange could explain the high rates of lactic acid production by malignant tumors, as first described by Warburg 60 years ago. Glycolysis is limited by the availability of inorganic phosphate and adenosine diphosphate (ADP), and thus, by the rate of breakdown of adenosine triphosphate (ATP) to ADP and P, by cellular ATPases (Racker, 1983). The increased Na\(^+\) influx associated with enhanced Na\(^+\)-H\(^+\) exchange in transformed cells could therefore promote glycolysis by stimulating the Na\(^+\),K\(^+\)-ATPase, which is highly sensitive to changes in intracellular Na\(^+\) concentration (Smith and Rozengurt, 1978). Furthermore, the elevation of intracellular pH resulting from activation of Na\(^+\)-H\(^+\) exchange would also stimulate lactic acid production, inasmuch as the rate-limiting enzyme in glycolysis, phosphofructokinase, is extremely pH sensitive (Tri-vedi and Danforth, 1966).

Organ Growth and Hypertrophy

Another implication of the important role of Na\(^+\)-H\(^+\) exchange in the response of cells to mitogenic stimuli is the possible involvement of this transport system in nonmalignant forms of cell growth and proliferation. For example, after loss of renal mass, the remaining renal tissue undergoes compensatory growth (Hayslett, 1979). Recent studies indicate that Na\(^+\)-H\(^+\) exchange activity is enhanced in plasma membrane vesicles prepared from remnant renal tissue after renal ablation in the dog or rat (Cohn et al., 1982; Harris et al., 1984). Whether stimulation of Na\(^+\)-H\(^+\) exchange is a general feature of such other examples of tissue growth and proliferation as cardiac or vascular smooth muscle hypertrophy remains to be examined. The possible effect of angiotensins to stimulate Na\(^+\)-H\(^+\) exchange as noted earlier is of interest in this regard.

Conclusions

The plasma membrane Na\(^+\)-H\(^+\) exchanger is a ubiquitous transport system that participates in diverse cell functions involving extrusion of H\(^+\) from the cell or uptake of Na\(^+\). Intracellular pH is probably the most important physiological factor regulating the Na\(^+\)-H\(^+\) exchanger, a property that en-
ables the antipporter to play a critical role in the regulation of intracellular pH. As a consequence of this role, the Na+-H+ exchanger functions as an important signal transducer for a broad spectrum of stimuli that alter cell function through changes in intracellular pH. As a transporter of Na+, the antipporter participates in the regulation of cell volume. The plasma membrane Na+-H+ exchanger helps mediate the transport of not only Na+ and H+, but also Cl− and organic anions across epithelia. The Na+-H+ exchanger thus plays a major role in determining the volume and ionic composition of the extracellular fluid and of various epithelial secretions.

Given the diversity of its physiological functions, it would not be surprising if the Na+-H+ exchanger participated in pathophysiological processes, as well. Very preliminary evidence raises the possibility that this may indeed be the case. Disorders of whole body acid-base balance have traditionally been considered to fall within the domain of nephrology. Clearly, the study of acid-base homeostasis at the cellular level is of great relevance to all disciplines. It is not altogether unreasonable to suggest that pharmacological manipulation of plasma membrane Na+-H+ exchange activity may eventually find clinical use in the treatment of a spectrum of diseases ranging from cancer to hypertension.

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