Hydrogen ion currents in rat alveolar epithelial cells

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ABSTRACT Alveolar epithelial cells isolated from rats and maintained in primary culture were studied using the whole-cell configuration of the "patch-clamp" technique. After other ionic conductances were eliminated by replacing permeant ions with *N*-methyl-D-glucamine methanesulfonate, large voltage-activated hydrogen-selective currents were observed. Like H^+ currents in snail neurons and axolotl oocytes, those in alveolar epithelium are activated by depolarization, deactivate upon repolarization, and are inhibited by Cd^{2+} and Zn^{2+}. Activation of H^+ currents is slower in alveolar epithelium than in other tissues, and often has a sigmoid time course. Activation occurs at more positive potentials when external pH is decreased. Saturation of the currents suggests that diffusion limitation may occur; increasing the pipette buffer concentration from 5 to 120 mM at a constant pH of 5.5 increased the maximum current density from 8.7 to 27.3 pA/pF, indicating that the current amplitude can be limited in 5 mM buffer solutions by the rate at which buffer molecules can supply H^+ to the membrane. These data indicate that voltage-dependent H^+ currents exist in mammalian cells.

INTRODUCTION

In this report, voltage-gated hydrogen ion currents are demonstrated in alveolar epithelial cells. Similar voltage-gated H^+ currents have been measured directly in invertebrate (snail) neurons (*Helix aspersa,* Thomas and Meech, 1982; *Lymnaea stagnalis,* Byerly et al., 1984) and axolotl oocytes (*Ambystoma,* Barish and Baud, 1984). A wide variety of H^+ conducting mechanisms exist in other cells. There is strong indirect evidence for a H^+ conductance in human neutrophils (Henderson et al., 1987, 1988); other examples are cited by Meech and Thomas (1987). Water channels of amphibian skin and urinary bladder appear to be permeable to protons and are blockable by heavy metals, but are not voltage-gated (Harvey et al., 1991). Renal epithelia apparently have H^+ conductive pathways (Sabolic and Burckhardt, 1983). The uncoupling protein of the inner mitochondrial membrane is a nonvoltage-gated protonophore, probably a carrier, which generates heat in brown fat by dissipating the mitochondrial electrochemical H^+ gradient (Nicholls and Locke, 1984; Klingenberg and Winkler, 1985). A H^+ conducting pathway in skeletal muscle sarcoplasmic reticulum membrane may function as a counterion transporter to facilitate Ca^{2+} flux (Meissner and Young, 1980). Indirect evidence exists for proton channels in carrot cells (Barr et al., 1990). Finally, the membrane-spanning component of H^+-ATPase, the F_0 complex, is believed to comprise a H^+ conducting pore (Soboll et al., 1988). Unitary H^+ currents through the CF_0-CF_1 subunit of chloroplast synthase have been reported (Wagner et al., 1989).

At a typical external pH (pH_o) of 7.4 and internal pH (pH_i) of 7.2, the Nernst potential for H^+ (E_H) is −12 mV. Because the resting membrane potential of most cells is more negative than this, the driving force for a H^+ conductance (g_H) is inward. Nevertheless, the voltage-dependence of the g_H in neurons, oocytes, and alveolar epithelial cells is such that only outward H^+ currents are clearly evident, and functions which have been proposed for plasma membrane H^+ conductances generally involve dissipation of intracellular acid loads under conditions in which pH_i falls substantially (Henderson et al., 1987), or during action potentials while the membrane is depolarized above E_H (Thomas and Meech, 1982; Byerly et al., 1984; Mahaut-Smith, 1989*a*).

Voltage-gated H^+ currents do not inactivate during prolonged depolarization (Byerly et al., 1984; Meech and Thomas, 1987; Mahaut-Smith, 1989*b*). Barish and Baud (1984) observed a variable droop in the H^+ current during long depolarizing pulses, and a small depolarizing shift in the observed reversal potential with long prepulses, which they attributed to depletion of H^+ ions near the membrane. Similar phenomena can be seen in alveolar epithelial cells, which are much smaller (typically 8–15 μm in diameter) than oocytes (2–2.2 mm in diameter; Barish and Baud, 1984). That the depolarizing shift in the observed reversal potential is due to depletion of H^+ from the intracellular solution is demonstrated by experiments in which this shift is reduced by high buffer concentrations in the pipette solution.

Type II pneumocytes produce and secrete pulmonary surfactant, which lowers the surface tension at the

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air-fluid interface in the lungs and thus reduces the tendency for atelectasis (alveolar collapse). Although the freshly isolated cell preparation is initially mainly type II cells, identifiable by their characteristic lamellar bodies, type II cells lose functional and morphological features within a few days in culture (Dobbs, 1990). After this they closely resemble type I alveolar epithelial cells (Cheek et al., 1989), the large thin flat cells which cover most of the alveolar surface. Analogous differentiation is believed to occur in vivo, as one function of type II cells is to resurface damaged pulmonary epithelium after injury (Adamson and Bowden, 1974; Evans et al., 1975). Although this process complicates the study of pneumocytes in primary culture, no cell line has proven to be a good model for the type II pneumocyte (Dobbs, 1990). H^+ currents were observed in cultured cells within a day after isolation as well as weeks later, so this conductance appears to be a property of both types of pneumocytes. A preliminary account of this work has been presented (DeCoursey, 1991).

METHODS

Type II alveolar epithelial cells were isolated from adult rats using enzyme digestion, lectin agglutination, and differential adherence, following procedures developed by Mason et al. (1977) and Simon et al. (1986), and modified by others and ourselves as described elsewhere (DeCoursey et al., 1988; Jacobs and DeCoursey, 1990). H^+ currents were studied in cells 1–25 d after isolation.

Conventional whole-cell patch clamp technique (Hamill et al., 1981) was used, as described recently (DeCoursey, 1990), at bath temperatures near 20°C. Data are presented without correction for junction potential or leak current. Most experiments were done with solutions based on *N*-methyl-D-glucamine (NMG^+) neutralized with methanesulfonic acid ($CH_3SO_3^-$). External solutions contained nominally 160 mM NMG^+ and $CH_3SO_3^-$ and 2 mM $CaCl_2$ and were titrated with NMG^+ or $CH_3SO_3^-$; the osmolarity was adjusted with distilled water in some cases (particularly the pH 10 solution, *see next paragraph*) to near 300 mOsm, so actual concentrations are variable. External solutions were buffered with 5 mM MES (2[*N*-morpholino]ethanesulfonic acid) for pH_o 5.5; Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), BES (*N,N-bis*(2-hydroxyethyl)-2-amino-ethanesulfonic acid), or TES (2-[*tris*(hydroxymethyl)methylamino]-1-ethanesulfonic acid) for pH_o 7.0–7.4; TAPS (3-[*tris*(hydroxymethyl)-methylamino]-1-propanesulfonic acid) for pH_o 8.1–8.3; or CAPS (3-[cyclohexylamino]-1-propanesulfonic acid) for pH_o 10. Buffers were purchased from Sigma Chemical Co. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). The most frequently used internal solution was 160 (mM) NMG^+, 1 $CaCl_2$, 5 MES, and 5 EGTA, titrated to pH 5.5 with $CH_3SO_3^-$. An internal solution with a higher buffer concentration, also titrated to pH_i 5.5 with $CH_3SO_3^-$, contained 119 MES, 119 NMG^+, 0.74 $CaCl_2$, 3.7 EGTA. H^+ currents were also observed with pipette solutions containing Na^+ or TEA^+ (tetraethylammonium) as the main cation.

The pKa of NMG^+ was found to be ~9.7 by titrating with HCl. This means that in the pH 10 solution most of the NMG molecules will be uncharged. If the uncharged form of NMG can cross the membrane, then local alkalinization inside the membrane might occur (e.g., Roos and Boron, 1981; Boron, 1983), essentially "short-circuiting" the transmembrane pH gradient. Alveolar epithelial cell membranes typically became quite "leaky" (low resistance) in pH_o 10 solutions.

RESULTS

H^+ selectivity

When all ions known to be permeant in voltage-clamped alveolar epithelial cells are absent from both intracellular and extracellular solutions (with the exception of 2–4 mM Cl^- left to avoid electrode polarization), slowly activating outward currents can be seen at positive potentials. Measurement of the reversal potential of these currents, V_{rev}, indicates that H^+ is the main charge carrier. The method of estimating V_{rev} is illustrated in Fig. 1, in which families of tail currents in the same cell at three pH_o are shown. External and internal pH, pH_o and pH_i, respectively, are given in the format $pH_o//pH_i$. The g_H was activated by a depolarizing prepulse and then the membrane was repolarized to various test potentials. V_{rev} was interpolated from plots of the instantaneous current amplitude during the resulting tail currents, which were fit to single exponentials. As pH_o was decreased from 8.3 to 7.0 to 5.5, V_{rev} changed from −83 to −18 to +31 mV in this cell. This is in the direction that the Nernst potential for H^+, E_H, changes in these solutions. However, observed values of V_{rev} were consistently more positive than E_H; for example, in symmetrical pH 5.5//5.5 solutions E_H is 0 mV but V_{rev} was +30 mV. Possible reasons for this discrepancy are explored in the following paragraphs. At some potentials the tail transients can be fitted by a single exponential. In other cases the fit to an exponential is less good or even quite poor. It is possible that the underlying deactivation process may be exponential but distorted by the effects of accumulation/depletion phenomena because such processes clearly occur in these experiments. For this reason it was not attempted to fit the tail current data carefully. Forcing the data to the best single exponential, it nevertheless can be stated that deactivation is faster at more negative potentials, with the dominant time constant ranging from several hundred milliseconds at more positive potentials to a few tens of milliseconds at more negative potentials.

Voltage-dependence of g_H

The main features of the H^+ currents in alveolar epithelial cells are illustrated in Fig. 2. Families of currents recorded during 4 s long depolarizing pulses in 20 mV increments are shown, for the same cell as in Fig. 1. The g_H is off at the holding potential of −60 mV, and the whole-cell input resistance under these conditions was very high, with <10 pA of inward current at −100 mV, except at pH_o 8.3, where the cell was somewhat leakier. H^+ currents turn on slowly during depolarizing pulses, often with sigmoid kinetics. On repolarization to

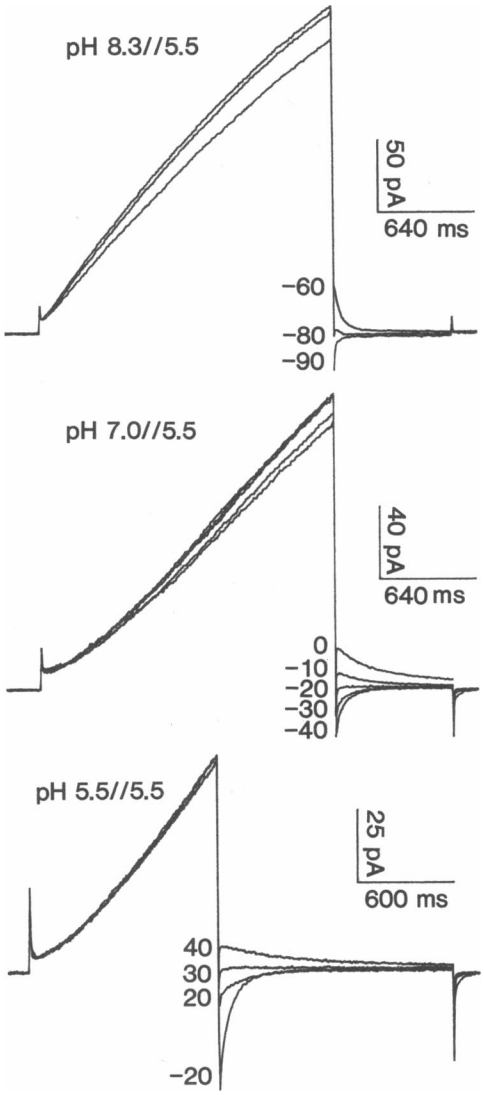

FIGURE 1 Measurement of tail currents and reversal potentials in an alveolar epithelial cell, with a pipette solution containing 5 mM MES at pH 5.5. The g_H was activated by a depolarizing prepulse to +40, +80, or +100 mV in pH_o 8.3, 7.0, or 5.5, respectively, from a holding potential of −60 mV. An interval of 10–20 s was allowed between pulses, but H^+ depletion is evident in several cases as the prepulse current was reduced. The tail currents shown in pH 5.5//5.5 reverse at +30 mV, but V_{rev} ranged from +26 to +44 mV when the prepulse duration and/or amplitude were decreased or increased, respectively. When bathed in 160 mM K^+ Ringer's solution, this cell had large K^+ currents of −4 nA at −20 mV. Diameter 15 μm, 31 pF input capacity, 20.5°C.

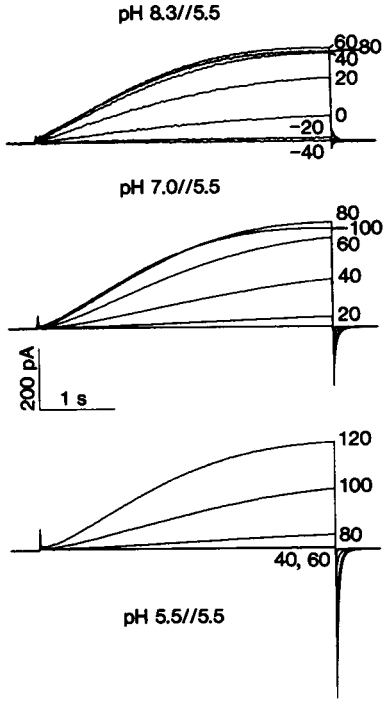

FIGURE 2 Whole-cell currents in the same cell as in Fig. 1 during voltage-clamp pulses 4 s long to the indicated potentials from a holding potential of −60 mV. Pulses were given in 20 mV increments with an interval of ~10 s between pulses. Calibration bars apply to all three families. There are large inward tail currents upon repolarization to −60 mV for pH_o 5.5, smaller inward tails in pH_o 7.0, and in pH_o 8.3 the tail currents are at first outward but after the pulse to +60 mV the tail reverses at −60 mV, and after the pulse to +80 mV the tail is clearly inward.

−60 mV, tail currents can be seen, reflecting the time course with which the g_H turns off, or deactivates. Deactivation is rapid compared to the slow rate of activation.

Although the H^+ currents appear qualitatively similar at various pH_o, the voltage at which the g_H is activated is more positive at lower pH_o. The effect of pH_o on the voltage dependence of activation is more clearly seen in Fig. 3, in which the currents at the end of the 4 s pulses in Fig. 2 are plotted. In the experiment illustrated, the g_H is activated at −20 mV in pH_o 8.3, at +20 mV in pH_o 7.0, and not until +80 mV in pH_o 5.5. As V_{rev} is shifted to more positive potentials by lowering pH_o, the voltage-dependence of activation is shifted by a roughly comparable amount, with the net result that only outward currents are clearly observed. In this respect, the g_H is reminiscent of inward rectifier channels, whose voltage-dependence shifts along with the K^+ reversal potential as the external K^+ concentration is changed. Inward rectifier channels carry mainly inward currents, but do carry small outward currents; whether the g_H can carry steady-state inward currents cannot be resolved from the present data. The inward currents in Fig. 3 are small and not detectably time-dependent, and presumably represent mainly leak current. Given this interpretation, the apparent reversal potentials in this figure reflect the voltage-dependence of H^+ current activation, rather

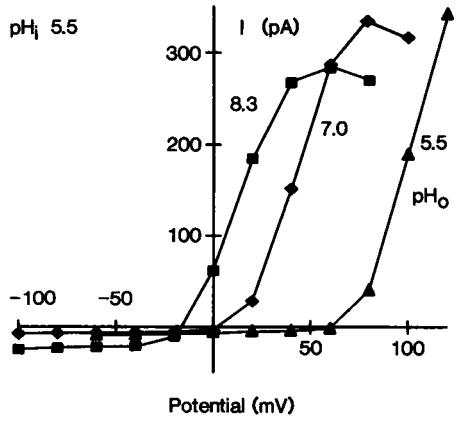

FIGURE 3 Current-voltage relationships for the families of currents in Fig. 2. Currents were measured at the end of the 4 s long pulses.

than the reversal potential of the g_H. Clear inward tail currents can be seen, so the H^+ conductance mechanism is capable of carrying inward current at least transiently.

H^+ current saturation

Two features in Fig. 2 indicate that the local H^+ concentrations may deviate from nominal values due to diffusion-limitation. First, the currents saturate during large depolarizing pulses. The potential at which saturation takes place is different at different pH_o. In pH_o 8.3 the currents at 40, 60, and 80 mV practically superimpose, while in pH_o 7.0 only the currents at 80 and 100 mV superimpose, and in pH_o 5.5 saturation is not evident up to 120 mV. In other experiments H^+ currents saturated also at low pH_o, at amplitudes similar to the maximum current at other pH_o in the same cell. Cells did not always survive the extreme positive voltage pulses necessary to observe saturation at pH_o 5.5. Although the potential at which the H^+ current saturates is different at different pH_o, the absolute magnitude of the current at saturation is roughly constant; that is in a given cell the maximum H^+ current amplitude seems to be limited. In the cell in Figs. 2 and 3 the maximum current was ~300 pA. The 10 s interval allowed between pulses in the experiment illustrated in Fig. 2 probably was not long enough to allow complete recovery from the effects of the previous pulse. However, the currents saturate with longer intervals between pulses as well. Current saturation might be due either to saturation of the membrane transport mechanism, or to limitation in the rate that buffer can supply H^+ to the membrane. The saturating current amplitude, estimated simply as the largest current observed in a given cell at any pH_o, varied from 100–450 pA in cells of various sizes with 5 mM MES in the pipette, and was correlated with the cell membrane area. This is a rough measurement, in that the pulse history affects the observed current magnitude. When the buffer concentration in the pipette was increased from 5 to 119 mM the maximum current density increased from 8.7 ± 2.8 pA/pF (mean ± SD, n = 10 cells) to 27.3 ± 4.5 pA/pF (n = 8) and little saturation was observed. These results indicate that at least with 5 mM buffer the translocation rate of the H^+ channel/transporter is not saturated, and therefore that the limiting process is the supply of H^+ to the membrane from the buffer.

The second indication of diffusion-limitation is in the tail currents. These generally conform to the expectation of the Nernst equation, being large and inward at −60 mV in pH_o 5.5, smaller and inward at pH_o 7.0, and outward for most of the pulses in pH_o 8.3; however, after the most positive pulse in pH_o 8.3 (the last pulse of the family) the tail at −60 mV reversed direction and became inward. Thus as the outward currents exhibited saturation V_{rev} shifted to substantially more positive values. Both the saturation of the current and the pulse-history-dependent shift in observed V_{rev} appear to be the result of depletion of H^+ from buffer molecules in the cell, but they reflect distinct depletion phenomena which occur at very different time scales. These ideas are explored further below under "*Reversal potential of H^+ currents*" and in the Discussion. V_{rev} depends on the free H^+ concentration, but this will decrease in accordance with the Henderson-Hasselbalch equation as buffer is depleted of H^+.

Block by polyvalent metal cations

H^+ currents in snail neurons and axolotl oocytes are inhibited by a variety of polyvalent metal cations (Thomas and Meech, 1982; Byerly et al., 1984; Barish and Baud, 1984; Meech and Thomas, 1987; Mahaut-Smith, 1989b; Byerly and Suen, 1989). Fig. 4 illustrates that H^+ currents in alveolar epithelial cells are similarly sensitive to such agents. Superimposed in the inset are currents during identical pulses to +60 mV in pH 8.1//5.7 in the absence or presence of 100 μM Cd^{2+}. Both the outward current and the tail current upon repolarization (not shown) are practically abolished. This inhibition was completely reversible. The effect of Cd^{2+} is not simple block. Plotted in Fig. 4 are peak current amplitudes from this experiment. The current-voltage relation appears to be shifted by 40–50 mV to more positive potentials in the presence of Cd^{2+} (■), compared with measurements before and after Cd^{2+} exposure (▲, ▼, respectively). In other experiments, when Cd^{2+} and Zn^{2+} were studied in the same cell, Zn^{2+} was found to inhibit H^+ currents more potently than did Cd^{2+}. Also in other cell types,

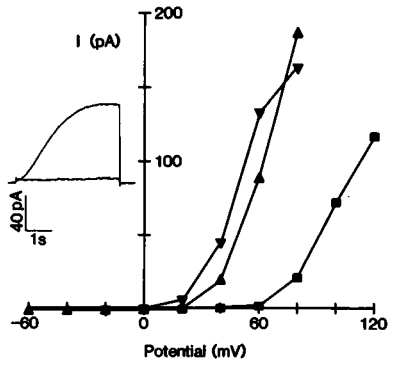

FIGURE 4 Inhibition of H^+ currents at pH 8.1//5.7 by Cd^{2+}. Currents measured at the end of 4 s pulses applied with 20 s intervals are plotted before (▲), in the presence of 100 μM Cd^{2+} (■), and after washout (▼). The inset illustrates currents at +60 mV in this experiment in the presence and absence of 100 μM Cd^{2+}.

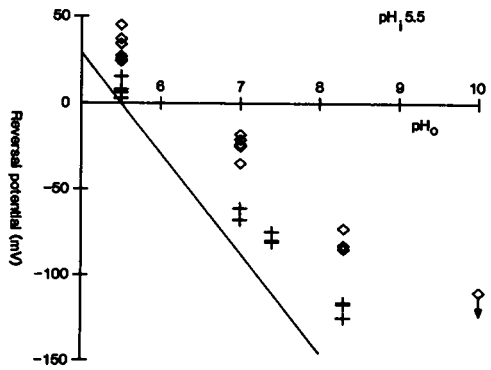

FIGURE 5 Observed reversal potentials in various pH_o always with pH_i 5.5, compared with the Nernst potential for H^+ shown as a line. At a given pH_o, each symbol is from a different cell. When more than one estimate of V_{rev} was made using different prepulses, the value of V_{rev} using the smallest or shortest prepulse is plotted, to minimize distortion due to H^+ depletion during the prepulse. The arrow on the point at pH_o 10 signifies that no reversal of current was observed; the value shown is the most negative potential at which outward tail currents could be resolved. The ability of pipette buffer to minimize local H^+ depletion at the inner side of the membrane is evident from the closer approach of V_{rev} in experiments with 119 mM MES buffer in the pipette (+) to the Nernst potential than values obtained with 5 mM MES in the pipette (◆).

Cd^{2+} shifts H^+ current activation to more positive potentials (Byerly et al., 1984; Barish and Baud, 1984).

Reversal potential of H^+ currents

Evidence suggesting that significant H^+ depletion may occur in these experiments was presented above, and it was suggested that this could account for the deviation of V_{rev} from E_H. Observed values of V_{rev} in a number of cells at various pH_o and always with pH_i 5.5 are plotted in Fig. 5. The diamonds represent experiments with 5 mM MES in the pipette. The V_{rev} values change approximately in parallel with E_H (line in Fig. 5), over a voltage range spanning ~140 mV. It seems clear that H^+ is the main charge carrier through the conductance described here. However, V_{rev} is well positive to E_H at all pH_o studied. This deviation is in the direction expected if H^+ were depleted from the cell as a result of the large outward current flowing during the prepulse, or alternatively if H^+ accumulated in an unstirred layer outside the membrane. To distinguish between these possibilities, experiments were carried out under identical conditions, but with 119 mM MES buffer in the pipette (plus signs in Fig. 5). In every case, V_{rev} in experiments with this higher buffer concentration was closer to E_H than when 5 mM buffer was used. This result strongly supports the interpretation that depletion of H^+ in the cell occurs as a result of outward current flow. By interpolation, the apparent pH_i after a depolarizing prepulse is ~6.3 with 5 mM MES and ~5.7 with 119 mM MES. These values correspond to a decrease in protonated buffer in the cell of 2.1 and 10 mM, respectively, calculated from the Henderson-Hasselbalch equation. Depletion of roughly this magnitude is reasonable considering the large H^+ currents flowing. The maximum H^+ current density with 5 mM buffer, 4×10^{-10} mol/cm^2 · s, corresponds to a depletion rate of 1.5 mM/s in a spherical cell 8 μm in diameter, and 0.44 mM/s in a 12 μm cell; the current density with 119 mM buffer was three times larger. As protonated buffer is depleted it will be replenished by diffusion from the pipette, which will occur more rapidly in a smaller cell. Because increasing the internal buffer concentration reduced the V_{rev} shift, this phenomenon must reflect H^+ depletion from the cell, rather than accumulation of H^+ in an external unstirred layer, although this might also occur to some extent.

With either concentration of buffer in the pipette, the V_{rev} values in Fig. 5 vary somewhat less steeply with pH_o than does E_H. The slope of the relationship between pH_o and V_{rev} is ~40 mV for a 10-fold change in H^+ concentration, with either concentration of pipette buffer, compared with 58 mV expected from the Nernst equation. In other preparations, the observed relationship between pH_o and V_{rev} is also less steep than that for E_H (Byerly et al., 1984; Barish and Baud, 1984; Thomas, 1988; Mahaut-Smith, 1989*a*). Although the traditional interpretation of such a result is that some other ion has finite permeability, Thomas' data are based on H^+ electrode measurements, and cannot be explained in this way. Mahaut-Smith (1989*a*) concluded that H^+ leakage dissipates the H^+ gradient near the membrane. The discrepancy in the present experiments increases at

high pH_o, suggesting that H^+ accumulation outside the membrane during outward current flow during the prepulse may contribute because a given H^+ current would have a larger effect on E_H at high pH_o. In the present experiments, however, measured values of V_{rev} could vary by 20 mV or more in a given solution, depending on the amplitude and duration of the prepulse, and inward tail currents at high pH_o were hard to resolve, so there is probably no point in overinterpreting this discrepancy. It is also possible that some of the other ions present have a finite permeability through the g_H mechanism. That V_{rev} varies with external H^+ concentration over a range exceeding 120 mV indicates that the alveolar epithelial cell membrane under these conditions is highly H^+ selective.

H^+ currents exist at physiological pH

The experiments described so far were done with pH_i 5.5, with the intention of facilitating the measurements by having a relatively large permeant ion concentration in the cell to enhance the current amplitude. Such a low pH might conceivably enable H^+ currents by modifying some membrane macromolecule to behave in a nonphysiological manner. Some experiments were done therefore using pipette solutions at near physiological pH_i, estimates of which in freshly isolated rat type II pneumocytes in vitro range 7.07–7.5 (Nord et al., 1987; Sano et al., 1988; Lubman et al., 1989). Fig. 6 shows a family of currents recorded in a cell with a pH_i of 6.9 bathed in pH_o 8.1. Slowly activating outward currents are evident at +20 mV and at more positive potentials. The H^+-selectivity of these currents was confirmed by measuring the reversal potential using the method illustrated in Fig. 1. V_{rev} was −61 mV, −3 mV, and +32 mV at pH_o 8.1, 7.0, and 6.4, respectively in this experiment, values close to E_H, −69 mV, −6 mV, and +29 mV. Similar currents were seen in several other cells with pH_i 6.9–7.4. Thus, H^+ currents are present in alveolar epithelial cells at pH_i within the physiological range.

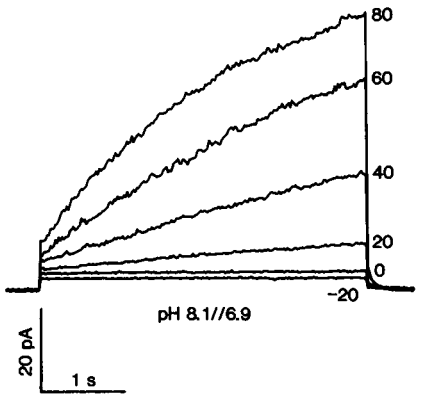

FIGURE 6 A family of H^+ currents in a cell with near neutral, i.e. physiological, pH_i at 6.9 and pH_o 8.1. The pipette solution contained 17 mM PIPES buffer (piperazine-*N*,*N*'-*bis*[2-ethane-sulfonic acid]). Note the outward tail currents after repolarization to the holding potential, −40 mV. The cell diameter was 16 μm and the input capacity was 17 pF.

DISCUSSION

A voltage-gated H^+ selective conductance of surprisingly large amplitude is present in the membranes of alveolar epithelial cells. In most respects this conductance resembles that described previously in snail neurons and axolotl oocytes. In all of these cells, the g_H is activated by depolarization with a threshold which shifts to more positive potentials as pH_o is lowered, the currents are inhibited by divalent metal cations, and the kinetics of deactivation are similar. Normalized to membrane area, as deduced from capacitance measurements, the g_H is of comparable density in snail neuron, 14.6 pA/pF (Byerly and Suen, 1989), and in alveolar epithelium, 27.3 pA/pF with 119 mM buffer. Whole-cell K^+ currents in snail neuron were 38.2 pA/pF at +30 mV, about twice as large as H^+ currents (Byerly and Suen, 1989). K^+ currents are 20–24 pA/pF at +20 mV, about the same amplitude as H^+ currents, in alveolar epithelial cells studied after comparable times in culture (Jacobs and DeCoursey, 1990). The most distinct difference between H^+ currents in different tissues is that the activation process is faster in snail neuron, with a time-to-half-peak of <25 ms at most potentials (Byerly et al., 1984; Mahaut-Smith, 1989*a*), and in axolotl, with a time constant up to ~300 ms (Barish and Baud, 1984), than in pneumocytes in which the current continues to increase throughout a 4 s pulse. In addition, activation follows an approximately exponential time-course in other cells (Byerly et al., 1984; Barish and Baud, 1984), whereas it is frequently sigmoid in pneumocytes. To the extent that diffusion limitation introduces a slowly decaying component due to H^+ depletion from the cell, the apparent rate of rise of the H^+ current would be increased. If H^+ accumulated externally, the voltage dependence of activation might be shifted progressively to more positive potentials during outward current flow, which would tend to slow activation. Given the similar membrane current density, however, such a phenomenon would be expected to occur in neurons as well. Evidently the g_H of alveolar epithelium simply activates more slowly than that in other tissues. The rapid activation of H^+ currents in neurons may reflect their proposed function during action potentials (Thomas and Meech, 1982; Byerly et al., 1984; Mahaut-Smith, 1989*a*).

Type II pneumocytes do not have detectable voltage-dependent inward currents (Jacobs and DeCoursey, 1990), and thus lack the apparatus necessary to sustain action potentials. The much slower kinetics of the g_H in these cells may reflect this physiological difference.

It is formally difficult to distinguish between outward H^+ currents and inward OH^- (or HCO_3^-) currents. However, the fact that the current saturated at about the same amplitude in a given cell at pH_o ranging from 5.5 to 8.3 or 10 strongly suggests that H^+ is the current carrier. H^+ is present inside the cell at a fixed concentration, whereas the concentration of OH^- externally under these conditions varies by several orders of magnitude. In addition, H^+ currents are large in pH 5.5//5.5 in which the concentration of H^+ is large whereas the concentration of OH^- is small. On the other hand, to the extent that the g_H seems limited by the buffer concentration rather than the free H^+ concentration, such arguments seem less convincing.

H^+ depletion during current flow

Consideration of the geometries, concentrations, and current amplitudes involved makes depletion of H^+ from the cell by H^+ currents seem quite likely. The amount of free H^+ in type II cells at pH_i 5.5 represents a total charge of 0.2–2 pC (picocoulombs) for a range of cell diameters 12–22 μm, whereas the quantity of charge carried out of the cell during maximum observed H^+ current ranges 0.1–1 pC per ms. It is obvious that practically all of the H^+ current observed must originate in H^+ bound to buffers and released continously during current flow. In six experiments with 5 mM pipette buffer, the total buffered charge in the cell due to exogenous buffer (MES with a pKa of 6.15 (Good et al., 1966) will be 82% protonated at pH 5.5) ranged from 0.35 to 2.2 nC, whereas the charge carried out of the cell during a long (10–20 s) pulse was 0.7–5.4 nC (nanocoulombs). The charge carried by H^+ was on average 3.1 times greater than the total in the cell. Either pipette buffer with bound H^+ diffuses into the cell rapidly enough to restore at least partially the buffer depleted during long pulses, or there are intrinsic buffers which remain in the cell even after an hour in the whole-cell configuration. In any case, significant depletion of total H^+ (free and buffered) in the cell seems inevitable. The pipette solution comprises an effectively infinite source of protonated buffer molecules, but given the geometries of pipettes and cells used the time constant for diffusional equilibrium of buffer between pipette and cytoplasm would range from 5 to 50 s, based upon both theoretical and empirically determined relationships (Pusch and Neher, 1988; Oliva et al., 1988). During long depolarizing pulses in experiments with 5 mM pipette buffer, the current drooped with time constants of this magnitude, from 3 to > 10 s.

The depletion of H^+ due to H^+ current flow can be observed by measuring V_{rev}, which reflects the bulk pH_i. The observed V_{rev} was consistently more positive then E_H, and the deviation of V_{rev} from E_H was substantially reduced when the pipette buffer was increased from 5 to 119 mM. Since currents of similar magnitude will deplete a fixed quantity of H^+, their effect on pH_i will be greater at low buffer concentrations, and hence V_{rev} will be more affected.

It is likely that H^+ depletion also occurs in a highly localized region near the membrane. H^+ currents saturated when the pipette buffer concentration was 5 mM, but not 119 mM. Transmembrane H^+ current will result in local depletion of H^+ from buffer molecules in a thin "reaction layer" near the membrane (see Delahay, 1954; Kasianowicz et al., 1987). The analogous problem of local depletion of Ca^{2+} ions at the inner surface of the membrane in the presence of mobile buffer during outward membrane transport has also been considered (Neher, 1986; Mathias et al., 1990). The severity of local depletion is reduced by increasing the buffer concentration. Taking the apparent rate constant for H^+ association with MES buffer as 0.6×10^8 M^{-1} s^{-1} (estimated from the rate that MES supplies protons to carbonic anhydrase, Jonsson et al., 1976), and the diffusion coefficient for H^+ as 8.65×10^{-5} cm^2/s at 20°C (Robinson and Stokes, 1959), equations A18–A21 of Mathias et al. (1990) give a time constant of local depletion of < 20 μs, a length constant of 0.4 μm, and a depletion of 6% (changing the local pH to 5.53) in 5 mM MES at the maximum observed H^+ current density. Thus, the reaction layer is small, develops more rapidly than could be resolved here, and does not change the local pH much for short pulses. Nevertheless, because the saturating current amplitude was correlated with cell membrane area, the limitation must be at or near the membrane rather than at the pipette tip, which would limit current according to pipette resistance, independently of cell size. Given that V_{rev} reflects bulk pH_i (refer to the previous paragraph), local H^+ depletion during current flow can be recalculated based on the effective pH_i derived from V_{rev} in Fig. 5. Now the local depletion in the reaction layer is 20% in 5 mM MES, and this would be even larger during families of long pulses (e.g., Fig. 2) than during the protocols used in V_{rev} measurements (Fig. 1) in which pulses were kept as small and short as possible. Given the assumptions used, local depletion seems to be a plausible explanation for the saturation of H^+ currents. In summary, H^+ current saturation and the deviation of V_{rev} from E_H both are the result of H^+

depletion, the former of rapid local depletion in the "reaction layer" near the membrane, and the latter of slower bulk depletion of H^+ from buffer molecules in the cell.

Intrinsic buffering

The current in a given cell saturated due to limitation in the rate at which buffer could supply H^+ to the membrane conductance mechanism. This saturation was obvious in most cells studied with 5 mM buffer. With 119 mM buffer, saturation was not apparent and greater than threefold larger currents were observed; however, the current amplitude was not increased 24 times as would be expected if the pipette buffer, MES, were the sole active buffer. One possibility is that there are intrinsic cytoplasmic buffers which are either fixed in organelle or surface membranes or which simply diffuse very slowly. If this were the entire explanation, then the effective intrinsic buffering capacity could be calculated by assuming that the limiting current increases in direct proportion to the sum of intrinsic and exogenous (pipette) buffer. This analysis gives an estimate for the intrinsic buffer as the equivalent of 49 mM MES at the pH studied. Intrinsic buffering power measurements in the literature, mostly in muscle or nerve, range from 10 to 80 mM $pH^{-1}\ l^{-1}$ (Roos and Boron, 1981). However, the limiting current values with high buffer concentrations are underestimates, because current saturation was not clearly detected up to potentials which damaged the membrane, so this estimate is likely too high. An alternative explanation is that whereas the g_H is not saturated with 5 mM buffer, with higher buffer concentration the maximum transport rate of the g_H mechanism is reached.

Channels or transporter?

Whether macroscopic H^+ currents are due to H^+ permeable ion channels or to some other mechanism is not clear. Byerly and Suen (1989) could not resolve single channel H^+ currents in snail neuron and concluded that any unitary current must be <0.004 pA. Decker and Levitt (1988) found that single channel currents carried by H^+ through gramicidin channels are limited by diffusion in the bulk solution at the channel mouth. At pH 3.75 the unitary current was <0.1 pA at +250 mV, but was increased to 1 pA by 2 M formic acid, which evidently provides H^+ ions to the mouth of the channel (Decker and Levitt, 1988). In light of this result, a report by Wagner et al. (1989) that unitary H^+ currents through the CF_0–CF_1 subunit of chloroplast synthase are relatively large, 3 pS and the same amplitude in symmetrical pH 5.5 or symmetrical pH 8.0 seems surprising, although the authors suggest a variety of mechanisms which could increase the local H^+ concentration near the channel mouth. A variety of single channel-like currents can be detected in alveolar epithelial cells (data not shown) but to date none has been identified as H^+ selective. The possibility that the H^+ current is carried by an electrogenic pump or other transporter cannot be immediately dismissed. H^+-ATPase can generate a proton current density of 2.5 $\mu A/cm^2$ in vacuoles (Hedrich et al., 1989) and 13 $\mu A/cm^2$ in *Neurospora* plasma membrane (Slayman and Gradmann, 1975), and the H^+/amino-acid symporter in fungal hyphae carries inward proton current of $\sim 1\ \mu A/cm^2$ (Kropf et al., 1984) These values are comparable with 8.7 or 27.3 $\mu A/cm^2$ in the present study (with 5 or 119 mM buffer, respectively, assuming the specific capacitance is 1 $\mu F/cm^2$). No ATP was included in the pipette solutions, however, and the g_H did not appear to run down during long experiments.

Possible functions of H^+ currents in alveolar epithelium

The present demonstration of H^+ currents in rat alveolar epithelial cells shows that voltage-dependent H^+ currents are present in mammalian cells, and that the currents are surpisingly large. A H^+ conductance in any cell must influence and may help to control pH_i, which in turn affects a great many cellular processes and physiological signals and events (reviews: Roos and Boron, 1982; Nuccitelli and Deamer, 1982; Moody, 1984; Häussinger, 1988). Considering only those features peculiar to type II alveolar epithelial cells, a main function is to produce and secrete surfactant. Whereas Sano et al. (1988) found no effect of secretagogues on pH_i, Chander (1989) has demonstrated that intracellular alkalosis stimulates surfactant secretion. Pulmonary surfactant is stored in lamellar bodies, which maintain a pH of <6.1 (Chander et al., 1986), and when surfactant is secreted the local alveolar surface must be acidified at least transiently. Compared with the arterial pH of ~ 7.4, the alveolar aqueous subphase, the extracellular liquid between the alveolar epithelium and the surfactant lining the alveolar airspace, is in fact quite acidic, at pH 6.69 in dogs (Effros and Chinard, 1969), 6.27 in fetal lambs (Adamson et al., 1969), and 6.92 in adult rabbits even when the pleural surface is exposed to solutions buffered to pH 6.75–7.5 (Nielson et al., 1981). The microscopic structure and aggregation of pulmonary surfactant are affected markedly by Ca^{2+} and pH (Efrati et al., 1987).

Pulmonary epithelium is a structural component of the diffusion barrier between blood and alveolar air, across which most of the body's acid is eliminated in the

form of CO_2, and consequently may be exposed to rapid and large fluctuations in pH_i. Carbonic anhydrase has been reported in rat alveolar epithelium (Sugai et al., 1981; Gros et al., 1988; cf. Lönnerholm, 1982). The pH_i of pig lungs drops from 7.47 to 7.11 or 6.37, after 15 min of anoxia or ischemia, respectively (Pillai et al., 1986). It can be imagined that if the g_H were activated by low pH_i, this mechanism could allow rapid dissipation of a large acid load. Alveolar epithelium has fluid and electrolyte transport functions, which differ in fetal and adult lung (Mason et al., 1982; Goodman et al., 1984; Cott et al., 1986; O'Brodovich et al., 1990). A proton-translocating ATPase has been demonstrated in the plasma membrane of rat alveolar epithelium (Lubman et al., 1989). Rat and sheep type II cells also have a Na^+-H^+ antiporter (Nord et al., 1987; Sano et al., 1988; Shaw et al., 1990).

Whether there might be a relationship between the specific functions of alveolar epithelium and H^+ currents, or whether H^+ currents are simply a little recognized feature of many different cell types is not clear at this point. The relationship between the H^+ conductance and these various functions could be better evaluated with more information regarding the behavior of the conductance under more physiological conditions. Small but well-defined H^+ currents can be observed at physiological pH_i (Fig. 6). Because the g_H appears to activate at potentials more positive than normal resting potentials, a key question is whether the g_H may be activated and carry small inward currents at potentials negative to E_H. Thomas (1988) has shown that both internal and surface pH change in a manner consistent with inward H^+ current for small depolarizations; but has ascribed these changes to indirect effects of Ca^{2+} influx which may expose external binding sites to H^+ causing surface alkalinization and cause internal acidification by displacing H^+ from intracellular binding sites (Thomas, 1989). Considering the concentration of H^+ in the cell, the outward H^+ currents seen experimentally in alveolar epithelial cells are extremely large and rapidly deplete intracellular H^+, and any physiological H^+ current in an intact cell would most likely be much smaller. Activation of even a relatively small fraction of the g_H would have a substantial effect on pH_i in an intact cell.

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