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invited review

Hypothesis: do voltage-gated H^+ channels in alveolar epithelial cells contribute to CO_2 elimination by the lung?

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DeCoursey, Thomas E. Hypothesis: do voltage-gated H^+ channels in alveolar epithelial cells contribute to CO_2 elimination by the lung? *Am. J. Physiol. Cell Physiol.* 278: C1–C10, 2000.—Although alveolar epithelial cells were the first mammalian cells in which voltage-gated H^+ currents were recorded, no specific function has yet been proposed. Here we consider whether H^+ channels contribute to one of the main functions of the lung: CO_2 elimination. This idea builds on several observations: *1*) some cell membranes have low CO_2 permeability, *2*) carbonic anhydrase is present in alveolar epithelium and contributes to CO_2 extrusion by facilitating diffusion, *3*) the transepithelial potential difference favors selective activation of H^+ channels in apical membranes, and *4*) the properties of H^+ channels are ideally suited to the proposed role. H^+ channels open only when the electrochemical gradient for H^+ is outward, imparting directionality to the diffusion process. Unlike previous facilitated diffusion models, HCO_3^- and H^+ recombine to form CO_2 in the alveolar subphase. Rough quantitative considerations indicate that the proposed mechanism is plausible and indicate a significant capacity for CO_2 elimination by the lung by this route. Fully activated alveolar H^+ channels extrude acid equivalents at three times the resting rate of CO_2 production.

pH; acid-base regulation; proton; pulmonary gas diffusion

VOLTAGE-GATED H^+-selective ion channels are present in a number of cells, including snail neurons, most mammalian phagocytes, and rat alveolar epithelial cells. Voltage-gated H^+ currents were discovered in 1982 in snail neurons by Roger Thomas and Robert Meech (92). The first direct measurement of H^+ currents in mammalian cells was in rat alveolar epithelial cells (13), where the H^+ current density is as large as that of voltage-gated K^+ currents. Specific functions for H^+ currents have been proposed in several cells (see PROPERTIES OF H^+ CHANNELS), but none has yet been proposed in pulmonary epithelium. The properties of H^+ channels appear ideally suited to extruding acid from cells, and a major function of the lung is to eliminate metabolically produced acid from the body, in the form of CO_2. It seems obvious that a possible function of H^+ channels in alveolar epithelium might be to help extrude acid into the alveolar subphase (the extracellular liquid lining the alveolar surface). Yet, until now this idea has not been proposed explicitly, largely because several objections could immediately be raised. *1*) It is well known that acid is extruded by the lung in the form of CO_2, and thus the need for an additional acid extrusion mechanism is not obvious. *2*) To produce outward H^+ current requires that the electrochemical gradient for H^+ be outward. If the extracellular pH (pH_o) is 7.4 and the intracellular pH (pH_i) is 7.2 [reported values in alveolar epithelial cells range from 7.07 to 7.5 (67, 75)], then the Nernst potential for H^+, E_H is −12 mV. If the resting membrane potential, measured in primary culture, is −40 mV [reported values range from −27 to −63 mV (6, 32)] then ~30-mV depolarization would be required to produce an outward electrochemical gradient for H^+. *3*) Finally, according to the traditional belief that small, uncharged molecules such as water and CO_2 are freely and rapidly permeant through cell membranes, there would be no need for another mechanism of CO_2 extrusion. On the other hand, alveolar epithelial cells express H^+ channels at a very high density, and we do not like to imagine Nature doing things for no good reason. Several factors suggest that it is time to evaluate the possibility that some part of normal acid

extrusion by the lung is mediated by H^+ channels. In brief, these are: *1*) CO_2 is less membrane permeant than has been assumed traditionally. *2*) Carbonic anhydrase II (CA II) is present in alveolar epithelial cells. Theoretical and experimental evidence suggests that carbonic anhydrase facilitates CO_2 extrusion by the lung ("facilitated diffusion") and that its deficiency results in respiratory acidosis. *3*) The transepithelial potential difference favors selective activation of H^+ channels in the apical membrane rather than the basolateral membranes. *4*) H^+ channels are expressed at a high level in mammalian alveolar epithelial cells. Their properties are ideally suited to the proposed role in CO_2 extrusion by the lung.

In essence, the present hypothesis extends the idea of facilitated diffusion by proposing that recombination of HCO_3^- and H^+ occurs in the alveolar subphase rather than inside the epithelial cell (which would necessitate CO_2 diffusing across the apical membrane). The purpose of this paper is to consider the possibility that H^+ channels are expressed at high levels in alveolar epithelial cells for the purpose of facilitating CO_2 extrusion by the lung. Final confirmation or refutation of this hypothesis will require further study, but it is hoped that this suggestion will stimulate and focus research into this question.

CO_2 PERMEABILITY OF CELL MEMBRANES IS NOT AS HIGH AS WE USED TO THINK

All of the acid extruded via the lungs is in the form of CO_2. Because of the traditional assumption that small neutral molecules such as CO_2 and H_2O permeate cell membranes freely and rapidly, involvement of H^+ channels seems superfluous. Recent observations indicate that this assumption needs to be reexamined. Nakhoul et al. (Ref. 71 and see also Refs. 10 and 81) found that the CO_2 permeability of *Xenopus* oocytes is increased significantly by the expression of the water channel aquaporin-1 and was substantially higher than the CO_2 permeability of lecithin-cholesterol bilayers (40). If the presence of water channels increases CO_2 flux, then the intrinsic membrane permeability of CO_2 clearly must be limited. These studies reinforce other evidence that the membrane permeability to CO_2, in certain cells at least, is exceedingly low (95). In tracer studies under a variety of conditions, the flux of CO_2 across the alveolar/capillary barrier displayed evidence of diffusion limitation at pH $>$ 8 but not at pH 7.4 (27). It seems a priori logical that it is in the best interests of the mammal to have a high CO_2 permeability in alveolar epithelium. However, limited CO_2 permeability coupled with CO_2 extrusion in the form of H^+ via H^+ channels (together with HCO_3^- extrusion) would impart strong outward rectification to the diffusion process. The voltage-gating mechanism of H^+ channels is tightly regulated by pH_o and pH_i, with the result that the channels open only when there is an outward electrochemical gradient for H^+, and hence only outward H^+ currents are activated under physiological conditions (5, 8, 16, 18, 55, 68, 91).

FACILITATED DIFFUSION OF CO_2

It has been known for some time that carbonic anhydrase is present in lung tissue. Some early studies reported localization in alveolar capillary endothelial cells (43, 63), but further study revealed that alveolar epithelial cells also express carbonic anhydrase (31, 37, 88). Several types of evidence indicate that facilitated diffusion of CO_2 occurs in intact lung tissue (reviewed in Ref. 37). Inhibiting carbonic anhydrase reduces the diffusion of CO_2 in vitro and in the lung (26, 57, 58). Interpreting these studies requires determining which effects are due to inhibition of carbonic anhydrase in red blood cells, where it plays a central role in the well-known "Cl^- shift" (e.g., Ref. 83). In addition, it is necessary to distinguish between effects on capillary endothelial cells, which express high levels of carbonic anhydrase, and extravascular spaces, which include epithelial cells. Enns and Hill (30) demonstrated that intracellular carbonic anhydrase is present in the lung and plays a role in facilitated CO_2 diffusion. By use of selectively permeable inhibitors, Heming et al. (44) determined that diffusion of CO_2 was inhibited only by extravascular carbonic anhydrase inhibition. It has been shown recently by Northern blot and immunohistochemistry that of four isozymes of carbonic anhydrase in the lung, the soluble enzyme CA II is present unambiguously in rat type II cells, both in situ and in vitro (31). Expression in type I epithelial cells could not be ruled out in light of their small cytoplasmic volume; furthermore, type II cells in culture continued to express CA II after differentiation into type-I-like cells (31). This localization is compatible with a role in facilitated elimination of CO_2 by the lung. Presence in type I cells would provide teleological support to the present proposal, because although the diffusion distance through type I cells is short, 0.1–0.5 μm (86), intracellular conversion of CO_2 to HCO_3^- and H^+ would obviate the need for CO_2 extrusion through the apical membrane.

Several additional lines of evidence suggest that human CA II plays a role in CO_2 extrusion by the lung. Hereditary CA II deficiency in humans results in severe acidosis, with both a renal component (87) and a respiratory component (78). Recent studies using genetic knockout to selectively eliminate CA II support this picture. Lien and Lai (61) demonstrated respiratory acidosis in mice genetically deficient in CA II, which they attributed to CA II deficiency of both red blood cells and alveolar type II epithelial cells. These authors proposed that CA II facilitates CO_2 extrusion by the lung by accelerating the recombination of HCO_3^- and H^+ to form CO_2 within alveolar epithelial cells. The resulting CO_2 then diffuses out through the apical membrane. Despite this suggestion by the authors, their data do not distinguish whether CO_2 recombination occurs within the alveolar epithelium or in the subphase. In any case, the results make it clear that simple diffusion of CO_2 down its partial pressure gradient does not eliminate CO_2 fast enough to avoid respiratory acidosis.

PROPERTIES OF H^+ CHANNELS

Voltage-gated H^+ channels are extremely selective for H^+ (21), are activated by membrane depolarization, and have a miniscule single-channel conductance, ~10 fS (14), roughly 1,000 times smaller than ordinary ion channels. Given the large macroscopic H^+ current (8, 13), each cell must express 10^5 to 10^6 H^+ channels. H^+ channels are opened by membrane depolarization, but the threshold voltage at which the H^+ conductance is first activated ($V_{threshold}$) depends strongly and linearly on the pH gradient ($\Delta pH = pH_o - pH_i$) across the membrane (18). Decreasing pH_i or increasing pH_o by one unit shifts $V_{threshold}$ by 40 mV (8, 18). In fact $V_{threshold}$ can be predicted from (8)

$$V_{threshold} = 20\ mV - 40\Delta pH \quad (1)$$

The result is that H^+ channels open only when there is an outward electrochemical gradient for H^+, and hence their function is evidently to extrude acid from cells. In snail neurons, H^+ channels may open during action potentials to compensate for the metabolic cost of excitation (5, 68, 92), or they may open due to local acidification near the membrane due to Ca^{2+}/H^+ exchange (5, 70). In human neutrophils and in other phagocytes, H^+ channels are activated during the respiratory burst (reviewed in Ref. 22), resulting in electrogenic H^+ extrusion that lowers extracellular pH and tends to repolarize the membrane potential (46–48, 72, 89). The evidence that voltage-gated H^+ channels contribute to this H^+ extrusion is strong (14, 16, 22, 54). In alveolar epithelial cells, H^+ currents are present at a relatively high density (13), exceeded only by that in eosinophils (35, 84, 85), neutrophils (15), and basophils (9). Here we propose that by extruding acid, H^+ channels contribute to CO_2 elimination by the lungs.

Several of the properties of H^+ channels in alveolar epithelium make them ideally suited to the proposed role in facilitating CO_2 extrusion. *1*) They appear to be present in the apical membrane. *2*) They are opened by cytoplasmic acidification. *3*) Their gating is controlled locally, so that the critical factors are local pH and the voltage across the apical membrane. *4*) Related to *3*, the transepithelial potential is oriented in the direction to enhance H^+ channel opening in the apical membrane but not in the basolateral membranes. *5*) Finally, there is no energetic cost to the cell of allowing H^+ efflux down its electrochemical gradient through H^+ channels. Of course the cell must first synthesize the H^+ channels and insert them into the apical membrane. In contrast, H^+ efflux via the H^+-ATPase consumes ATP directly, and H^+ efflux via the Na^+/H^+-antiporter indirectly consumes energy by dissipating the Na^+ gradient, which must be restored by the Na^+ pump.

HYPOTHESIS

Figure 1 illustrates the essential features of a hypothetical mechanism in which H^+ channels contribute to the elimination of CO_2 by the lung. Once CO_2 enters the alveolar epithelial cell, it is converted to H^+ and HCO_3^- by carbonic anhydrase. H^+, after combining with mobile buffer, and HCO_3^- diffuse across the cell to the apical membrane (upper pathway in Fig. 1). This facilitation of CO_2 diffusion within alveolar epithelium by carbonic anhydrase has been proposed previously (30, 44, 61). However, it is not essential to the present proposal. Conceivably, the main function of carbonic anhydrase in alveolar epithelium is to convert CO_2 to HCO_3^- and H^+ just inside the apical membrane, providing a local gradient to drive both HCO_3^- and H^+ extrusion (lower pathway in Fig. 1). In either case, at the apical membrane H^+ is extruded through voltage-gated H^+ channels, and HCO_3^- exits passively through anion channels or perhaps via Cl^-/HCO_3^- exchange (56, 76). If Cl^-/HCO_3^- exchange were the preferred mechanism, the Cl^- in the alveolar subphase would be replenished by Cl^- efflux through anion channels. One attractive feature of the proposed extrusion of HCO_3^- together with H^+ is that the simple act of extruding HCO_3^- would acidify the subcompartment just inside the apical membrane. Lowering local pH_i would enhance the outward ΔpH and promote activation of the H^+ conductance. The coextrusion of HCO_3^- and H^+ are thus cooperative. Furthermore, their coextrusion would be electroneutral. Once HCO_3^- and H^+ arrive in the alveolar aqueous subphase, the layer of liquid lining the epithelial surface, they spontaneously recombine to form CO_2, which enters the alveolar gas phase. The H_2O formed at the same time would be reabsorbed osmotically. In the rest of this paper the plausibility of the required elements of this hypothesis are evaluated.

EVALUATION OF THE HYPOTHESIS

Can H^+ Channels Handle the Job?

Fully activated, the H^+ conductance of alveolar epithelial cells extrudes acid ~100 times faster than any other membrane transporter, including Na^+/H^+ antiport, Cl^-/HCO_3^- exchange, and H^+-ATPase (16). Human metabolism produces 250 ml CO_2/min (60), and the total alveolar surface area is 75 m^2 (97). If all of the CO_2 produced by metabolism were extruded by H^+ channels, this would require an efflux rate of 9.6 pA/pF, assuming a specific capacitance of 2.5 $\mu F/cm^2$ (23). The maximum H^+ current density in rat alveolar epithelial cells is 27 pA/pF (13). It is unlikely that the H^+ conductance is fully activated in vivo, and it seems probable that the bulk of CO_2 extrusion occurs simply by diffusion across the membrane, but this calculation shows that the H^+ conductance has the capacity to extrude very large quantities of acid and could in theory extrude three times more acid than produced by basal metabolism.

The magnitude of H^+ channel expression in rat alveolar epithelial cells is impressive. The H^+ current density during a large depolarization is 20–30 pA/pF (8, 13), which amounts to several hundred pA in the whole cell membrane. H^+ currents are comparable in size to voltage-gated K^+ currents in these cells (23, 62, 80), although the intracellular concentration of H^+ is

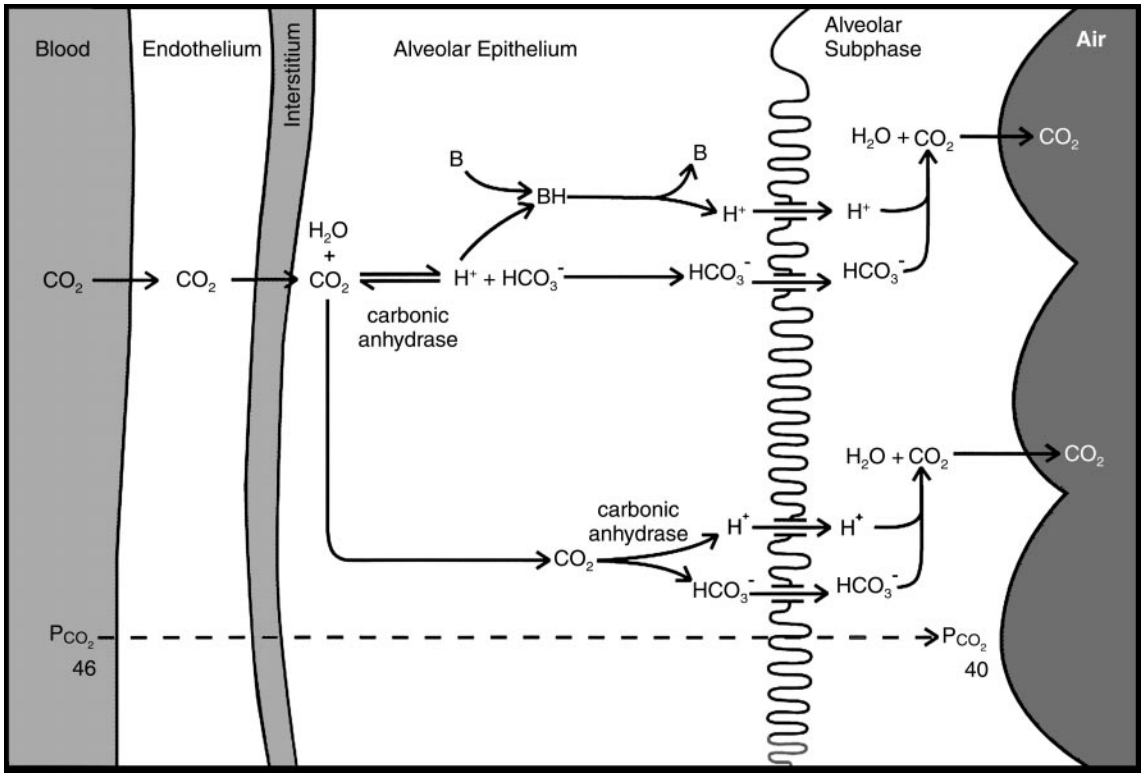

Fig. 1. Diagram illustrating the essential features of the proposed mechanism for acid extrusion by H^+ channels. Briefly, CO_2 leaves the blood and crosses the endothelial cell layer to reach the alveolar epithelium. Diagram is not to scale: diffusion paths through the endothelial cell, interstitium, and epithelial cell are all of roughly equal thickness (97). There is evidence that carbonic anhydrase-catalyzed facilitated diffusion may contribute to CO_2 movement across endothelial cells (26, 63). Carbonic anhydrase II, present in cytoplasm of alveolar epithelial cells, catalyzes the conversion of CO_2 (and H_2O; not shown) to HCO_3^- and H^+ (via H_2CO_3; also not shown). These ions diffuse across the cell, the H^+ bound to mobile buffer (B). H^+ leaves by permeating voltage-gated H^+ channels in the apical membrane, and HCO_3^- leaves through anion channels (or perhaps by Cl^-/HCO_3^- exchange). Extruded HCO_3^- and H^+ recombine to form CO_2 and H_2O in the aqueous subphase, a thin layer of liquid lining the epithelial surface. CO_2 then enters the gas phase and H_2O is reabsorbed. The lower pathway is similar, except that CO_2 diffuses through the cell and is converted to HCO_3^- and H^+ near the apical membrane to provide a local source near the efflux channels. See text for more details of the proposed mechanism.

$\sim 10^{-7}$ M, one million times smaller than that of K^+, $\sim 10^{-1}$ M. Because the single-channel current is ~1,000 times smaller for H^+ channels than for K^+ channels, there are evidently ~1,000 times more H^+ channels in the cell membrane.

Are H^+ Channels Present in the Apical Membrane?

The hypothesis requires that H^+ channels are present in the apical membrane of alveolar epithelial cells. Assuming that alveolar epithelial cells cultured on glass are polarized with their apical side up, this requirement is fulfilled because cell-attached patches and excised patches of this exposed membrane consistently express H^+ currents (17). Evidence supporting this intuitively logical orientation of cultured alveolar epithelial cells (basolateral membrane adhering to substrate, apical membrane facing the culture media) is that dome formation has been observed in confluent monolayers (33, 34, 69). These domes reflect fluid accumulation under the monolayer as a result of the fluid-absorptive function of adult alveolar epithelium. Further support for this orientation of the monolayer is that transepithelial potentials have the "correct" apical-negative bias (11, 69). In addition, the apical surface exhibits microvilli (24), characteristic of type II cells in situ.

It should be cautioned that most studies of alveolar epithelial cells in culture have been performed on type II cells, in part because isolating type I cells has been an intractable problem. Type II cells differentiate into type I cells in vivo after alveolar epithelial injury, and a process like this takes place in vitro. Thus it seems reasonable to consider that a confluent monolayer of type II cells provides the best available model for transepithelial transport in the alveolus.

Is the Electrochemical Gradient for H^+ Ever Outward?

At "normal" pH and resting membrane potential, the electrochemical gradient for H^+ is inward, and H^+ channels are closed. This is generally desirable, because if H^+ channels opened when ΔpH was inward, the result would be acid loading. To activate H^+ currents and extrude acid, an outward gradient is necessary. Two critical factors that apply in the in vivo lung promote preferential opening of H^+ channels in the apical membrane of alveolar epithelial cells: local con-

trol of H^+ channel gating and a favorable transepithelial potential difference (PD).

H^+ Channel Gating is Controlled Locally

The cooperative regulation of H^+ channel gating by pH_o, pH_i, and membrane potential requires only that an outward electrochemical gradient exist in the immediate vicinity of the channel. The sensitivity of H^+ channels in excised patches of membrane to both pH_o and pH_i was the same as in whole cell measurements (17). In addition, H^+ channel gating reflected local pH changes due to Na^+/H^+ antiport (15). Therefore, acidification of the cytoplasm just inside the apical surface due to HCO_3^- extrusion will promote H^+ channel opening.

Transepithelial Potentials Favor Apical H^+ Extrusion

Although alveolar epithelial cells studied in vitro are usually patch clamped when they are roughly spherical and unconnected to neighboring cells (conditions which provide for optimal electrical recording), the cells exist in vivo in tight monolayers. Like other epithelia, alveolar epithelia generate a transepithelial potential, with the apical surface negative to the basolateral surface. As a result, the transmembrane potential in an epithelial cell is not uniform but differs at the apical and basolateral surfaces. This is in the correct direction for promoting H^+ extrusion selectively across the apical membrane. A key factor is that each H^+ channel can sense only the potential across the membrane in which it is located and can sense only the local ΔpH. Even if the average ΔpH is inward, local submembrane acidification can activate H^+ channels locally, resulting in H^+ extrusion.

Is the Transepithelial Potential Large Enough to Activate H^+ Extrusion Through Channels?

Measurement of the alveolar transepithelial PD is complicated greatly by tissue geometry. In situ measurements are complicated by the possibility of cross talk with the PD across airway epithelium (79). Tracheal and bronchial PD values differed both from each other and from one mammalian species to another (4). The canine tracheal PD averaged −30.8 mV (4). In attempts to measure the alveolar PD, the observed value in a fluid-filled lung can be dominated by that across the airways (1). On the other hand, the alveolar PD might be underestimated because the probe does not reach the alveoli (79). Reported values of the transepithelial PD in intact lung tissue are −4.3 mV (lumen negative) in fetal sheep (79), −3.5 mV in rabbits (73), and −4.7 mV in adult rat (1). In cultured type II alveolar epithelial cells grown to confluence, an apical-negative PD of a few millivolts develops across the monolayer (11, 69). This result confirms that the origin of at least some of the PD arises from alveolar epithelium. When electrically tight monolayers are formed from adult rat alveolar epithelial cells, the PD is −9.7 mV (7). This larger value probably represents a closer approximation to in vivo properties. PD as high as −35 mV have been reported in A6 cell monolayers (25). There is considerable uncertainty about the precise value of the transepithelial PD, the resting membrane potential, and the local pH near the membrane in the in vivo alveolar epithelium, and in fact all of these may change continuously.

Reported values of the resting membrane potential of cultured alveolar epithelial cells are −27 mV (6) and −63 mV (32). The extent to which either value reflects the in vivo value is unclear, because the epithelium is composed mainly of type I rather than type II cells, and there is a transepithelial PD. The existence of the transepithelial PD means that the apical and basolateral membranes have different membrane potentials, and there is no single correct value for the whole cell membrane. Type II cells in primary culture, both early after isolation when they retain type-II-like properties, as well as weeks later when they flatten, lose their lamellar bodies and resemble type I cells and express depolarization-activated delayed rectifier-type K^+ channels but not inward rectifier K^+ channels (23, 62, 80). In general, cells with only delayed rectifier K^+ channels tend to have more positive resting membrane potentials than cells expressing inward rectifier channels (22). To the extent that the resting membrane potential is set by K^+ channels, it will be near their threshold for activation. The most common variety of delayed rectifier K^+ channel in rat alveolar epithelial cells, the type "*n*" or "low-threshold" K^+ channel (probably Kv1.3), has a threshold at −40 to −30 mV (12, 23, 62, 80). During the first few days in culture, 21–32% of type II cells express a different voltage-gated K^+ channel (type "*l*" or low-threshold, probably Kv3.1), which has a more positive threshold at −10 to −20 mV (23, 80). There may be two populations of alveolar epithelial cells in vivo, with different resting membrane potentials, reflecting their different K^+ channels. All things considered, it appears that any combination of transepithelial PD, local pH gradients beyond bulk values, or epithelial cell membrane depolarization approaching ~10–30 mV would suffice to drive H^+ channels to their threshold for opening.

Facilitated Diffusion

The idea that carbonic anhydrase facilitates the diffusion of CO_2 by converting it into HCO_3^- and H^+ is a venerable one (29, 96), and experimental evidence suggests that it contributes to CO_2 diffusion in the lung (30, 49). Net CO_2 diffusion is facilitated by formation of HCO_3^- because the concentration of CO_2 in tissues is much lower than that of HCO_3^-. Alveolar capillary blood has P_{CO_2} of ~46 Torr, and given its solubility in plasma [0.5095 ml $CO_2 \cdot l^{-1} \cdot$ atomosphere^{-1} (94)], the concentration of CO_2 is 1.4 mM. The intracellular concentration of HCO_3^- is an order of magnitude higher than this, depending on pH_i, and thus conversion of CO_2 to HCO_3^- greatly amplifies the concentration of diffusible species. CO_2 diffusion is facilitated only if both HCO_3^- and H^+ diffuse across the cell. In contrast with HCO_3^-, free H^+ is present at very low concentration. The importance of availability of mobile buffer for H^+ in mediating facili-

tated CO_2 diffusion has been demonstrated clearly (38, 39). Although H^+ diffuses five times faster than any other cation [$D_H \approx 10^{-4}$ cm^2/s (82)], its concentration is so small that diffusion of free H^+ (as H_3O^+) is negligible. Essentially all diffusion of H^+ occurs as protonated buffer (BH). In this context, the presence of buffer has two effects, one favorable to our hypothesis, and one less so. Flux across a membrane is greatly enhanced by the presence of buffers in the nearby unstirred layers (41), because buffer effectively increases the concentration of the transported species and thus short circuits the delays due to diffusion. However, the presence of buffer reduces the effective diffusion coefficient of H^+ (53). Mobile buffers (such as HCO_3^-, $H_2PO_-^4$, or CO_3^{2-}) simply diffuse like other small molecules (such as CO_2) with a diffusion coefficient $D_{BH} \approx 1 \times 10^{-5}$ cm^2/s (38, 39), which is 10-fold slower than free H^+. In contrast, fixed (immobile) buffers greatly lower the effective diffusion coefficient (53). Mobile buffers can partially compensate for this effect. For example, if mobile and fixed buffers both have pK_a = 6, then, according to the analysis of Junge and McLaughlin analysis (*Eq. 15* of Ref. 53), the effective diffusion coefficient for H^+ ($D_{DH,eff}$) depends on the relative proportions of fixed and mobile buffer ([F] and [M], respectively). For [F] = 10 [M], $D_{H,eff} = 0.1D_{BH}$; for [F] = [M], $D_{H,eff} = 0.5D_{BH}$; and for [F] = [M]/10, $D_{H,eff} = 0.9D_{BH}$. The buffering capacity (93) of alveolar epithelial cells grown in monolayers is 25–29 mM/pH unit between pH_i 6.8 and 7.4 and increases to 45 mM/pH unit at pH_i 6.6 and to 77 mM/pH unit at pH_i 6.4 (64). This value includes both fixed and mobile buffers other than HCO_3^-. An exceedingly indirect estimate of the fixed or slowly diffusing component of buffer capacity, is β = 15–21 mM/pH unit at $pH_i \approx 6.3$, based on the magnitude of buffer depletion due to H^+ currents in rat alveolar epithelial cells at different exogenous mobile buffer concentrations (13). The main intracellular mobile buffers include HCO_3^- and phosphate (45), which at physiological pH_i are present in cytoplasm at tens of millimolar. Therefore a very rough equality between fixed and mobile buffer concentrations in epithelial cells seems reasonable, and hence $D_{H,eff}$ is roughly one-half that of CO_2 or $\sim 0.5 \times 10^{-4}$ cm^2/s. The slightly lower diffusion coefficient is more than offset by the much higher concentration of mobile buffer in the cell. It has been estimated that, in cells containing carbonic anhydrase, more than one-half of the CO_2 flux occurs by facilitated diffusion (39).

In traditional models of facilitated diffusion, HCO_3^- recombines with H^+ to form CO_2, which then diffuses through the membrane. In the present proposal HCO_3^- and H^+ pass through the apical membrane of alveolar epithelium and recombine to form CO_2 in the alveolar subphase. The recombination of HCO_3^- and H^+ is a very rapid, diffusion-limited protonation reaction, with a rate constant of 4.7×10^{10} $M^{-1} \cdot s^{-1}$ at 25°C (28). However, the slower equilibration between $H_2CO_3 \leftrightharpoons CO_2 + H_2O$ has a time constant of 4.75 s under conditions in the alveolar subphase, pH 6.92 (74) and 37°C, calculated according to *Eq. 4* and the rate constants of Gros et al. (37). Would this reaction proceed fast enough to produce significant CO_2? The fluid in the alveolar subphase contains 11 mM HCO_3^- (73). An early estimate of the volume of fluid in the alveolar subphase was 10–50 pl/alveolus (98), but the true volume may have been underestimated due to dehydration (73). An elegant study using rapid freezing and low-temperature microscopy gives an average depth of the subphase of 0.2 μm at 80% of total lung capacity (3), which, multiplied by the total alveolar surface area [75 m^2 (97)], gives an aqueous subphase volume of 15 ml for the entire lung. Using Roughton's (83) parameter values and the subphase HCO_3^- and pH measurements of Nielson and co-workers (73, 74), the forward rate of uncatalyzed conversion of HCO_3^- to CO_2 would be: $k_v[HCO_3^-][H^+]/K_a = 70\ (11 \times 10^{-3})(10^{-6.92})/(2 \times 10^{-4}) =$ 463 μM/s, which, for a 15-ml subphase volume is 6.9 μmol/s. This is 4% of the total metabolic rate of CO_2 production (186 μmol/s). Independently of whether H^+ channels supply the H^+ necessary for this reaction, it evidently takes place to some extent and appears to contribute to CO_2 extrusion by the lung. The absence of non-HCO_3^- buffers in the subphase fluid would tend to accelerate the approach of the HCO_3^- dehydration reaction to equilibrium (36). If carbonic anhydrase were present in the subphase or bound to the apical membrane surface, this would speed the recombination reaction sufficiently to account for most of the CO_2 extruded by the lung. However, existing data contradict this localization (26, 27). That local concentrations of the relevant molecular species near the membrane may differ substantially from bulk values seems quite likely, in light of the large continual flux across the apical membrane of alveolar epithelial cells. For example, the local concentration of H^+ at 1 Å from the distal mouth of a H^+ channel conducting 2 fA of H^+ current would be $\sim 1.9 \times 10^{-6}$ M or pH 5.7 (calculated using *Eq. 152* of Ref. 2). The rate of spontaneous recombination with HCO_3^- to form CO_2 at this pH is 7.2 mM/s or 108 μmol/s for the entire subphase. This latter value is more than one-half the normal rate of CO_2 production by the body. Obviously these calculations involve many uncertainties and arbitrary assumptions, and more direct measurements would be valuable.

HCO_3^- Extrusion is Required

To produce net electroneutral CO_2 extrusion, HCO_3^- must be extruded from the apical membrane at the same rate as H^+. The simplest possibility is that HCO_3^- exits through anion channels in the apical membrane. Cl^- currents are nearly ubiquitous and are present in adult rat alveolar epithelial cells in primary culture (E. R. Jacobs, V. V. Cherny, T. E. DeCoursey, unpublished observations). Indirect evidence suggests that Cl^- channels are present in the apical membrane of adult rat alveolar epithelial cell monolayers (52). Anion channels are notoriously nonselective, and in many cases conduct HCO_3^- about as well as Cl^- (42, 50, 51, 90). Continuous HCO_3^- efflux through anion channels requires an outward driving force. HCO_3^- efflux is favored

by an outward HCO_3^- concentration gradient and by a membrane potential negative to its Nernst potential, $E_{HCO_3^-}$. Assuming that pH_i is 7.2 and the intracellular $[CO_2] \leftrightarrow [HCO_3^-] + [H^+]$ reaction is near equilibrium, $[HCO_3^-]_i$ is 17.6 mM. Because $[HCO_3^-]$ in the subphase is 11 mM (73), there is an outward concentration gradient of HCO_3^- and $E_{HCO_3^-}$ is +12.6 mV. A resting potential negative to $E_{HCO_3^-}$ will drive HCO_3^- efflux. For continuous passive efflux of HCO_3^- and H^+ through channels to occur simultaneously, the membrane potential across the apical membrane must be between E_H and $E_{HCO_3^-}$.

Alternatively, HCO_3^- might exit via the Cl^-/HCO_3^- exchanger, which is present in adult mammalian alveolar epithelial cells in vitro (56, 76). However, the Cl^-/HCO_3^- exchanger appears to be located exclusively in the basolateral membranes of rat alveolar epithelial cells in monolayers (66). If the basolateral localization extends to human alveolar epithelium in vivo, this would preclude participation of the Cl^-/HCO_3^- exchanger in the proposed mechanism of CO_2 extrusion.

EXPERIMENTAL TESTS

The hypothesis presented here should be evaluated experimentally. Several specific tests are suggested. The main result of the exploration of the hypothesis is that the feasibility of H^+ channels eliminating most of the CO_2 produced in the body in the form of H^+ is restricted by the slow rate of CO_2 formation from H^+ and HCO_3^- in the aqueous subphase. Because the subphase lacks carbonic anhydrase activity (26, 27), instilling exogenous carbonic anhydrase should enhance CO_2 efflux. In the converse experiment, exogenous carbonic anhydrase applied into the airways greatly enhanced CO_2 influx across the lung from alveolar air to perfusate, presumably by converting HCO_3^- to CO_2 (26).

Other elements of the hypothesis should be examined experimentally, as well. It would be useful to determine exactly where H^+ channels are located. Are they expressed exclusively in the apical membrane or also in basolateral membranes? Are they present in both type II cells and type I cells? Type I cells comprise 90–95% of the alveolar surface (60) and ought to participate if this mechanism is to play a major role in CO_2 elimination. It is thus an important observation that type II cells continue to express CA II in culture even after they differentiate into type-I-like cells (31). Similarly, that type II cells in culture for weeks continue to express high levels of H^+ channels (8, 13, 19) suggests that type I cells also express these channels. Because type II cells are precursors of type I cells, e.g., during repair of epithelial damage, it is generally assumed that cultured type II cells are the best available model for alveolar transport processes that in vivo would largely involve type I cells, but this assumption should be tested if possible.

An obvious experimental question is whether blocking the H^+ conductance alters CO_2 extrusion by the lung and secondarily produces respiratory acidosis. Testing this idea is complicated by the anatomic inaccessibility of the alveoli and by the absence of selective inhibitors of H^+ channels. The classical inhibitor is $ZnCl_2$, which inhibits H^+ current at lower concentrations than K^+ channels in alveolar epithelium (20) but also inhibits most ion channels and binds to many proteins. If the role of H^+ channels requires HCO_3^- efflux via Cl^- channels, then blocking these channels should indirectly inhibit CO_2 efflux.

ALTERNATIVE FUNCTIONS

If H^+ channels do not contribute significantly to normal CO_2 extrusion, why are they there? In general, H^+ channels contribute to pH_i regulation, because they are activated and extrude H^+ when there is an outward electrochemical gradient for H^+. This role as an acid-relief valve is analogous to the role played by H^+ channels in other cells. The H^+ conductance is activated during the respiratory burst of phagocytes (22, 46–48) and during recovery from acute acid loads in osteoclasts (77). The importance of pH_i homeostasis in alveolar epithelium has been discussed extensively (65). In the lung, H^+ channels may be activated during severe acid or CO_2 loading of alveolar epithelium, such as exercise, when the rate of CO_2 production can increase by more than an order of magnitude.

Although the hypothesis may not be correct, the fact remains that each alveolar epithelial cell expresses several hundred thousand H^+ channels. Presumably these channels perform some useful function. This density of H^+ channel expression is greater than in any cell besides leukocytes (16), suggesting that alveolar epithelial cells have a greater need for this mechanism of acid dissipation than most other cells. Therefore, several other possible functions will be mentioned.

An advantage to CO_2 extrusion via H^+ channels is that their tightly regulated gating results in only outward current. They would act as rectifiers, allowing only CO_2 efflux. During the normal breathing cycle, the alveolar P_{CO_2} varies by >2 Torr (59), a large range considering the entire P_{CO_2} diffusion gradient from blood to air is, at most, 5–6 Torr. CO_2 efflux via H^+ channels would prevent backdiffusion of CO_2 from air to tissues during early inspiration, when the alveolar P_{CO_2} is highest.

Due to gravity in an erect individual, the P_{CO_2} in the normal lung varies from 42 Torr at the base to 28 Torr at the apex (99). Because H^+ channel-mediated CO_2 extrusion is apparently limited by the backreaction of $CO_2 \rightarrow H^+ + HCO_3^-$, then H^+ channels might facilitate CO_2 extrusion at the top of the lung where the ventilation/perfusion ratio is high and P_{CO_2} is low. This would maximize CO_2 elimination by tending to reduce regional variation in P_{CO_2}.

Another possibility is that H^+ channels regulate the pH of the aqueous subphase, which is more acidic than typical extracellular fluid, and whose pH appears to be tightly regulated (74). Because there is a transepithelial PD, H^+ extrusion through H^+ channels will lower the pH of the subphase until the chemical gradient balances the apical membrane voltage gradient. If this were the case, then in principle the transepithelial PD

could be determined from the alveolar epithelial cell membrane potential and the observed pH gradient.

In summary, the present consideration of the hypothesis that H^+ channels contribute to CO_2 extrusion by the lung does not establish that this happens in vivo but shows that it is sufficiently feasible to merit exploring.

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