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Topical Review

Voltage-activated Hydrogen Ion Currents

T.E. DeCoursey[1], **V.V. Cherny**[1,2]

[1]Department of Physiology, Rush Presbyterian St. Luke's Medical Center, 1653 West Congress Parkway, Chicago, Illinois 60612
[2]Frumkin Institute of Electrochemistry, Russian Academy of Sciences, Moscow, Russia

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Introduction

Discovered in snail neurons by Thomas and Meech in 1982 [151], voltage-activated H^+-selective currents have been found in an increasing number of species and cells, including human phagocytes and skeletal muscle. The properties of the H^+ currents are similar in all of these preparations. The H^+ conductance, g_H, is undetectably small at large negative potentials, and activates in a time-dependent manner during depolarizing voltage pulses, apparently like other voltage-gated ion channels. The estimated single channel conductance is quite small, ~10 fS, and thus a carrier mechanism cannot be formally excluded. The g_H is highly selective, with no detectable permeability to other ions and a relative permeability $P_H/P_{Na} > 10^6$. Protons probably traverse the membrane by hopping across a hydrogen-bonded chain within an integral membrane protein, although the molecule responsible is entirely unknown at present. The voltage dependence of H^+ channel gating is modulated by pH on both sides of the membrane such that only outward H^+ currents are observed at fixed membrane potentials. Activation of the g_H would therefore alkalinize the cytoplasm in an intact cell. The fully activated g_H alkalinizes small cells two orders of magnitude faster than other pH regulating transporters, at no metabolic cost to the cell. Voltage-activated H^+ channels may serve as a safety valve in situations of excessive metabolic acid production.

The goal of this review is less to summarize the literature than to explore critically some of the ideas which have developed in this growing field. We hope to establish, as far as possible based on existing information, the fundamental properties of the g_H with an aim of understanding its mechanism. One focus is on practical experimental complications which result from the very low concentration of H^+ ions which carry these currents. We mention specific functions only briefly; more detailed reviews of proposed roles of H^+ currents have appeared [43, 104, 112]. We do not discuss voltage-independent mechanisms of proton conductance (i.e., nongated proton conductance through membranes) which have been reviewed elsewhere [36].

Conventions

The term g_H will refer exclusively to the voltage-activated H^+ conductance, and will exclude voltage-independent H^+ "leak" currents, whether selective or not. Although the molecular nature of the mechanism responsible for the g_H is not known, H^+ currents will be described as mediated by channels, for convenience and because there is no evidence for another type of mechanism. We consider the currents as reflecting outward H^+ movement, rather than inward OH^- movement, although as will be discussed this distinction is not firmly established. External pH, pH_o, and internal pH, pH_i, are given in the form $pH_o//pH_i$, as in pH 7//6.

H^+ Channels May Be Widely Distributed

After a decade in which the g_H was studied only in invertebrates or amphibia, recent studies have focused on mammalian, especially human cells. The cells and

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Correspondence to: T.E. DeCoursey

Table 1. Species and tissue distribution of H^+ currents

Cell type	Species	Maximum H^+ current (pH_i) (pA/pF)	Reference
Neuron	*Helix aspersa*	4.5 (~6.8)	113
	Lymnaea stagnalis	14.6 (5.9)	24
Oocyte	*Ambystoma*	8.4 (~7.2)	9
Epithelium:			
Alveolar	Rat	27.3 (5.5)	38
Lung A549	Human	~2 (5.5)	[a]
Prostate PC-3	Human	~4 (5.5)	[a]
Phagocytes-macrophages:			
Monocyte-derived	Human	1.1 (6.0)	78
Peritoneal	Mouse	~30[b] (6.0)	87
Alveolar	Rat	~10 (6.5)	[a]
THP-1	Human	22 (5.5)	92
Phagocytes-granulocytes:			
Neutrophil	Human	17[c] (6.0)	39
HL-60	Human	133[b] (5.5)	42
K-562	Human	~5 (6.0)	[a]
Skeletal muscle (myocyte)	Human	~10[b] (5.5)	15

Maximum H^+ current is the largest H^+ current measured in a given cell, usually ~150 mV positive to V_{rev} (normalized to surface area), reported $g_{H,max}$ values are converted to current at V_{rev} + 150 mV. In some studies where typical values or cell size were not specified, estimates were made from data in figures, etc., and are preceded by a tilde (~), as are values from surveys including a small number of cells. All studies were done at room temperature (20–25°C). [a]V.V. Cherny and T.E. DeCoursey, *unpublished data.* [b]The extracellular solution contained EGTA and no Ca^{2+}. [c]Measured in TEA^+ solution; recent values in TMA^+ solution containing EGTA are >100 pA/pF (pH_i5.5).

species in which H^+ currents have been reported are listed in Table 1. A systematic exploration of which cell types express H^+ currents (and which do not) would contribute to the development of ideas about the function of this conductance. The existence in human neutrophils of H^+ channels resembling those in snail neurons was proposed in 1987 by Henderson, Chappell and Jones on the basis of pH_i measurements [70]. This was the first cell in which a proposed function of voltage-activated H^+ currents preceded their direct measurement [39]. Several lines of evidence suggest that the g_H is activated during the respiratory burst in phagocytic cells. The predominance of epithelial and phagocytic cells in Table 1 most likely reflects the interests of the investigators, although it is possible that the presence of H^+ currents in these cells may be related to their exposure to environments of relatively uncontrolled pH. The H^+ current amplitudes in Table 1 are somewhat arbitrary because the conditions of the measurements varied substantially, but genuine differences occur. The high H^+ current density in neutrophils and granulocyte-differentiated HL-60 cells may reflect their need to cope with an acute acid load during the respiratory burst.

In general, H^+ currents are of roughly comparable magnitude to other ionic currents. H^+ currents may be overlooked in cells studied in "physiological" solutions because at normal pH activation occurs at fairly positive potentials, but H^+ currents can be observed in the presence of other currents [12, 22, 23, 39, 40, 107].

There is indirect evidence for a voltage-dependent g_H in several other cell membranes: the OK (opossum kidney) cell line [56], principal cells of frog skin [105], and leech Retzius neurons [50]. Also intriguing is that the membrane of *Elodea densa* becomes highly permeable to H^+ at high pH_o [114]. These suggestive studies need to be followed up with direct voltage-clamp studies to confirm the nature of the g_H present. In retrospect, the positive shift at low pH_o of the resting potential [135] or of V_{rev} [106], often cited as evidence of a proton conductance in skeletal muscle, is not consistent with the properties of the voltage-activated g_H in myotubes [15] or in other cells, which would not be activated at the potentials used. Numerous studies indicating H^+ permeability of other cell membranes, which did not address the question of voltage dependence are not listed. A voltage-independent H^+ "leak" would have radically different effects on a cell than the g_H described here, whose pH and voltage dependence are essential to its function.

Properties of Voltage-gated H^+ Currents

H^+ Channels Are Extremely Selective

The voltage-activated g_H is extremely selective for protons over other ions. The reversal potential of H^+ currents, V_{rev}, can be measured by standard "tail current"

procedures. V_{rev} is not significantly changed when any of the physiological ions, including Na^+, K^+, Cl^-, and Ca^{2+} is introduced or removed and replaced with TMA^+ (tetramethylammonium$^+$), TEA^+ (tetraethylammonium$^+$), NMG^+ (N-methyl-D-glucamine$^+$), Cs^+, Li^+, aspartate$^-$, or $MeSO_3^-$ (methanesulfonate$^-$) [9, 15, 40, 42, 87]. Under some conditions, external Na^+ shifts V_{rev} to more positive potentials [9, 40]; however, this is due to indirect effects of Na^+-H^+ antiport rather than Na^+ permeation of the H^+ pathway (*see* "Interactions with the Na^+-H^+ Antiporter"). The largest observed deviations of V_{rev} from the Nernst potential for protons, $E_H = (RT/F)\ln([H^+]_o/[H^+]_i) \simeq (pH_i\text{-}pH_o)$ (58.2 mV) at 20°C, indicate that the H^+ permeability, P_H, calculated using the Goldman-Hodgkin-Katz voltage equation [74], is $>10^6$ greater than that of Na^+ or any other ion and $>10^7$ greater than that of TMA^+. In spite of this high selectivity, in most studies the observed V_{rev} does not follow E_H perfectly when pH_o or pH_i is varied. Typically, V_{rev} changes ~40 mV/pH or less [15, 38, 42, 87, 107], although Thomas and Meech [151] found that pH_i after prolonged voltage-clamp depolarizations varied precisely with E_H in *Helix* neurons, and Barish and Baud [9] observed a 54.3 mV/pH_o slope in oocytes which is close to the Nernst prediction of 58.2 mV. In general, the agreement seems better near neutral pH_i, or when the transmembrane pH gradient is small. Deviations of V_{rev} from E_H most likely reflect imperfect control of pH rather than permeability to other ions. In conclusion, the g_H is not detectably permeable to other ions.

Whether H^+ currents reflect proton efflux or OH^- influx is not a trivial question. It seems safe to rule out HCO_3^- influx as responsible because its removal does not greatly affect the g_H [115]. Several types of evidence have been used to support the idea that the outward currents reflect H^+ efflux. (i) Lowering pH_i (i.e., increasing $[H^+]_i$) increases the outward current and conductance [*see* Fig. 2; 22, 42, 87, 107]. (ii) Inward tail currents appear to be larger at low pH_o (high $[H^+]_o$) [9, 22, 104]. (iii) The maximum slope conductance is larger at low pH_o in *Ambystoma* oocytes [9]. (iv) In alveolar epithelial cells perfused with low but not high buffer concentrations (5 mM *vs.* 119 mM MES) the outward current saturated at the same amplitude over a wide range of pH_o [38]. If OH^- influx mediated the current then current saturation due to depletion of the current-carrying species should be less at high pH_o. However, none of these arguments is conclusive. (i) Lowering pH_i by 1.4–2.0 units increases $[H^+]_i$ 25–100-fold, yet $g_{H,max}$ (*see* Eq. 1 below) is increased only by 1.4–2.3 fold [22, 42]. In fact, the near constancy of $g_{H,max}$ over a wide range of pH_i is perhaps more remarkable. (ii) Inward tail currents may appear small at high pH_o because channel closing is more rapid at large negative potentials and thus harder to resolve. (iii) In other cells low pH_o does not affect or reduce $g_{H,max}$ ([22, 38, 87]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). (iv) This argument presumes that H^+ current saturation was the result of depletion of the charge carrier. If instead it were due to pH_i changes during pulses, then H^+ efflux and OH^- influx would have equivalent effects. It should be mentioned that points i and ii equally complicate the hypothesis that OH^- is the current carrier. Therefore, existing data do not firmly establish that H^+ rather than OH^- is the charge carrier for what we will nevertheless persist in describing as H^+ current.

If conduction through H^+ channels occurs by a "Grotthuss" mechanism[1], in which protons jump from one water molecule to another (*see* "How do H^+ Channels Work?"), then this question becomes moot. Protons move through water or ice by jumping from one water molecule to another forming a hydronium ion (H_3O^+) at each jump. Conversely, hydroxyl ions (OH^-) jump by stealing a proton from an adjacent water molecule. The net result of outward movement of H_3O^+ or inward movement of OH^- through a channel is precisely the same, the transfer of one proton across the membrane in the outward direction. The actual transfer of an "excess" proton (from H_3O^+ to H_2O) may be faster than the transfer of a "defect" proton (from H_2O to OH^-) in water or ice [27, 33, 34, 46, 98], but this distinction may not hold for a single-file row of water molecules in a narrow channel, and anyway may be a virtually unmeasurable difference. H^+ (H_3O^+) efflux and OH^- influx would be distinguishable only if these ions physically traverse the channel by hydrodynamic conduction as discrete ions (without interacting chemically with the water molecules inside), or if H^+ permeation involves at least one step in which water does not participate (*see* "H^+ Channels May Not Be Water-filled Pores").

H^+ Channels Exhibit Voltage-dependent Gating

Over a wide range of conditions, only outward H^+ currents can be detected at maintained voltages. This strong outward rectification of the *steady-state* g_H-voltage relationship is due to a voltage-, time-, and pH-dependent gating process, not to intrinsic rectification of the open channel current. Open H^+ channels conduct inward as well as outward current because transient inward "tail" currents are observed upon repolarization from a depolarizing pulse which activated the g_H. Whether the instantaneous H^+ current-voltage relation rectifies in accordance with the Goldman-Hodgkin-Katz current equation has not been systematically explored,

[1] Two centuries ago Freiherr von Grotthuss (variously spelled) proposed that conduction of all electrolytes took place by a mechanism which was similar, but not strictly analogous, to that now applied to H^+ conduction [98].

although this equation fits the data with a small pH gradient [15].

H^+ channels close at potentials negative to E_H, and open with a probability that increases with depolarization. Fitting the g_H-voltage relation with a Boltzmann function:

$$g_H(V) = g_{H,max}/(1 + \exp[(V_{1/2} - V)/k]), \quad (1)$$

where $g_{H,max}$ is the limiting conductance, $V_{1/2}$ is the half-activation potential, and k is a slope factor, provides an imperfect approximation of the voltage dependence of the g_H (Figs. 1*B* and 2*B*). The value for $V_{1/2}$ depends strongly on pH_o and pH_i as will be discussed next. The slope factor, k, appears to be fairly constant at various pH_i and pH_o and ranges from 7–14 mV [9, 15, 22, 39, 40, 107], although a steeper slope at higher pH_o was observed in newt [9]. This slope factor corresponds with the movement of 1.8–3.6 equivalent gating charges across the membrane, and is moderate in comparison with other ion channels whose gating typically moves 2–6 equivalent charges. Because of uncertainties regarding the precise value and the constancy of pH near the membrane (*see* "Practical Considerations") fine details in the observed g_H-V relation must be interpreted cautiously.

The Voltage Dependence of Gating is Modulated by pH_o and pH_i

Lowering pH_o shifts the voltage-activation curve of the g_H to more positive potentials in all cells in which this property has been studied (Fig. 1). This shift is reminiscent of the effect of pH_o on most voltage-gated ion channels, which usually has been attributed to neutralization of fixed negative charges at the external surface of the membrane, on or near the channel mouth [*discussed in* 74]. However, Byerly, Meech and Moody [22] noted that the 46 mV shift in the g_H-V relation between pH_o 7.4 and 6.4 was larger than the maximum shift predicted by Gouy-Chapman-Stern theory, 37 mV/unit pH [52], and suggested that external protons interact directly with H^+ channel gating. In addition, the activation kinetics were slowed at low pH_o more than could be accounted for by a simple shift of the voltage dependence of gating, inconsistent with a simple electrostatic mechanism [22].

One wonders whether the g_H-V relation shifts in parallel with E_H, or simply in the same direction. The magnitude of the shift of g_H-V with pH_o is typically less than the shift of E_H, but the measured V_{rev} also shifts less than E_H [9, 15, 22, 38, 40, 87, 107]. It is possible that the local pH near the channel does not correspond with the bulk pH, and that the channel itself may behave as a perfect pH electrode. A systematic comparison of the effects of pH on these parameters would be valuable. The bulk of existing data suggests that the voltage dependence of the g_H responds to pH_o in a limited manner, i.e., the effect saturates at high pH_o [9, 22, 38, 87], as can be seen in Fig. 1. This behavior may indicate that after one or more modulatory sites on or near the channel are deprotonated, a further increase in pH_o has no additional effect.

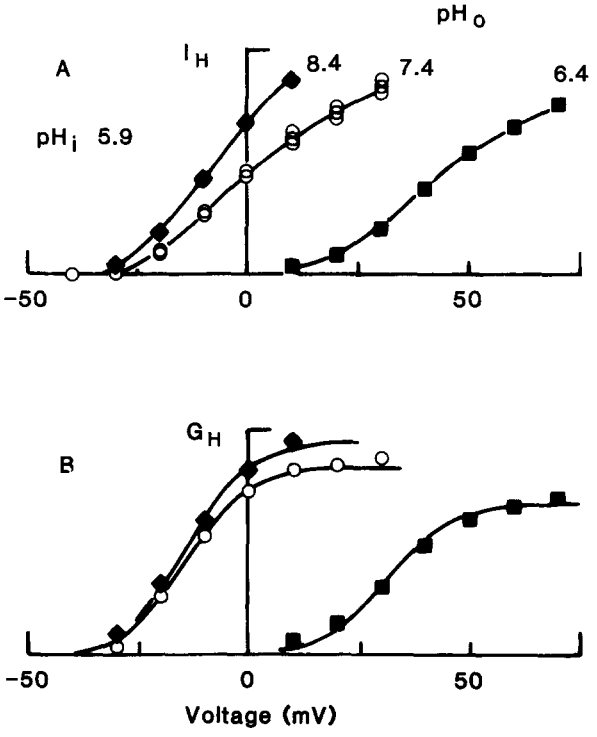

Fig. 1. Effect of pH_o on H^+ currents in snail neurons, *Lymnaea stagnalis*. (*A*) Steady-state current-voltage relation at pH_o 8.4 (◆), 7.4 (○), and 6.4 (■), in a neuron perfused with pH_i 5.9 solution (Cs aspartate, EGTA and MES). External solution hyperosmotic Tris saline, holding potential, V_{hold}, was −50 mV. (*B*) Chord conductance for the currents in *A*, with $g_{H,max}$ 0.48, 0.42, and 0.34 μS at pH_o 8.4, 7.4, and 6.4, respectively, and $V_{1/2}$ −14, −14, and +32 mV. Cell diameter 100 μm, calibrations are I_H 50 nA and G_H 0.5 μS. Adapted with permission from [22].

Lowering pH_i shifts the voltage dependence of g_H activation to more negative potentials (Fig. 2). As for changes in pH_o, changes in pH_i generally shift the g_H-V relation less than E_H [22, 40, 42, 87, 107, 149]. Byerly et al. [22] proposed that H^+_i bind to acidic groups at the inner side of the membrane at low pH_i, altering the surface potential, and hence the potential sensed by the H^+ channel. This pH_i sensitivity, in combination with the pH_o sensitivity just discussed, results in activation of the g_H occurring only at potentials positive to E_H, so that only outward current is observed. The modulation by pH_i lends itself well to the proposed function of the g_H as an acid extrusion mechanism.

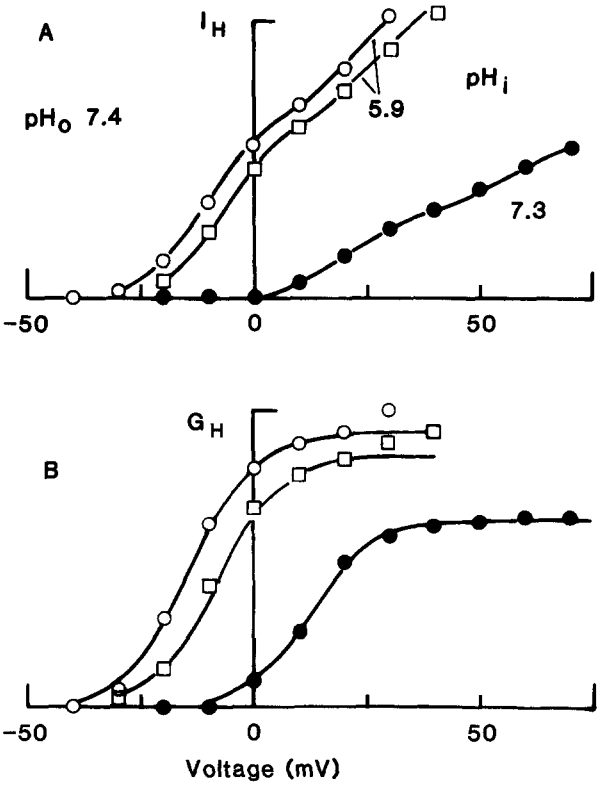

Fig. 2. Effect of pH_i on H^+ currents in internally perfused snail neurons. (*A*) The steady-state outward current is plotted for pulses to the command potentials on the abscissae in the same neuron at pH_i 5.9 before (○) and after (□) perfusion with pH_i 7.3 (●), all at pH_o 7.4. External solution hyperosmotic Tris saline, internal solution Cs aspartate, EGTA, and MES (pH 5.9) or HEPES (pH 7.3). (*B*) Chord conductance-voltage relationship calculated from the data in *A*, using the same symbols. The curves show the best fit of the data with Eq. (1), after fixing $g_{H,max}$ at 0.60 μS (○) or 0.55 μS (□) for pH_i 5.9 and at 0.41 μS for pH_i 7.3 (●). The midpoint, $V_{1/2}$, was −14 or −8 mV at pH_i 5.9 and +12.5 mV for pH_i 7.3, and the slope factor, *k*, was fixed at 7 mV. Cell diameter 120 μm, calibrations are I_H 60 nA and G_H 0.65 μS. Adapted with permission from [22].

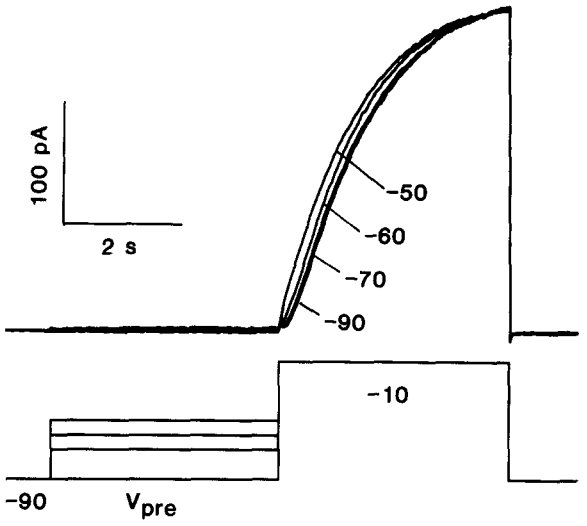

Fig. 3. Dependence of H^+ current activation kinetics on prepulse potential. A rat alveolar epithelial cell at pH 8.0//6.5 was held at −90 mV, stepped for 4 sec to the indicated prepulse potentials, and then an identical depolarizing pulse to −10 mV applied (*see pulse diagram below*). The test current had a distinctly sigmoid rising phase following large negative prepulses, which was diminished when the prepulse was more positive. Time-dependent H^+ current was detectably activated during the prepulse to −50 mV and reached 1.6 pA, which corresponds with 0.6% of $g_{H,max}$ measured in this cell. External solution TMA^+ $MeSO_3^-$ with 100 mM TRICINE, 1 mM EGTA, and 3 mM $CaCl_2$. Internal (pipette) solution TMA^+ with 100 mM BIS-TRIS, and 1 mM EGTA.

H^+ Channel Gating Is Complex

Despite many similarities between H^+ currents in various cells, there are distinct differences in gating kinetics—the rates of channel opening and closing. Activation of H^+ currents during depolarizing pulses is rapid in neurons, with a time-to-half-peak of 25 msec just above threshold decreasing to <5 msec at more positive potentials, comparable to the rate of K^+ channel activation in these cells [22, 107]. The activation time constant is an order of magnitude larger in axolotl, 100–300 msec [9]. In mammalian cells H^+ currents activate more slowly, with half-maximal current attained at >1 sec during small depolarizations [15, 38, 42, 87]. H^+ currents in human neutrophils continue to rise for as long as three minutes before reaching an apparent steady-state value during small depolarizing pulses [39], as can be seen in Fig. 5.

Barish and Baud [9] described the g_H of newt with a two-state gating mechanism:

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

in which both activation (channel opening) and deactivation (channel closing) occurred with an exponential time course, the time constant of current relaxation, $\tau = 1/(\alpha + \beta)$, was the same for activation and deactivation at a given potential, and τ had a bell-shaped voltage dependence with a maximum near the midpoint of the steady-state voltage-activation curve. In contrast, the kinetics of g_H gating in mammalian cells cannot be described by a two-state model. Activation kinetics have two rising exponential components in myotubes [15], and in alveolar epithelium and phagocytes are clearly sigmoid [38, 39, 40, 87]. Figure 3 illustrates that the sigmoidicity of H^+ current activation in a rat alveolar epithelial cell was reduced by first stepping the membrane to more positive potentials, even though little or no detectable H^+ current was activated during the prepulse. This behavior suggests that the channels pass through at least two closed states before opening:

$$C_2 \rightleftharpoons C_1 \rightleftharpoons O.$$

At large negative potentials most channels are in state C_2, and upon depolarization they first pass through state C_1 before opening (state O). Hodgkin and Huxley [77] proposed a mechanism like this to account for the delayed (sigmoid) activation kinetics of both Na^+ and K^+ currents in nerve membrane, and analogous prepulse-dependence of delayed rectifier K^+ current activation kinetics is well known [32]. At potentials near threshold, H^+ channels enter a "pre-activated" or "primed" state (C_1) from which subsequent depolarization results in channel opening with less delay. Additional evidence consistent with this type of model is that the rate of channel opening is slowest just above threshold, and becomes progressively faster over the entire voltage range in which channels open (as opposed to being slowest near $V_{1/2}$), similar to the behavior of many delayed rectifier K^+ channels. This model may be oversimplified—additional states may be present. It is doubtful that H^+ current gating will be modeled as extensively as that of other ion channels because uncertainties introduced by the exceedingly low permeant ion concentration, buffer effects, and unstirred layer effects complicate the interpretation of kinetic subtleties (*see* "Practical Considerations"), and single channel currents may be undetectably small.

The rate of H^+ current deactivation has been less thoroughly studied than activation. It is clear that deactivation is faster at more negative potentials, and is generally comparable with or faster than activation in the same preparation [9, 15, 22, 38, 39, 87, 107]. In addition, the time constants shift along the voltage axis to some extent when pH_o is altered ([22]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). Clearly, more work on this property is needed.

Polyvalent Metal Cations Inhibit H^+ Currents

One identifying characteristic of all voltage-activated H^+ currents described to date is their sensitivity to inhibition by Cd^{2+} and Zn^{2+} at concentrations of 1 mM or lower. Precise comparisons of effective concentrations are difficult because of the binding of these cations by various anions. Whether the divalent form or another chemical form of these ions, e.g., $Cd(OH)^{1+}$, is active has not been explored. Other polyvalent cations which inhibit H^+ currents include: La^{3+}, Al^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Hg^{2+}, Be^{2+}, and Ba^{2+} ([15, 24, 113, 151]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). Ca^{2+}, and to a lesser extent Mg^{2+}, inhibits H^+ currents in human myotubes [15], but Ba^{2+}, Ca^{2+}, or Mg^{2+} did not detectably affect H^+ currents in snail neurons [24]. Possible effects of Ca^{2+} should be considered in interpreting studies of H^+ currents in nominally Ca^{2+}-free solutions; some investigators routinely add EGTA to external solutions [42, 87]. EGTA may minimize contamination by foreign metal cations; we find that H^+ currents are larger and activate at more negative potentials in external solutions containing EGTA even when free Ca^{2+} is held essentially constant (V.V. Cherny and T.E. DeCoursey, *unpublished data*), but it is possible that EGTA has other effects.

At moderate concentrations polyvalent cations shift the g_H-V relation to more positive potentials, slow H^+ current activation, and reduce $g_{H,max}$ [9, 15, 22, 38, 39, 42, 87, 108]. Polyvalent cations exert quite similar effects on other voltage-gated ion channels [74]. These effects are often considered in terms of shifts of the voltage dependence of the conductance. Huxley, Frankenhaeuser and Hodgkin [49] suggested that calcium might bind to and neutralize negative charges on the outside of the membrane, thus altering the potential sensed by the voltage-sensing element of Na^+ or K^+ channels. Alternatively, some investigators have described these same effects in terms of voltage-dependent block [15, 113]. It is possible that polyvalent cations interact with the same sites on the channel as external H^+, and that occupancy of these sites by either H^+ or by metal cations shifts the voltage dependence, i.e., the effect of these cations may be equivalent to lowering pH_o. Cd^{2+} bound near the outer mouth of the channel may reduce $g_{H,max}$ by lowering the local H^+ concentration as a result of electrostatic repulsion. Several other channels exhibit this behavior [57]. However, the slowing of activation by Cd^{2+} or Zn^{2+} is greater than can be explained by a simple shift of the g_H-V relation [9, 22, 87]. By analogy with similar effects of divalent cations on other ion channels [54, 74], Byerly et al. [22] proposed that Cd^{2+} slowed g_H activation by direct interaction with channel gating.

There Are No Good Organic Inhibitors

A variety of organic agents *partially* inhibit H^+ currents. TEA^+ inhibits H^+ currents in some preparations [15, 22, 113] and shifts g_H activation towards more positive potentials (V.V. Cherny and T.E. DeCoursey, *unpublished data*), but it is possible to record robust H^+ currents in the presence of isotonic TEA^+ in the external solution [9, 107] or in symmetrical TEA^+ solutions [39]. The H^+ currents in these latter studies might have been larger if TEA^+ had not been present, but clearly TEA^+ cannot be considered an effective blocker. Inhibitory effects of TEA^+ may be ascribable to impurities [104], or to adsorption of TEA^+ to negative charges near H^+ channels.

Weak bases which detectably reduce H^+ currents include D600 (100 μM methoxyverapamil), 4-amino-

pyridine (10 mM) [113], amiloride (100 μM) [40], rimantadine, and amantadine (0.1–1 mM) (V.V. Cherny and T.E. DeCoursey, *unpublished data*). These agents may interact directly with H^+ channels, but one must also consider the possibility that the apparent inhibition is the result of changes in local pH due to permeation of the nonionized form of the molecule through the membrane. The inhibition by 4-aminopyridine was attributed to its permeation increasing the intracellular buffering power [113]. Weak acids [61] or bases [29] which permeate membranes in uncharged form can significantly alter local pH in unstirred layers near the membrane due to protonation/deprotonation reactions. Amiloride decreased the H^+ current at a given potential and shifted the voltage dependence of the g_H to more positive potentials [40]. Neutral amiloride (pKa 8.8 [93]) molecules that enter the cell may be rapidly protonated, thus depleting H^+_i near the membrane, and consequently decreasing H^+ currents both by reducing the electrochemical gradient and by shifting the voltage dependence of the g_H to more positive potentials. In summary, no potent organic H^+ channel blockers are known, and those that have been described may act by changing local pH rather than by binding to and occluding the channel.

H^+ Currents Are Not Carried Through Other Ion Channels or Transporters

A question frequently asked is "Do H^+ currents pass through some other (better known) type of ion channel?" with the implication that H^+ currents would be less interesting if this were the case. The most common voltage-activated channel in cells with H^+ currents is the "delayed rectifier" K^+ channel. The voltage dependence, activation kinetics, and to a limited extent pharmacology, of H^+ and K^+ currents in snail neurons are somewhat similar [22]. However, Byerly and Suen [24] demonstrated that the amplitudes of H^+ and K^+ currents in isolated membrane patches were not correlated. In alveolar epithelial cells H^+ and K^+ currents share a qualitatively similar voltage dependence, but have grossly different activation kinetics, K^+ currents inactivate while H^+ currents do not, and 100 nM charybdotoxin has no effect on H^+ currents (V.V. Cherny and T.E. DeCoursey, *unpublished data*), whereas the K^+ currents are inhibited with a K_i of ~1 nM [82]. H^+ and K^+ currents can be seen simultaneously in alveolar epithelial cells, and under these conditions charybdotoxin abolishes the K^+ current without affecting the H^+ current (V.V. Cherny and T.E. DeCoursey, *unpublished data*). A variety of other inhibitors have little or no effect on H^+ currents at concentrations which abolish current through certain ion channels: 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS, 20 μM) [113]; amiloride (100 μM), apamin (0.3 μM) [108]; nifedipine (1 μM) [15]; venturicidin (10 μM), charybdotoxin (100 nM), noxiustoxin (100 nM), phencyclidine (200 μM), mefenamic acid (100 μM 2-[(2,3-dimethylphenyl)amino]benzoic acid), anthracene-9-carboxylic acid (1 mM) (V.V. Cherny and T.E. DeCoursey, *unpublished data*); partial inhibition by DIDS (100 μM 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) was reported [78]. Another reason that H^+ currents could not be carried through K^+ channels is that the surface density of H^+ channels required to account for the observed currents is several orders of magnitude higher than the density of K^+ channels in these cells. Finally, H^+ currents are not somehow "caused" by abnormal pH or ionic conditions. Like all ion channels, they are best studied under conditions which minimize the contributions of other channels, but H^+ currents can be seen at normal pH_o and pH_i, and in the presence of normal ionic constituents.

A classical discriminator between ion channels and other membrane ion transporters such as carriers or pumps is that channels are capable of much higher transport rates: $>10^8$ ions/second [74] and 10^9 H^+/second in gramicidin [3]. In the physiologic pH range, the putative single channel H^+ current may be only ~1–2 fA (at 100 mV), which corresponds with ~10^4 H^+/sec. This transport rate is well within the range reported for carriers and enzymes [74], and unless single voltage-activated H^+ channel currents can be measured directly at lower pH where their conductance might be much larger (*see* Fig. 6), a channel mechanism cannot be considered firmly established. On the other hand, the maximum rate of H^+ efflux per cell via the g_H is much greater than that for other common membrane transporters (Table 2). The existence of a number of biological transporters, which under some circumstances can function as proton conductors or transporters, raises the question whether the H^+ channel is really an altered state of one of these molecules. However, the proton channel of CF_0 loses its H^+ selectivity when its subunits are isolated [141]. The following transporter inhibitors have little or no effect on H^+ currents: SITS (20 μM), carbonyl cyanide-*m*-chlorophenyl hydrazone (CCmP 20 μM), *N,N′*-dicyclohexylcarbodiimide (DCCD 10–100 μM), oligomycin (2–10 μg/ml) [113]; *N*-ethylmaleimide (NEM 2 mM), diethylpyrocarbonate (1 mM), amiloride (100 μM) [108]; strophanthidin (2 mM), amiloride (small inhibition at 100 μM), dimethylamiloride (10 μM), venturicidin (10 μM), and DCCD (200 μM) ([40]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). H^+ currents can be recorded in the presence or absence of internal ATP [42, 87], evidently ruling out the possibility that they are the result of H^+-ATPase activity. The voltage-activated H^+ channel is thus most likely a distinct molecular entity.

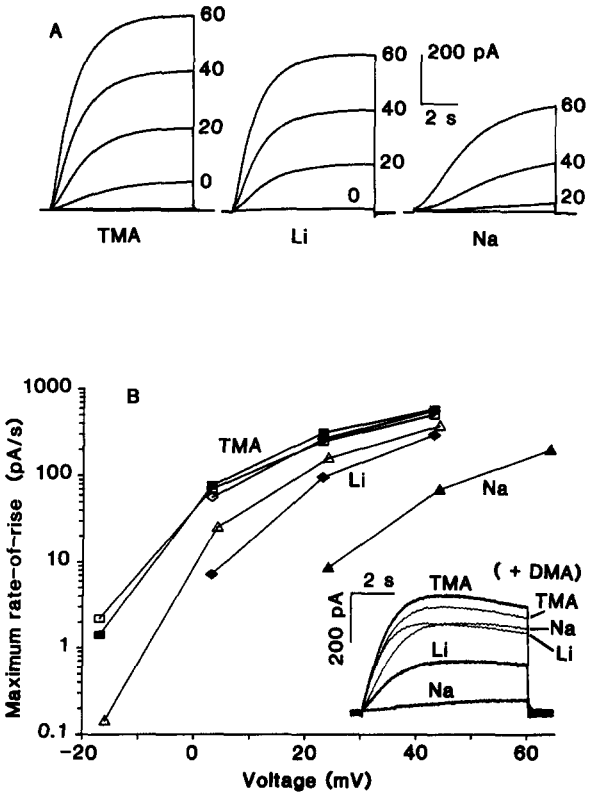

Fig. 4. (*A*) Families of H^+ currents in an alveolar epithelial cell in TMA^+, Li^+, or Na^+ solutions, all at pH 7.0//5.5. Currents are superimposed for pulses from a V_{hold} of −60 mV to the indicated potentials (mV). Pipette solutions contained NMG^+ with 119 mM MES buffer. Calibration bars apply to all families. (*B*) Maximum rate-of-rise of H^+ currents in TMA^+ (■), Li^+ (◆), or Na^+ (▲) at pH 7.0//5.5 in a different cell. Open symbols indicate measurements in the same solutions, with 10 μM DMA added to inhibit Na^+-H^+ antiport. Because of concern that depletion effects might distort the g_H-V relationship (*see* "Practical Considerations"), we plot instead the maximum rate-of-rise of H^+ currents, I_H'. I_H' occurs early in the pulse, well before the current reaches its maximum amplitude, and after a relatively small net H^+ efflux, and is therefore less subject to distortion from current-dependent pH_i increases. The I_H'-V relationship shifts along the voltage axis in an approximately parallel manner when pH_o or pH_i is changed. The *inset* shows currents recorded in this experiment during identical pulses to +20 mV from a V_{hold} of −40 mV, in the three solutions, as indicated. The lighter lines show currents in these solutions after addition of 10 μM DMA. Note that DMA increased the currents in both Li^+ and Na^+ solutions nearly to that in TMA^+. Adapted with permission from *The Journal of General Physiology* [40] by copyright permission of the Rockefeller University Press.

H^+ Efflux Is Not Coupled to the Movement of Other Ions

Anion substitution itself does not affect H^+ currents or V_{rev} markedly, with similar currents recorded in Cl^-, aspartate$^-$, or methanesulfonate$^-$ solutions [15, 39, 42, 87]. HCO_3^- removal does not greatly affect the g_H [113]. Similarly, monovalent cations have little effect on H^+ currents, although Na^+ and Li^+ indirectly modulate H^+ current gating by serving as substrates for the Na^+-H^+ antiporter [40], as discussed below. H^+ currents are not affected by changes in $[Ca^{2+}]_i$ between 0.1–10 μM [22].

H^+ Channels Are Biological pH Meters: Interactions with the Na^+-H^+ Antiporter

The high sensitivity of H^+ channels to pH in the physiological range can be exploited to use the g_H as a local pH meter. The voltage dependence of the g_H may reflect the local pH near the channel and the reversal potential the transmembrane pH gradient [40].

Figure 4*A* shows that Na^+, and to a smaller extent Li^+, suppresses the g_H in alveolar epithelial cells. We postulated that Na^+-H^+ antiport might account for this effect if $Na^+{}_o$ for $H^+{}_i$ exchange depleted $H^+{}_i$ faster than could be replenished by diffusion of protonated buffer from the pipette [40]. In the whole-cell configuration of the patch clamp technique, ongoing membrane transport can significantly alter the intracellular ionic composition, with the main barrier to diffusion being the pipette tip [109]. If pH_i is increased in Na^+ solutions by Na^+-H^+ antiport, the voltage-activation curve and V_{rev} should shift to more positive potentials, just as when pH_i is increased via the pipette solution. These effects were observed, and were substantially reversed by amiloride or the more specific Na^+-H^+ antiport inhibitor dimethylamiloride (Fig. 4*B*). To a first approximation, the effects of Na^+ and Li^+ on the g_H were interpretable as simple shifts of the voltage dependence, analogous to the shifts observed when pH_o or pH_i is changed. The inhibitory effect of $[Na^+]_o$ on H^+ currents was largest under conditions most favorable to Na^+-H^+ antiport, namely a large inward Na^+ gradient and a large outward pH gradient (pH 7.0//5.5), and were practically eliminated when the proton gradient was outward (pH 6.0//7.0), i.e., under conditions unfavorable to antiport. Calculations using a simple compartmental diffusion model showed that a plausible rate of Na^+-H^+ antiport could alter pH_i given the geometries involved [40]. Thus, Na^+ does not directly affect H^+ currents.

Physiological Modulation of the g_H

Chronic Regulation of H^+ Currents

Progesterone induces maturation of *Ambystoma* oocytes, during which pH_i increases and the amplitude of H^+ currents decreases [12]. The H^+ current increases several-fold in HL-60 cells induced to differentiate by dimethylsulfoxide [134]. These long-term effects may be examples of up- or downregulation of channel expression.

The g_H is Activated Acutely During the Respiratory Burst

Activation of a proton conductance in human neutrophils by phorbol esters [70, 71, 88, 124] and by arachidonic acid [69, 89] was proposed based on pH_i measurements using fluorescent dyes. Arachidonic acid elicits superoxide release and is released by phagocytes upon stimulation by substrates for phagocytosis such as latex beads, or by phorbol esters which also trigger superoxide release. Figure 5 illustrates that voltage-activated H^+ currents in human neutrophils are enhanced by arachidonic acid over a time course of several minutes [39]. The enhancement is comprised of a shift of the voltage dependence of activation to more negative potentials and an increase in $g_{H,max}$ [39]. As a result, the H^+ currents are larger at any given potential. The enhancement of H^+ currents by arachidonic acid was confirmed recently in murine macrophages, in which several unsaturated fatty acids enhanced H^+ currents with potency related to their ability to stimulate NADPH oxidase [86]. One possible explanation of these effects is that unsaturated fatty acids have been shown to make the membrane surface potential more negative [28], which would alter the effective membrane potential sensed by the H^+ channel.

During the "respiratory burst" phagocytes release superoxide, which is converted into various bactericidal oxygen species. Because NADPH oxidase is electrogenic, this process results in intracellular H^+ generation, which tends to reduce pH_i and depolarize the membrane potential [70–72, 88, 124]. Under normal ionic conditions, Na^+-H^+ antiport is rapidly activated and prevents large changes in pH_i, although it is electroneutral and therefore cannot dissipate excess charge in the cell. In the absence of Na^+, however, pH_i falls dramatically and the resting potential can depolarize beyond 0 mV [124]. Under this condition, activation of the g_H can be demonstrated, and addition of Cd^{2+} or Zn^{2+} exacerbates the depolarization and pH_i decrease [70, 71]. It seems clear that the g_H is activated by agents which promote the respiratory burst in the absence of other pH-regulating systems (69–72, 88, 89, 124, 125]. Less well established is the extent to which H^+ channels contribute to H^+ extrusion when other mechanisms are present. The resolution of this question may await discovery of potent and selective inhibitors of the g_H.

In chronic granulomatous disease NADPH oxidase is genetically defective and incapable of generating superoxide, severely compromising the body's defenses against bacterial infection. In an intriguing study of neutrophils from patients with chronic granulomatous disease, the g_H was found to be dramatically reduced while two other pH_i-regulating systems studied were normal [125]. The g_H was evaluated indirectly by pH_i-sensitive dyes, and direct exploration of the mechanism of the defect is of great importance. It is possible that the g_H is normal, but that its activation is defective in chronic granulomatous disease.

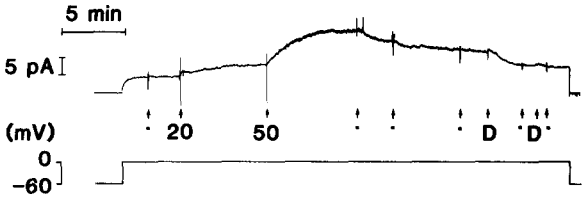

Fig. 5. H^+ currents in human neutrophils are enhanced by arachidonic acid (AA). The command voltage is shown below the whole-cell membrane current. At the arrows, the indicated concentration of AA (in μM) was added; dots indicate a bath change with AA-free solution. The outward current increased over the course of a few minutes at 20 μM AA, and 50 μM AA had a more pronounced effect. The enhancement was partially reversible upon washout of the AA (dots). At the arrows labeled *D*, dimethylsulfoxide (the vehicle for AA) was added as a control at twice the concentration used for the largest AA concentration. Internal and external solutions contained TEA^+ $MeSO_3^-$ at pH 7//6 with 20 mM buffer. The enhanced currents in the presence of AA were H^+ currents because they exhibited time-dependent activation during depolarizing pulses, V_{rev} remained near E_H, and they were inhibited by Zn^{2+}. Adapted with permission from [39].

How Do Proton Channels Work?

H^+ Channels Resemble Other Voltage-Gated Ion Channels

Are H^+ currents carried through ion channels analogous to those for other ions? This question cannot be answered at present because the identity and structure of the H^+ "channel" is not known, but it is possible to speculate about the extent to which H^+ channels may resemble other channels. Naturally occurring ion channels are large proteins usually comprised of several subunits which assemble in the membranes of cells. Each subunit typically includes up to a dozen putative membrane-spanning segments connected by loops which along with terminal segments may extend well beyond the membrane. Voltage-gated Ca^{2+}, Na^+, and K^+ channels all have four domains of six membrane-spanning regions [25]. Ions permeate in single file through a narrow region filled with water molecules [*discussed in* 74]. Neither ions nor water molecules can pass each other within this narrow region of the channel. H^+ currents share many features common to a number of other ion channels: the probability of channel opening is strongly dependent on membrane potential, during voltage pulses the conductance is activated or deactivated (i.e., turned on or off) with a time course which becomes more rapid at large positive or negative

potentials, the channels are selectively permeable, the currents do not depend on the presence or concentration of other ions, current flows passively and does not require ATP. Single H^+ channel currents have not been resolved, and apparently are too small to be detected directly using currently available technology [15, 24, 39].

Many Nonselective Channels Conduct Protons

A variety of membrane proteins which are permeable to monovalent cations also can be shown to conduct H^+ at low pH. These include gramicidin A [76], voltage-gated Na^+ channels [13, 118], certain synthetic oligopeptides [2, 97], the MotA protein of flagellar motors [16], the transmembrane domain of the M2 protein of influenza virus [45], the phoE porin channel [62], and amiloride-sensitive Na^+ channels [53, 130]. The ADH-activated water channel, evidently a water-filled pore, has been reported to conduct H^+ detectably at low pH [55, 65, 66]. However, vasopressin did not enhance the H^+ conductance at pH 6.5 [143], and a different water channel, aquaporin CHIP-28, after purification and reconstitution into bilayers was not detectably permeable to H^+ or other cations at neutral pH [154]. The uncoupling protein of mitochondria in brown fat effects proton translocation, but also conducts Cl^- so the conductive species may be OH^- (as nuclear oxygen) rather than protons [127]. Figure 6 illustrates the single channel proton current amplitude for a 100 mV driving force for a variety of proton permeable membrane proteins. The H^+ conductance of each of these channels is substantially greater than that of other cations, suggesting that protons rather than the larger hydronium ions (H_3O^+) are the conductive species, and that conduction most likely occurs via a mechanism in which protons hop from one water molecule to the next in a water-filled pore [119]. This type of H^+ conduction mechanism, called variously a "water-wire," "Grotthuss" mechanism, or prototropic transfer [98], is invoked to explain the anomalously high conductivity of protons in bulk solution [14, 81]—protons diffuse ~5 times more rapidly than other monovalent cations [136]. Proton permeation by hopping occurs without displacing the water in the pore, whereas a cation must traverse the single-file region of the pore in conjunction with the movement of a number of water molecules, as has been shown by streaming potential measurements in gramicidin channels [101]. Given this mechanism, one might expect that proton conductance would be an inescapable property of any cation channel which is normally filled with water molecules. In fact, many types of ion channels can conduct protons, but demonstration of this property usually requires low pH because the channel density is too low to observe H^+ currents at physiological pH. If protons permeate by a water-wire type mechanism, then other permeant ions could "block" proton conduction by interrupting the chain of water molecules. This effect has been demonstrated for gramicidin A channels, in which Na^+ blocks H^+ current [67].

The unitary conductance of the channels in Fig. 6 is a remarkably consistent function of pH. Over a wide range, the unitary H^+ conductance is directly proportional to $[H^+]$, indicated by the slope of the line in Fig. 6. The single channel H^+ current saturates at very high $[H^+]$, at 4 M HCl in gramicidin [3] and at 2 M HCl in two synthetic channels [41], reflecting the maximum rate of H^+ permeation through the channel pore. At pH > 2 the unitary conductance is diffusion limited, i.e., it is determined entirely by the rate at which protons reach the channel mouth [37]; in fact, the H^+ conductance of gramicidin-containing bilayers is proportional to $[H^+]$ over the pH range 4.0–8.5 [60]. Extrapolation of these data suggests that the single channel H^+ current would be <1 fA at pH 6. Estimates based on H^+ current variance measurements are in this range (▲, ▼ in Fig. 6), consistent with the possibility that voltage-gated proton channels are water-filled pores in which protons permeate by a water-wire mechanism.

Are Highly Selective Channels also Water-filled Pores?

Another functionally defined class of molecules exhibits much more selective H^+ conduction. The F_0 subunit of the H^+-ATPase of bacteria, mitochondria, and chloroplasts can function as a proton channel [*reviewed by* 79, 84, 115, 139]. The F_0 subunit from yeast mitochondria incorporated into bilayers exhibited single channel currents which were detectable only at pH < 3, were linearly related to proton concentration, and had an open probability which was only weakly voltage dependent [138], behavior like that of the other channels in Fig. 6. In contrast, the unitary conductance of the F_0 subunit from spinach chloroplasts, CF_0, in bilayers was independent of pH between 5.6–8.0 (dotted horizontal line in Fig. 6) [5, 153]. The pH-independent conductance through CF_0 appears to differ from that of mitochondrial F_0 and the other channels in Fig. 6. The extremely high selectivity of these channels, $P_H/P_{Na} > 10^7$ [5, 84], indicates that they effectively exclude other ions. The problem presented is how and whether such extreme selectivity can be achieved by a water-filled pore, given that many ions are smaller than water molecules.

Voltage-gated H^+ Channels May Have a Small, pH-independent Conductance

The unitary conductance of the voltage-activated H^+ channel estimated from current fluctuation analysis is consistent with that expected by extrapolating measurements at lower pH of presumably diffusion-limited currents in other channels (Fig. 6). However, this view

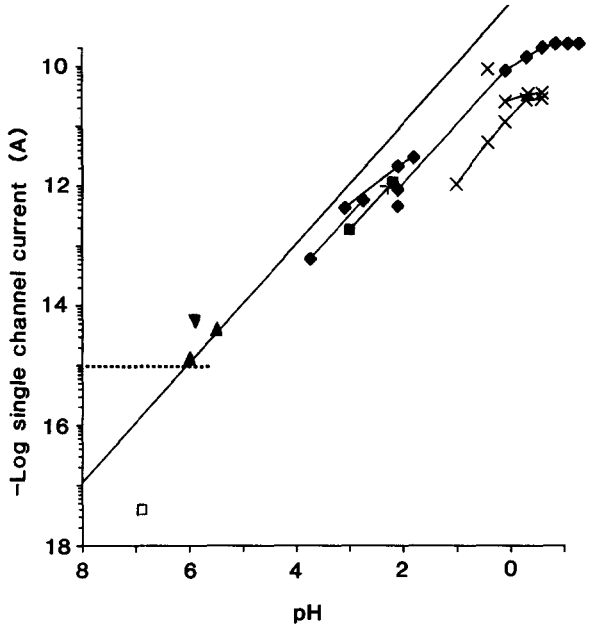

Fig. 6. Single channel proton currents in a variety of types of proton-conducting membrane proteins. All reported currents or conductances have been converted into the current expected for a 100 mV driving force; where HCl concentrations were given, pH was calculated according to the activity coefficient for HCl [136]. The line has a slope of +1, as expected if conductance is directly proportional to $[H^+]$, and shows the access resistance to a 4 Å diameter pore based solely on free H^+ concentration, and without considering the effects of buffers, surface charges, etc. (*see text* for further discussion). Data points from the same study are connected by lines. Gramicidin (◆) from [3, 76, 100, 126]. The predominant conductance levels are plotted of three synthetic channels formed from 21 amino acid subunits (X) composed entirely of serine and leucine [97], or with an α-aminoisobutyric acid substitution [41]. The two lower conductance channels were proton selective and predicted to be ~1 Å in diameter, while the larger channel was predicted to be ~8 Å in diameter and conducted other cations as well. Although the influenza M2 protein (+) is not very selective [45, 133], H^+ conductance is its hypothesized function [68]. The F_0 proteolipid (■) has a relative H^+ permeability $>10^4$ that of K^+ [138]. Recent estimates (dotted line) indicate that the CF_0 H^+ conductance is at least 10 fS [140], highly selective, and independent of pH between 5.6–8.0 [5]. Higher estimates for CF_0 have been also reported [5, 153], but require special mechanisms to supply enough protons to the channel [84; *see also* "How Large Can Single-Channel H^+ Currents Be?"]. The MotA estimate (□) is based on pH changes and is thus indirect [16], as are the CF_0 estimates. The values for the voltage-activated H^+ channel are based on H^+ current fluctuations, and represent an upper limit (▼) where excess noise was not detected [24] or best estimates (▲) [15, 39].

is complicated by several factors. (i) Voltage-gated single-channel H^+ currents have not been successfully recorded in the low pH range used to demonstrate unitary H^+ currents through other molecules (V.V. Cherny and T.E. DeCoursey, *unpublished data*), hence there is no evidence that voltage-activated H^+ channel currents would be larger at lower pH. H^+ permeation through the channel, rather than entry into the pore, may be rate-limiting already at physiological pH. (ii) Fluctuation analysis is indirect, and requires making assumptions about gating which may be incorrect. In spite of the slow macroscopic current relaxations, single channels might open and close at frequencies above the recording range. (iii) Extrapolation of the $[H^+]$-current relationship measured in other channels at low pH may not be valid for greatly lower H^+ concentrations, if for example buffering or hydrolysis increases the supply of protons to the channel [90]. (iv) Finally, the macroscopic g_H does not vary with pH_i in proportion to free H^+ concentration, suggesting that the unitary conductance is in fact practically pH_i independent in the physiological pH range, like the reported behavior of CF_0[2] [5, 84, 153]. With optimal technique including quartz microelectrodes, RMS noise at 100 Hz is ~5 fA [99], so that 1–2 fA single channel H^+ currents would not be resolved convincingly. In summary, although the small conductance estimated for the voltage-activated H^+ channel is compatible with values extrapolated for various water-filled pores, the apparent pH independence of the conductance suggests a different mechanism.

How Large Can Single Channel H^+ Currents Be?

Diffusion of the conductive species from the solution to the mouth of the channel provides an effective limit to single channel conductance [10, 74, 94]. If we assume a pore diameter between 1 Å, estimated by Lear et al. [97] for a synthetic proton-selective channel, and 4 Å, the diameter of the nonselective gramicidin A pore, the diffusional component of the access resistance would limit the unitary H^+ current to 0.3–1.0 fA at pH 6, assuming that free protons diffuse to the channel with $D = 8.65 \times 10^{-5}$ cm^2/sec at 20°C [136]. Interactions between water molecules and the protein walls may further reduce the H^+ conductance of a water-filled pore [62]. Protonated buffer molecules, present at several orders of magnitude higher concentration than free H^+, will to some extent short-circuit the diffusional resistance of bulk solution and replenish protons near the channel [90]. The access limitation due only to buffer diffusion (if $D = 5 \times 10^{-6}$ cm^2/sec) for 100 mM buffer at its pK limits the current to 0.75–3.0 pA. This value represents an upper limit which ignores free H^+ diffusion limitation, buffer deprotonation rates, proton accumulation on the distal side of the pore, slowing of the apparent diffusion constant by immobile cytoplasmic buffers [4, 85], and the resistance of the channel itself; the actual limit probably lies between 0.3 fA and 3 pA. The lower end of this range seems most likely because

[2] Voltage-gated H^+ channels and CF_0 differ in pharmacological sensitivity, as discussed under "H^+ Currents Are Not Carried through Other Ion Channels or Transporters."

(a) the H^+ conductance of gramicidin-containing bilayers is proportional to free $[H^+]$ over the pH range 4.0–8.5 even in well-buffered solutions [60], and (b) saturation at large positive potentials [*cf.* 6] of voltage-activated H^+ current was observed with low but not high buffer in the cell [38]. The H^+ current through gramicidin A channels was increased up to 12–fold by 2 M formic acid, although the mechanism appeared to involve binding of formic acid to the channel rather than simple buffering [37]. Several other mechanisms could enhance at least transiently the proton concentration near a channel, including local concentration by negative surface charges [110] or charges on the channel protein [57, 83], and buffering by the membrane itself [59, *but cf.* 91], in which case anionic lipid headgroups might facilitate rapid proton translocation in the plane of the membrane [64]. Voltage-activated H^+ currents can be sustained for minutes if bulk pH changes are prevented, and therefore any mechanism invoking a rapidly consumable local supply of protons is not viable.

H^+ Channels May Not be Water-filled Pores

Two fundamental experimental observations for both CF_0 and voltage-activated H^+ channels are their extreme selectivity for H^+ over other cations and the insensitivity of the conductance to pH. Both properties can be accounted for if H^+ permeation occurs through a "proton wire" or hydrogen-bonded chain formed by amino acid side chains in membrane proteins [121, 122]. A variety of protein side chains can efficiently transport protons via hydrogen-bonded chains [155]. The proton wire might comprise a water-filled channel which only partially spans the membrane, with a small region of hydrogen-bonded peptide. The discontinuous water bridge would ensure impermeability to other cations, because protons but not other ions could jump across the membrane by such a mechanism. Proton transport through a hydrogen-bonded chain requires two steps, the transport step (proton hopping) and then a turning (rotational reorientation) of the (e.g.) hydroxyl groups to allow access to the next proton [35, 46, 122, 147]. This type of mechanism might be imagined to result in a lower unitary conductance than a water-filled pore, one which might saturate at a pH-independent limit even in the physiological pH range. The series of hopping and turning steps required for a proton to cross a chain of protein side groups spanning the membrane was estimated[3] to require ~2.75 μsec for a 1 mV driving force [121], which corresponds with an upper limit of 3.4 pA single channel current at 59 mV assuming that channel permeation is rate-limiting, whereas the unitary H^+ current through water-filled gramicidin is 140 pA at 59 mV at saturation [3]. The turning step is slower than the H^+ hopping step, i.e., is rate-determining, in water [34], and is even slower in ice [27], and might be further slowed by constraints on rotation of water or side groups inside a channel [62]. Asymmetrical proton transfer events would also slow conduction considerably [98]. Initiation of turning depends on the pH-independent breaking of hydrogen bonds between waters, thus the conductance could be independent of pH [120]. Additional properties of H^+ channels (voltage-dependent gating, slow gating kinetics, and the modulation of gating by pH_o and pH_i), are all more readily explained by a hydrogen-bonded chain formed in a membrane protein rather than in a water wire defect between membrane lipids.

In summary, a number of different membrane proteins behave as proton channels with moderate to extremely high selectivity. Most of these channels are entirely or partially filled with water molecules. The unitary conductance appears in most cases to be proportional to $[H^+]$ at $pH > 0$, consistent with a "water-wire" mechanism in which protons permeate a water-filled channel by hopping from one water molecule to the next. It is possible that certain highly H^+-selective channels including the voltage-gated H^+ channel may operate by a proton-wire mechanism in which protons jump across a hydrogen-bonded chain formed at least partially of side groups within an integral membrane protein.

H^+ Channels Are Abundant

In cells in which the relative amplitudes of H^+ and K^+ currents have been compared, the current density is similar [24, 38] or the H^+ current is 10 times larger [42]. Because the single channel conductance of H^+ channels is three orders of magnitude smaller than that of K^+ channels, there must be a large number of H^+ channels. In studies in which unitary H^+ currents were estimated, H^+ channel densities were >11, 12, and 170 channels/μm^2, respectively, in *Lymnaea* neurons [24], human myotubes [15], and human neutrophils [39]. Na^+ and K^+ channels at the node of Ranvier are >10 times more densely packed, at 1,000–2,000 channels/μm^2, and rhodopsin proteins in a crystalline two-dimensional array are packed at 89,000/μm^2 [74]. Thus, the estimated density of H^+ channels in the membrane is high but not unreasonably so, and could conceivably be increased up to ~3 orders of magnitude before reaching a level of physical implausibility.

How Do H^+ Channels Sense the pH?

The voltage dependence of the g_H shifts as either pH_o or pH_i is altered, such that only outward H^+ currents can

[3] This order-of-magnitude estimate was based on the assumption that the mobilities of ions and faults in a hydrogen-bonded chain across a membrane are comparable with those in ice, and should be viewed in the context of considerations discussed in [122].

be detected. Byerly et al. [22] pointed out that although the effects of pH on the voltage dependence of g_H activation are in the direction expected for simple neutralization of negative surface charges, the magnitude of these effects requires a direct (allosteric) interaction of H^+ with channel gating. Allosteric regulation of the rate of Na^+-H^+ antiport by pH_i is a well-known example of this type of mechanism [8]. The only voltage-activated ion channel with a comparably strong dependence on permeant ion concentration is the inwardly rectifying K^+ channel, whose voltage-dependent gating shifts in parallel with E_K when the external K^+ concentration is varied [63, 132, 144]. Both H^+ and K^+ channels exhibit time- and voltage-dependent gating modulated by permeant ions such that in the steady-state each conductance rectifies strongly. Two main classes of models have developed during several decades of speculation on how inward rectifier channels sense the K^+ concentration: blocking particle models [7, 63, 75, 146, 152] and electrochemical gating models [31, 80, 131, 132]. Could a blocking particle model account for the pH dependence of g_H gating? A cytoplasmic moiety, perhaps negatively charged in deference to the voltage dependence of gating, might occlude the channel at negative but not positive potentials, but this would not explain the modulation by pH_i. Another possibility is that a "tail" of channel protein could extend into the external solution and occlude the channel except when the electrochemical gradient favored H^+ efflux. The mechanism might be current dependent rather than voltage dependent, and thus the particle need not be charged, but should tend to relax back to a position which prevents conduction. An electrochemical gating mechanism is another possibility, of which two general classes can be imagined: (1) allosteric regulation (by protonation) of a voltage-dependent gating mechanism in a pre-existent integral membrane channel, and (2) stabilization of the open channel state by protonation of internal sites and stabilization of the closed configuration by protonation of externally accessible sites. In one specific form of this latter mechanism several charged channel subunits might be aligned parallel to the external membrane surface at large negative potentials, with protonation of externally accessible sites stabilizing the closed configuration. Depolarization would tend to reorient the subunits perpendicular to the surface by pulling the negatively charged (deprotonated) end of the molecules toward the inner membrane surface, where the same or different negatively charged sites would be protonated to stabilize the open configuration of the channel. Alamethicin gates by voltage-induced reorientation and aggregation of several molecules [47]; a model for inward rectifier K^+ channel gating proposed by Ciani et al. [31] invokes preformed subunit aggregates which reorient across the membrane; and convincing evidence exists that synthetic H^+-permeable channels form in this manner [30].

The nature of H^+ permeation suggests additional types of gating mechanisms. For example, proton pumps have been modeled as hydrogen-bonded chains interrupted by an active site or proton "injector" which consists of a protonation site whose pK may be altered significantly by conformational changes in the protein molecule [123]. Movement or stress on part of the channel resulting from changes in the membrane electric field could be imagined to alter the pK of such an active site to complete the hydrogen-bonded chain across the membrane.

It is possible that H^+ "channels" actually function more like carriers. The pH_o and pH_i dependence of the voltage-activation curve of the g_H could be mediated by bilateral "access channels" or "ion wells" analogous to those proposed for H^+-ATPase [96, 115] and Na^+/K^+-ATPase [44, 51, 73]. Protonation sites might exist in "proton wells" located within the membrane field in channel-like closed structures with a carrier-like mechanism effecting the translocation of H^+ across the central barrier. Because a conformational change may expose the H^+ binding site(s) to the solution on the opposite side of the channel, this mechanism can be defined as a carrier [95]. Further quantitative descriptions of the pH_o and pH_i dependence of H^+ channel gating are needed to clarify the range of reasonable models.

Practical Considerations

The nature of the g_H and of protons results in several types of experimental complications. Some of these complications are also encountered in the study of other types of ion channels, but usually to a lesser extent. Our goal here is to explore the order of magnitude of the problem under various experimental conditions, and to discuss practical implications for experimental design.

Which Are the Best Impermeant Ions To Use?

To study H^+ currents in isolation from other contaminating conductances, it is desirable to use solutions devoid of ions permeant through other ion channels. It is also prudent to avoid substrates for other types of transporters, especially those which alter pH such as Na^+-H^+ antiport. Cations which have been used for this purpose include Cs^+ [15, 24, 42, 87] and the larger molecules TMA^+ [40], TEA^+ [9, 39, 107], and NMG^+ [15, 38, 113]. TEA^+ not only is impermeant through most channels but blocks K^+ channels. However, at high concentrations it can inhibit H^+ currents ([15, 22, 113]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). The pH of NMG^+ solutions tends to change over time and must be checked frequently. We have not yet discovered problems with TMA^+. Anions which have

been used include: aspartate$^-$ [15, 24, 42, 87], gluconate$^-$ [113], and $MeSO_3^-$ [38–40], although buffer is a major anion in many studies. Aspartate$^-$ solutions are notorious breeding grounds for bacteria. Large ions have lower conductivities than smaller ions, thus the pipette resistance and consequently access resistance in the whole-cell configuration tend to be larger. Because of their lower mobilities, these ions also can result in significant liquid junction potentials [11]. Optimal solutions for recording H^+ currents have not yet been established and may vary among preparations.

Is the Permeant Ion Concentration Known?

A prerequisite for quantitation of ion channel behavior is knowledge of the permeant ion concentrations on both sides of the membrane. The complications arising when the permeating ion accumulates or is depleted in a small space such as the periaxonal space in squid [48] or in the t-tubules of skeletal muscle [1] are well known for K^+ channels, and unstirred layer effects have been exhaustively reviewed [10]. Frankenhaeuser and Hodgkin [48] dismissed the possibility that, in the absence of a barrier to diffusion (i.e., the Schwann cell), accumulation of K^+ near the membrane would affect their data, because an action potential would change the local $[K^+]_o$ only 0.05 mM, a negligible increase. However, 0.05 mM $[H^+]$ corresponds with pH 4.3, which illustrates that the rules of the game are different when H^+ is the current carrier. We observe a moderate increase in H^+ current when the extracellular buffer concentration is increased (V.V. Cherny and T.E. DeCoursey, *unpublished data*). In addition to depletion/accumulation phenomenon in external unstirred layers, the cellular compartment represents an obvious and significant limitation in the control of pH near the membrane. In snail neurons 90–120 μm in diameter, internal perfusion with buffered solutions applied via a suction pipette one-third the cell diameter effectively controlled pH_i (measured with a pH electrode) only at high buffer concentration (100 mM) and then only after 8–10 min [23]. At [buffer] < 20 mM, pH_i changed very slowly and did not reach that of the pipette solution, which Byerly and Moody [23] attributed to the intrinsic buffering power of cytoplasmic buffers. Similarly, pH_i measured by a pH-sensitive dye equilibrated faster in small (2–8 pF) cells with 100 mM than with 10 mM buffer in the pipette solution (within ~1 min at high buffer), and 100 mM but not 10 mM buffer brought pH_i to the value in the pipette solution [42]. The inability of low buffer concentrations to completely alter pH_i is somewhat surprising. The presence of immobile or slowly diffusing intrinsic cytoplasmic buffers should slow the equilibration of pH_i toward the pH of the pipette solution [85, 109], but eventually pH_i should attain the value of the pipette solution unless there is ongoing net acid/base transport across the cell membrane. Possible mechanisms include simple H^+ leakage through the membrane, any of the transporters listed in Table 2 for which permissive ionic conditions exist, or the presence of weak acids or bases which are membrane permeant in uncharged form and can thereby dissipate a pH gradient [*reviewed by* 17, 111]. As a practical consideration, using as high buffer concentration as possible seems prudent, although cells seem less happy under these conditions ([22]; V.V. Cherny and T.E. DeCoursey, *unpublished data*). Byerly et al. [22] found that adding sucrose to make the external solutions hypertonic improved the survival of snail neurons perfused with high buffer.

Large H^+ Currents Increase pH_i

During H^+ currents, pH_i increases on a time scale of minutes in large snail neurons [113, 148, 151], and on a time scale of seconds in small cells dialyzed by the whole-cell configuration of the patch-clamp technique [38, 42, 87]. Figure 7 illustrates that during very long and large depolarizing pulses, severe "droop" is observed in small cells perfused with only 5 mM buffer. A second identical voltage pulse applied after 30 sec elicited a much smaller and more slowly rising current, behavior expected if pH_i were substantially higher than during the first pulse. H^+ current droop was greatly diminished when the pipette buffer concentration was increased to 119 mM, implicating depletion of protonated buffer from the cell and a consequent rise in pH_i as the cause [38]. When measured under conditions where depletion would be minimized (e.g., during small pulses, in cells with small H^+ currents, or with high buffering), no detectable inactivation of the g_H occurs in any preparation [9, 15, 22, 38, 39, 87, 107, 113, 151]. Direct measurement of pH_i by pH microelectrodes or fluorescent dyes confirms that H^+ efflux during large prolonged outward currents greatly increases pH_i [42, 87, 107, 113, 151]. The outward H^+ current following HCl injection decayed with a time constant of 1.4 min in neurons, slightly faster than the rate that pH_i increased, but both phenomena correspond with the same H^+ efflux rate and clearly reflect the same process [113]. Droop of H^+ current in smaller alveolar epithelial cells had a time constant on the order of ~10 sec (at 5 mM pipette buffer), consistent with the smaller cell volume [38].

The fact that activation of H^+ currents can rapidly raise pH_i presents a practical difficulty for the experimentalist, but dramatically confirms the idea that the g_H can function in intact cells as an efficient mechanism of H^+ extrusion during recovery from an acid load. Simple considerations illustrate that H^+ currents ought to change pH_i as observed. In Fig. 7 the peak current,

~170 pA, would dissipate the entire charge due to free H^+ in the cell (5.6×10^{-18} mol) in ~3 msec. Obviously, essentially the entire conductive H^+ efflux is the result of buffer molecules releasing protons near the membrane to replace those which leave the cell. The charge extruded by the H^+ current during the pulse, 2.4 nC, was several times larger than the total charge associated with exogenous buffer molecules in this cell, 0.68 nC, suggesting that a significant quantity of intrinsic cellular buffer remained even after perfusion with the pipette solution [38]. In cells perfused with 119 mM MES pipette solution, also at pH 5.5, distinct but less pronounced "droop" occurred during long pulses; although the maximum H^+ currents normalized for membrane capacity (i.e., surface area) were about three times larger, the charge transferred during these pulses was generally smaller than the total charge associated with the buffer [38]. In summary, the magnitude of H^+ currents is such that depletion of free H^+ near the membrane will occur practically instantaneously, and depletion of protons from cytoplasmic buffers, with buffering power comparable to that of intact cells [137], can occur within a few seconds. A 9 sec pulse can increase pH_i by 1 unit even with 100 mM buffer [87]. The enormous capacity of the g_H to alkalinize small cells is evident by inspection of Table 2: the maximum rate of pH_i change is 1–2 orders of magnitude larger for H^+ currents than for any other pH-regulating transporter.

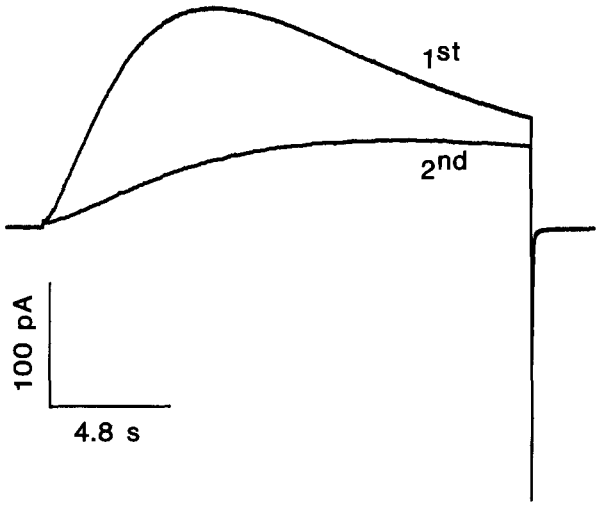

Fig. 7. During large prolonged depolarizations H^+ currents sometimes decay with time. This "droop" is not due to inactivation, or closing of channels, but is the result of depletion of protonated buffer from the cell. The H^+ current in this alveolar epithelial cell reached a maximum and then slowly decayed to half its peak value during a 20 sec pulse to +100 mV (at pH 5.5//5.5, with 5 mM MES buffer in the pipette). After ~30 sec an identical depolarizing pulse elicited a much smaller current, indicating that recovery from depletion of protonated buffer in the cell was far from complete. Note that the rate of rise of H^+ current during the second pulse was much slower, which is expected if pH_i were substantially higher at the start of the second pulse. That the peak outward current during the second pulse did not attain the amplitude at the end of the first pulse may indicate that (i) additional depletion occurred during the rising phase of the second pulse, or (ii) the conductance was activated to a smaller extent during the second pulse than at the end of the first pulse. The latter possibility is intriguing because it implies that the allosteric modulation of H^+ current by pH_i takes place on a time scale which is slow compared with the rate of channel permeation and of local proton-buffer reactions. In other words, the droop in current during the first pulse was due to depletion of H^+ and protonated buffer and hence a reduced driving force, but the probability of channel opening did not decrease immediately. Cell diameter 15 μm, series resistance 10.3 MΩ, V_{hold} −60 mV. This experiment was described in general terms in [38].

Practical Effects of Depletion

The depletion of protons from buffer as a result of H^+ currents alters the appearance of H^+ current data in ways which are to some extent predictable. When the H^+ currents during large depolarizations exhibit droop, it is certain that pH_i was increased substantially by the H^+ efflux; pH_i can change somewhat during H^+ currents even when droop is not evident. Since full recovery from H^+ current-induced pH_i changes requires 1–3 min for small cells, similar to the time required for the initial equilibration of pH_i upon break-in [42, 87], quite long interpulse intervals are needed for full recovery. Nevertheless, extreme compulsion may be unwarranted for routine measurements, considering that the current waveform during a single pulse may be distorted by depletion even if the system was stable at the start of the pulse. Current droop is less apparent in larger cells, such as myotubes (input capacity 182 pF [15]), presumably because the surface/volume (or g_H/volume) ratio is lower, i.e., there is a larger volume of buffered solution in the cell to sustain pH_i. On the other hand, much longer equilibration times are required to change pH_i in large cells [23] because of the longer diffusion distances involved.

H^+ depletion during pulses can lead to overestimation of the rate of H^+ current activation (i.e., too small τ or $t_{1/2}$). Determination of the "steady-state" or equilibrium value of H^+ current is also compromised. A slow droop superimposed on a gradually increasing current can produce a pseudo-equilibrium. Nevertheless, this error should be minimized by a small g_H/volume ratio, and in large cells the g_H-V relation appears roughly comparable with that in smaller cells. Slow activation kinetics in many cells complicates evaluation of the voltage dependence of activation, because even 4–10 sec pulses are insufficient for full equilibration at all potentials [15, 38–40], and thus the peak H^+ current does not provide a true steady-state estimate of the g_H attainable at a given potential. Both the slope factor, k, and midpoint, $V_{1/2}$), of the measured g_H-V relation can depend on pulse duration for this reason. During small

depolarizing pulses, the H^+ current in human neutrophils continues to rise for as long as three minutes before finally reaching an apparent steady-state value [39]. Even in this case, however, it was demonstrated that the g_H is activated in a graded manner by depolarization [39].

A Boltzmann function (Eq. 1) does not always describe real g_H data well; g_H-V data do not always exhibit saturation ([22]; V.V. Cherny and T.E. DeCoursey, *unpublished data*), and depletion can cause artificial saturation. The establishment of $g_{H,max}$ during large depolarizations, and consequently large H^+ efflux, is especially questionable. Conductance-voltage relationships reveal the voltage dependence of channel opening probability only if the instantaneous (or single channel) current-voltage relationship is linear, but any rectification can be "corrected" by measuring the tail current amplitude at a fixed potential after prepulses. This method does not correct for the effects of depletion during large pulses, however. If the tail current is outward, the apparent g_H-V relation will be artificially steepened; if the tail current is inward, the slope will appear flatter. Finally, this discussion is not intended to be discouraging. By understanding the limitations of the data we hope simply to avoid its overinterpretation. We have already summarized many properties of H^+ currents which in spite of any complications have been established firmly.

Functions of H^+ Currents

The cooperative modulation of the g_H by pH_o, pH_i, and by depolarization which result in its activation only at membrane potentials positive to E_H, makes it ideally suited to extrude acid from cells. H^+ current is electrogenic and H^+ efflux would also hyperpolarize the resting potential, which in turn would turn off the g_H. The main question then is not what effects activation of the g_H would have, but rather when the g_H is activated in intact cells in their normal environment. Specific proposed functions of the g_H in different cells are beyond the scope of this review, and have been reviewed elsewhere [104, 112]; here we will consider general principles which are relevant to evaluating possible functions.

Does the g_H Conduct Inward Current?

H^+ channels clearly can conduct inward currents, because inward "tail currents" can be detected. The more critical question, carefully explored by Thomas [150], is whether the g_H may be activated at normal resting potentials, conducting small inward currents at potentials negative to E_H, which would constitute a chronic acid load. Only outward steady-state currents have been detected at all combinations of pH_o and pH_i studied to date. Any steady-state inward current must be very small.

Local pH May Activate H^+ Currents

It may not be necessary for the average pH_i to decrease in order to activate the g_H [15, 113]. A transient decrease in pH near the membrane might suffice to shift the voltage dependence of activation to more negative potentials and thus activate the g_H, as proposed by Meech and Thomas [113]. Because of its local regulation, the g_H could be selectively activated at the apical or basolateral surfaces of a single cell. During action potentials, Ca^{2+} influx through voltage-activated Ca^{2+} channels would increase H^+_i due to Ca^{2+}/H^+ exchange [113, 142] or due to displacement of H^+_i from common intracellular binding sites [150]. In cells perfused with a solution of low buffering power, the Ca^{2+} ionophore A23187 enhanced H^+ currents [78], suggesting that Ca^{2+} influx can lower local pH_i enough to activate the g_H. Many cells exhibit $[Ca^{2+}]_i$ oscillations or spikes, whose role in signaling is under active study. pH_i may vary in a time-dependent manner as well, for example as a result of displacement of H^+_i by Ca^{2+} from shared binding sites. The g_H would tend to be activated when local pH_i is lowest. In conclusion, spatial or temporal pH fluctuations may activate the g_H in situations not predictable from time-averaged, bulk pH measurements, for example, by fluorescent dyes.

Cells Have Many Acid Extrusion Systems

An essential capability of all cells is to extrude acid equivalents. Cellular metabolism generates acid which must be disposed of to maintain pH_i. Mammalian cells maintain pH_i within a fairly narrow range, usually 6.8–7.5 [137], higher than if H^+ were passively distributed. For pH_o 7.4, the corresponding E_H ranges -37 to $+6$ mV at 37°C. Most cells maintain a more negative membrane potential than this, so any H^+ permeability would result in inward H^+ current. Any H^+ "leak" into the cell or metabolically generated acid must be actively extruded to maintain pH_i. Cells closely regulate pH_i because virtually all cellular processes are affected by pH—enzymes have pH optima, most ion channels are modulated by protons in various ways ranging from block to alterations in voltage dependence, selectivity, or single channel conductance [74, 116, 137]. A large body of evidence indicates that pH_i is set at a different point in cells activated (e.g., by mitogens, growth factors, secretagogues) or perturbed (e.g., during volume regulation) in various ways [21, 26, 58, 117]; that is to say pH_i changes are correlated with various altered states of cell function.

A variety of membrane transporters in cells alter pH_i, some of which are listed in Table 2. The large

Table 2. Transporters regulating pH in small cells

Transporter	Maximum rate (pH_i) (pH/min)	Energy cost (ATP use)	Electrogenic?	Effect on pH_i (normal mode)	References
Na^+/H^+ exchange	0.13–0.23 (6.3–6.5)	Indirect	No	Increase[a]	20, 128, 145
Na^+-HCO_3^- symport	0.12 (7.0)	Indirect	Yes	Increase[b]	103
Cl^-/HCO_3^- exchange	−0.08 (7.8)	Indirect	No	Decrease[a]	18, 129, 145
H^+-ATPase	0.09 (6.6)	Direct	Yes	Increase	102
H^+ channels	6.0 (6.0)	Free	Yes	Increase	87

These values are taken from alveolar epithelial cells and phagocytes, small 8–15 μm diameter cells, in order that the surface-to-volume ratio be reasonably uniform. The pH_i value at which the measurement was made is given in parentheses. The effect on pH_i is for normal operation in these cell types; [a]some of these transporters can be reversed experimentally. [b]Normal mode operation in kidney decreases pH_i [19]. Only selected transporters are listed; not included are Na^+/HCO_3^--Cl^-/H^+ exchange, K^+/H^+ exchange, or Na^+ cotransport with various organic acids and bases.

number of mechanisms reflects both the importance of pH_i regulation, and that different mechanisms operate under different conditions. The effects of some of the transporters on pH_i may be secondary to other primary functions, such as fluid and electrolyte transport across epithelia. The possibility of modulating any of these systems also gives the cell a number of possible ways of coordinating its response to various situations. Under normal physiological conditions, all of the mechanisms in Table 2 are functionally oriented so that their action results in increased or decreased pH_i. By virtue of the normally inward Cl^- gradient, Cl^-/HCO_3^- exchange tends to acidify the cell, and facilitates recovery from artificially imposed alkaline loads [129, 145]. The Na^+-H^+ antiporter, driven by the usually large inward Na^+ gradient, increases pH_i and permits recovery from acid loads. The g_H mechanism is oriented in the cell membrane so that it conducts only outward H^+ current, and thus its activation results in cellular alkalinization.

Examination of Table 2 illustrates the utility of the g_H in regulating pH. The g_H is entirely passive, and incurs no direct or indirect energy cost to the cell. Na^+-H^+ antiport passively loads the cell with Na^+, requiring the eventual expenditure of ATP to pump out the Na^+ (via Na,K-ATPase). The capacity of the g_H to change pH_i is phenomenal. The fully activated g_H can change pH_i at a rate of ~6 U/min, nearly two orders of magnitude faster than other pH_i-regulating transporters. Clearly, activation of only a fraction of the g_H would suffice to rapidly dissipate any sudden acid load that a cell might experience.

Future Directions

The variety of cells known to have voltage-activated H^+ channels is increasingly rapidly. The behavior of H^+ currents is remarkably similar in the disparate tissues and species where they have been described. The sensitivity to pH_o and pH_i shared by all voltage-activated H^+ channels described thus far make it clear that activation of this conductance will result in extrusion of cytoplasmic acid. The major question in determining the function of this conductance is to define precisely the conditions under which it is activated. In general, the g_H is likely quiescent under normal conditions and may be activated during metabolic or environmental stress. A promising line of study in this regard is the activation of the g_H during the respiratory burst in phagocytic cells. The molecular basis for the H^+ conductance is at present completely unknown. There are a number of intriguing parallels between H^+ channels and several other membrane proteins, but at present it would be premature to draw any conclusions. It seems clear that H^+ current is not carried through other types of ion channels, is not coupled to the movement of any other ion, and is not likely carried through another membrane transporter, but instead is mediated by a distinct molecular entity.

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