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FOUR VARIETIES OF VOLTAGE-GATED PROTON CHANNELS

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1. ABSTRACT

The properties of voltage-gated proton channels are reviewed, with an emphasis on comparing different cells. Characteristic properties of these unique ion channels are shared by all members of this family. By definition, these channels are voltage-gated, opening upon depolarization of the membrane. Gating is tightly regulated by pH_o and pH_i resulting in only outward current. H^+ channels are inhibited by Cd^{2+} and Zn^{2+}. Proton channels are extremely selective - no other ions are detectably permeant. H^+ currents increase at low pH, but only about two-fold/Unit decrease in pH_i. Single channel currents have not been measured, and are likely very small (a few fA). Kinetic properties vary dramatically, suggesting categorization of known H^+ channels into four isoforms. Type ***n*** channels in snail neurons have extremely rapid gating kinetics, opening and closing in a few milliseconds. Type ***o*** channels in newt oocytes and type ***e*** in epithelial cells gate more slowly. Type ***e*** channels close with a double exponential time course. Type ***p*** channels, found mainly in phagocytes, have the slowest gating kinetics, under certain conditions requiring many seconds to open. This tentative classification will evolve, as more information becomes available.

2. INTRODUCTION

Voltage-gated proton channels were first described relatively recently in snail neurons (1,2). Shortly afterwards they were found in axolotl (newt) oocytes (3). Almost a decade after their discovery, voltage-gated H^+ channels were described in mammalian cells (rat alveolar epithelium, 4). Since then, they have been reported in a steadily increasing variety of cells, including human cells. The exquisite sensitivity of the gating mechanism to both pH_o and pH_i results in opening of these channels only when the membrane potential is positive to the Nernst potential for protons, E_H. As a direct consequence of this regulated gating, these channels carry only outward currents. Thus, it is generally accepted that a major function of these channels is acid extrusion from cells, especially under conditions of high metabolic activity (reviewed by 5,6). Strong evidence suggests that H^+ channels open during the 'respiratory burst' in phagocytes, where they help to dissipate the accumulation of positive charge (protons) in the cell due to the activity of the electrogenic NADPH oxidase (7-9; reviewed by 10).

3. FEATURES SHARED BY ALL VOLTAGE-GATED PROTON CHANNELS

3.1. Voltage-dependent gating

By definition, voltage-gated channels have an open probability that depends strongly on membrane potential. Depolarization increases the open probability of H^+ channels by at least 3-4 orders of magnitude, and probably more (11). The open probability at voltages negative to E_H is close to zero. Even if the inward 'leak'

current were considered to be purely H^+ selective (which itself is not supported by the data), the calculated membrane permeability to H^+, $P_H < 10^{-4}$ cm/s (11), which is already lower than some estimates of the P_H of simple bilayer membranes (12). When one asks whether H^+ channels may be related to other membrane proteins that have the ability to conduct proton current (*e.g.*, the F_0 component of H^+-ATPases, bacteriorhodopsin, viral proteins, *etc.*), a major difference is that these other transporters do not exhibit voltage-dependent gating. Transport of H^+ may be voltage-dependent because of Ohm's Law, and thus many proton-transporting entities will exhibit voltage-dependent H^+ fluxes (13,14), but to our knowledge, true voltage-dependent gating has not been described for any other H^+ selective transporter.

3.2. The voltage dependence of gating is modulated strongly by pH_o and pH_i

A quintessential feature of voltage-activated H^+ currents is their dependence on pH_o and pH_i. Increasing pH_o or lowering pH_i shifts the voltage dependence of activation of the H^+ conductance, g_H to more negative potentials. This pH dependence of gating has been demonstrated in every cell in which H^+ currents have been described: snail neurons (*Lymnaea*, 2; *Helix*, 15), *Ambystoma* oocytes (3), frog oocytes (16), frog proximal tubule (17), rat alveolar epithelium (4), mouse macrophages (18); mouse microglia (19), CHO cells (20), rabbit osteoclasts (21), murine mast cells (22), human neutrophils (23), human THP-1 monocytes (24), HL-60 cells (25), human eosinophils (26), and a variety of cell lines (5). In a systematic study spanning a large range of pH_o and pH_i (including the physiological range), Cherny *et al.* (27) found that internal and external H^+ were of equivalent efficacy over a large concentration range encompassing physiological values. Increasing pH_o or decreasing pH_i by one Unit shifts the voltage-activation curve by 40 mV toward more negative values. In other words, the position of the voltage-activation curve can be predicted from the pH gradient (pH_o - pH_i) rather than by pH_o or pH_i independently. This pH sensitivity results in the g_H activating 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.

This strong outward rectification of the steady-state H^+ current-voltage relationship is due to the 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 that activated H^+ channels. Most investigators have been unable to demonstrate steady-state inward H^+ current, but extrapolation of the behavior described in alveolar epithelial cells (11,27), would predict inward currents when the pH gradient is large and negative. One report describes a small inward H^+ current under this condition, namely pH_o 6.5 and pH_i 8.0, or a gradient of -1.5 Units (17).

3.3. Block by polyvalent cations

Voltage-gated H^+ channels are among the few remaining ion channels that have not yet been cloned and sequenced. One reason for this is that there are no highly selective and potent inhibitors of H^+ channels. Other channels have been purified by using high-affinity binding by channel blockers, generally peptides. The only known inhibitors of H^+ channels, other than organic inhibitors of weak potency whose effects may have other explanations, are polyvalent cations. The most frequently used inhibitors are Cd^{2+} and Zn^{2+} applied to the bathing solution. Voltage-gated H^+ currents in every cell in which they have been described are inhibited by these cations. Combining results from all studies in which the potency of two or more polyvalent cations were compared (1,4,19,28-32), results in the following "consensus" potency sequence:

$$Cu \approx Zn > Ni > Cd > Co > Mn > Ba, Ca, Mg \approx 0$$

In addition to these divalent cations, the trivalent La also inhibits potently. There is a minor discrepancy for the weaker divalents: Bernheim *et al.* (31) reported that Ca and Ba inhibit more than Mg, Eder *et al.* (19) found that Ba but not Ca inhibits, and Byerly & Suen (29) reported no effect of Ca, Ba, or Mg. We see no difference between Ca and Mg (27). Block by polyvalent cations has not been characterized thoroughly, and these apparent disagreements may reflect different experimental conditions or genuine differences among varieties of H^+ channels.

3.4. Extremely high selectivity

Selectivity is determined by measuring the reversal potential, V_{rev}, in solutions of different ionic composition. V_{rev} can be measured using conventional "tail current" measurements. Measured V_{rev} values are then compared with the predictions of the Nernst equation, which for the case of H^+ is:

$$E_H = \frac{RT}{F} \ln \frac{[H^+]_o}{[H^+]_i}$$

where R is the gas constant, T is the absolute temperature, and F is Faraday's constant, and $[H^+]_o$ and $[H^+]_i$ are the extracellular and intracellular H^+ concentration, respectively. At room temperature, 20°C, E_H changes 58 mV/Unit change in pH_o (if pH_i is constant, as is usually the case for whole-cell measurements). In most studies of H^+ currents, the measured V_{rev} changes less than this: 23-35 mV/Unit (2[pH_i 5.9];15,18), or ~40-45 mV/Unit (4,17,19,21,25,31), but is sometimes in the range 49-58 mV/Unit (2[pH_i 7.3],3,11[D_2O],20,24,26,27,32). The latter values are close to a Nernstian slope. The deviations in each case are almost certainly the result of imperfect control over the pH near the membrane rather than evidence that other ions are permeant through H^+ channels. When the intracellular buffer concentration is increased from 5-10 mM to 100-120 mM V_{rev} agrees much better with E_H (4,22) and bulk pH_i measured by fluorescent pH-sensitive dyes approaches its nominal value more closely (25). Furthermore, when either cations (K^+, Na^+, Li^+, TEA^+, TMA^+, NMG^+, Cs^+, Ca^{2+}) or anions (Cl^-, aspartate$^-$, glutamate$^-$, methanesulfonate$^-$, isethionate$^-$) are substituted, there is no detectable change in V_{rev} (3,15,18,19,22,23,25,26,31,33,34). If one assumes that all of the deviation of V_{rev} from E_H is due to finite permeability of another cation, then the relative permeability of the channel to H^+ is still very high, $>10^6$ higher for H^+

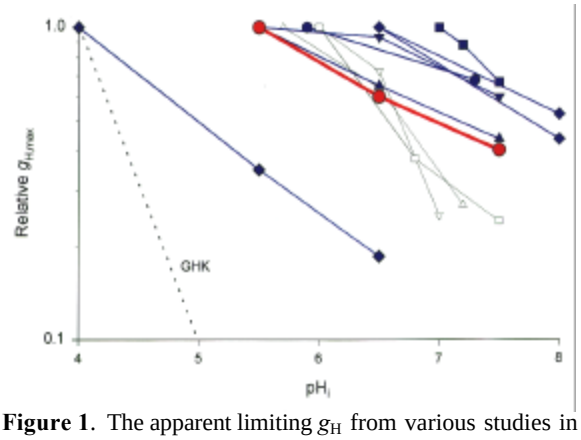

Figure 1. The apparent limiting g_H from various studies in which pH_i was varied (usually at constant pH_o) is plotted. Data are normalized to the largest value reported in each study, and data from each study are connected by lines. Most studies used whole-cell voltage-clamp and calculated the chord conductance, g_H, from the current measured at the end of a rectangular voltage pulse. In many cases, these pulses were not long enough for steady-state current to be achieved, and sometimes the inflection in the current (between concave upwards and convex) had not clearly occurred by the end of the pulse. In many cases, the voltage range explored (especially at higher pH_i) was not extended to positive enough voltages to activate the g_H fully, and in some cases the inflection in the g_H–V relationship was not reached. The data plotted as open symbols grossly underestimate $g_{H,max}$ at high pH_i on both counts (insufficient depolarization and pulse duration). Solid symbols are more reliable, but in most cases will tend to underestimate $g_{H,max}$ at high pH_i somewhat. The most reliable study used inside-out patches so the measurements were done at different pH_i in the same membrane (larger ●, darker line). The dotted line shows the slope if $g_{H,max}$ were directly proportional to the H^+ concentration (10-fold decrease/Unit increase in pH_i). Symbols: (□) 18; (Δ) 32; (∇) 26; (●) 2; (■) 15; (▲) 25; (◆, right-most data sets) 17; (▼) 27; (◆, left-most data set) 24; (larger ●) 35

(11,18,24,25,26,31,33). Because most sources of error tend to reduce P_H/P_{cation}, the true value is likely higher. In fact, there is no evidence that anything other than H^+ (or D^+) can permeate this channel.

3.5. H^+ current amplitude increases at lower pH

It is formally difficult to distinguish whether the current is carried by H^+ or by other "proton-equivalents" such as OH^- (moving in the opposite direction). The Nernst potentials for H^+ and OH^- are the same; therefore, V_{rev} measurements do not distinguish between them. Several types of evidence support the conclusion that it is indeed H^+ that carries the current. These include deuterium isotope effects (11), generally any evidence that suggests that the H^+ channel is not a water-filled pore but conducts protons across a hydrogen-bonded-chain (5,11,27,35,36), and the fact that lowering pH_i (hence, increasing $[H^+]$ and decreasing $[OH^-]$) increases the conductance. This latter point is examined in more detail in figure 1. The apparent limiting g_H from studies in which pH_i was varied (usually at constant pH_o) is plotted.

If H^+ currents were determined by diffusion of H^+ to the channel, or if permeation were governed by the Goldman-Hodgkin-Katz current equation (37,38), g_H would increase in direct proportion to the proton concentration $[H^+]$, *i.e.*, 10-fold/Unit decrease in pH_i as indicated by the dotted line in figure 1. The observed slope is less than this in every study. In the few studies that approach this slope (*indicated with open symbols and lighter lines*), the pulses were too short to approach steady-state current or the voltage range did not extend positive enough to approach a limiting value, or both. (One reason to use short pulses is that in cells with large H^+ currents there are severe problems with depletion of H^+ and protonated buffer from the cell, and long pulses exacerbate this problem). The more reliable data (*filled symbols*) uniformly indicate approximately a two-fold increase in $g_{H,max}$ per Unit decrease in pH_i. The most direct way to measure this property is to employ the excised, inside-out patch configuration, in which the bath solution faces the intracellular surface of the membrane, and pH_i can be changed repeatedly. In the single existing systematic study using this approach (*large circles*), $g_{H,max}$ increased only 1.7-fold/Unit decrease in pH_i over the range pH_i 5.5-7.5 (35). Less complete data in another study using patches suggests that this relationship holds for pH_i as low as 4.0 (24).

3.6. Extremely small single-channel conductance

Single voltage-gated H^+ channel currents have not yet been detected in any preparation, and it is improbable that they are large enough to be observed directly using present technology. The amplitude of unitary currents can be estimated by analyzing H^+ current fluctuations. In two of three such studies little or no additional noise was detected, and the unitary current was estimated to be <90 fS at pH_i 5.5 (31) and <47 fS at pH_i 5.9 (29). In human neutrophils, excess fluctuations were observed at voltages that activated H^+ currents and the single-channel conductance was estimated to be ~10 fS at pH_i 6.0 (23). The signal-to-noise ratio was poor in all of these studies, so the estimates must be considered approximate. This conductance corresponds with unitary H^+ currents of only a few fA, about three orders of magnitude lower than most ion channels, and in the range of currents that might be produced by ion carriers.

3.7. Are H^+ channels really ion channels or something else?

We use the term "channel" because H^+ is conducted passively down its electrochemical gradient, H^+ permeation is not coupled with the movement of any other ion, ATP is not required, and H^+ currents display time- and voltage-dependent gating that closely resembles that of ion channels. The mechanism of permeation probably differs significantly from that of other ion channels, as has been discussed at length elsewhere (5,11,27,35,36). Once the molecular structure of the channel is known, this question may become answerable.

Table 1. Summary of differences among varieties of H^+ channels

	PROTON CHANNEL TYPE			
	n (neuron)	**o (oocyte)**	**e (epithelial)**	**p (phagocyte)**
Tau_{act}	fast	medium	slow	slower
Sigmoid activation	?	no	pronounced	yes
Tau_{tail} components	1	1	2	1
Tau_{tail}	fast	medium	medium	medium/slow
$[Ca^{2+}]_i$ enhances	no	-	slight?	yes/no/maybe
Cells expressing	snail neuron	axolotl	rat alveolar epithelium, frog proximal tubule	macrophage, neutrophil, CHO, THP-1, microglia, myotube, HL-60

This table summarizes what appear to be significant differences between the behavior of H^+ channels in different cells, under comparable conditions. Not all parameters have been studied in every type of cell listed. The species and cells in each category and sources of this information are: ***n***, snail neuron (2); ***o***, axolotl oocyte (3); ***e***, rat alveolar epithelium (4,11,27,41), frog proximal tubule (17); ***p***, mouse or rat macrophages (18,41), human neutrophils (5,23), human skeletal myotubes (31), mouse microglia (19), human eosinophils (26), human THP-1 cells (24), HL-60 cells (25,41), CHO cells (20). Few studies report actual time constants, so most of the estimates were done by eye by the author on data in published figures.

4. DIFFERENCES AMONG TYPES OF H^+ CHANNELS

There is growing evidence that H^+ channels are not identical in all preparations, but differ in several respects. These differences are summarized in table 1. The proposed nomenclature is likely to be ephemeral and is destined to being pre-empted by molecular terminology once this channel has been cloned, but for the present may help to organize existing information.

4.1. Activation kinetics

In general, H^+ currents become activated much more slowly in mammalian cells (4) than in snail neurons (2). In many cells, activation has a non-exponential time course (4,17,20,23,24,26,27,31) that cannot consistently be fit with a Hodgkin-Huxley gating parameter raised to any fixed exponent (27), complicating the extraction of a time constant. Therefore, measurements of time-to-half-peak current (2,4,18) or maximum rate-of-rise of H^+ currents (27,33) have been used. We have finally adopted the practice of fitting an exponential after a delay (11,20,24,35). Even though the fit is not perfect in many situations, it is very useful to have a well-defined and clearly understandable, if somewhat arbitrary, parameter that reflects the kinetics of gating. The wide range of conditions used in various studies precludes direct numerical comparisons of this time constant, tau_{act}. Thus, table 1 gives tau_{act} in vague terms, where 'fast' means a time constant of a few milliseconds in neurons (2), 'medium' means a few hundred milliseconds (3), and 'slow' means seconds. Because tau_{act} depends strongly on pH_o, pH_i, voltage, and temperature, this comparison should be considered approximate.

4.2. Sigmoidicity of activation time course

Although H^+ currents in newt oocytes activate with exponential kinetics (Barish & Baud, 1984), in most mammalian cells the activation time course is sigmoidal (4,20,23,24,26,27). An exception is human skeletal myotubes, in which activation follows a double exponential time course (31). H^+ currents in rat alveolar epithelium exhibit a Cole-Moore effect (39), that is, the degree of sigmoidicity is increased when pulses are applied from more negative membrane potentials (5). Sigmoid activation kinetics classically were used to describe the turn-on of voltage gated Na^+ and K^+ channels in squid axons, leading to the mathematical formalisms by Hodgkin and Huxley (40) that correctly predicted the participation of several subunits of these channels in gating. It is our impression that the sigmoidicity of activation is more pronounced in alveolar epithelial cells than in phagocytes. However, this property is not easy to quantify, and there is almost nothing in the literature to permit comparisons with other types of channels.

4.3. Tail current kinetics

The rate of channel closing or deactivation can be determined by observing the decay of tail currents. Unfortunately, the time constant, tau_{tail}, has not been reported systematically in most studies of H^+ currents, and thus the comparison of this parameter relies almost exclusively on 'eyeballing' data in published figures. It is clear that tail currents decay much more rapidly in snail neurons (2) than in any mammalian cell. It is also clear that tail currents are faster by about an order of magnitude in alveolar epithelial cells than in human neutrophils or CHO cells (11,23,24,27,41). Time constants, tau_{tail}, are a few milliseconds in type ***n*** channels in snail neurons (2), 10-100 msec in type ***o*** and ***e*** channels (3,17,27), and on the order of seconds in type ***p*** channels in human neutrophils (23), CHO cells (20), THP-1 cells (24), and skeletal myotubes (31). Some type ***p*** channels have tau_{tail} with 'medium' kinetics, HL-60 cells (25) and mouse macrophages (42).

4.4. Tail current components and their voltage- and pH-dependence

The tail current decays with two clear components at voltages positive to V_{rev} in alveolar epithelial cells (type ***e*** channels), the slower component being much more strongly dependent on pH_o than the faster component (27). In contrast, tail currents in phagocytes (24,42) and in CHO cells (20) decay monoexponentially at all voltages and tau_{tail} is independent of pH_o. Few studies have addressed the question whether tau_{tail} depends on pH_i

– a small dependence on pH_i may occur in alveolar epithelium (11,27) and in mouse macrophages (42).

4.5. $[Ca^{2+}]_i$ dependence of H^+ currents

There are several reports that increased $[Ca^{2+}]_i$ enhances the H^+ current in phagocytes (26,32,43). In contrast, there was no effect of raising $[Ca^{2+}]_i$ from 10^{-7} M to 10^{-5} M in snail neurons (2). $[Ca^{2+}]_i$ effects were reported in whole-cell studies, and much of the result depends on comparisons of different groups of cells, and the use of weakly buffered pipette solutions. In some studies at least part of the Ca^{2+}-activated conductance appeared to include a Cl^- component (32,43). Thus, the results are not as convincing as one would like. In the most carefully executed study, increasing $[Ca^{2+}]_i$ from 0.1 µM to 1 µM increased the H^+ current amplitude by < 2-fold (26). In a few attempts to explore this question using inside-out patches of membrane, we have been unable to distinguish conclusively between a small effect and no effect. Indirect evidence in human neutrophils suggests that increased $[Ca^{2+}]_i$ has no effect on either g_H or its activation by fMLP (44). It is abundantly clear that H^+ channels are not Ca^{2+}-activated in the usual sense.

5. PERSPECTIVE

There are several distinct differences between the H^+ channels in different cells. Other apparent differences require further clarification. It seems likely that at least four isoforms of the H^+ channel protein will be isolated.

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