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pH regulation and beyond: unanticipated functions for the voltage-gated proton channel, HVCN1

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Abstract

Electrophysiological studies have implicated voltage-gated proton channels in several specific cellular contexts. In neutrophils, they mediate charge compensation associated with the oxidative burst of phagocytosis. Molecular characterization of the hydrogen voltage-gated channel 1 (HVCN1) has enabled identification of unanticipated and diverse functions: HVCN1 not only modulates signaling from the B-cell receptor following B-cell activation and histamine release from basophils, but also mediates pH-mediated activation of spermatozoa, as well as acid secretion by tracheal epithelium. The importance of HVCN1 in pH regulation during phagocytosis was established by surprising evidence indicating its first-responder role. In this review, we examine recent findings from a functional perspective, and discuss the potential of HVCN1 as a therapeutic target for autoimmune and other diseases.

Introduction

The voltage-gated proton channel (HVCN1, also called H_v1 and VSOP) was a mysterious protein for a number of years. Proton currents were identified in snail neurons and amphibian oocytes in the 1980s [1–3], in mammalian cells in 1991 [4], and in human cells in 1993 [5–7]. However, the gene encoding the channel was not discovered until 2006 when two groups cloned human and murine HVCN1 genes [8,9], prompting a wave of new studies on voltage-gated proton channel function and regulation. Historically, proton currents have been associated with the NADPH oxidase in phagocytic cells, where they mediate rebalancing of charges across the plasma membrane to allow optimal NADPH oxidase-mediated reactive oxygen species (ROS) production [10–13]. Availability of an HVCN1-deficient mouse line has facilitated further functional characterization of HVCN1, opening new avenues for the investigation of its roles in other cell types. We now know that the proton channel is expressed in such diverse cell types as neutrophils, basophils, B cells,

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spermatozoa and airway epithelial cells and that it performs specific functions in each of them. This review will highlight the significance of such functions and the emerging potential to exploit HVCN1 as a therapeutic target in applications such as allergy, autoimmune diseases and contraception.

Key electrophysiological properties of the proton channel

Consideration of the electrophysiological properties of voltage-gated proton channels leads to the conclusion that their fundamental function is acid extrusion from cells [14]. Proton channels are perfectly selective for protons. Furthermore, they are ion channels, not carriers or exchangers, and acid extrusion occurs independently of other ionic concentrations [1,2,14]. Voltage-gated proton channels open upon membrane depolarization, with a sigmoid time course. However, their open probability depends strongly on pH on both sides of the membrane. The threshold voltage at which proton channels begin to open was determined empirically to be $40(\text{pH}_o - \text{pH}_i) + 20$ mV [15]. In practical terms, this means that at symmetrical pH, the channel opens 20 mV positive to the Nernst potential for protons, E_H , that increasing pH_o or decreasing pH_i by one unit shifts the entire g_H - V (proton conductance-voltage) relationship by -40 mV, and that at all physiologically achievable pH, the channel opens only when the electrochemical proton gradient is outwards. Thus, proton channels are designed to open only when this will result in acid extrusion from cells.

Proton channels have been found in a multitude of cell types within two dozen species, based on both direct voltage-clamp evidence and gene homology [16]. In electrophysiological studies, widely used to provide unequivocal evidence of expression, proton channel function is characterized in the plasma membrane. However, indirect evidence supports the presence of proton channels in phagosomes [17] and in the Golgi complex [18]. When overexpressed in HeLa cells, HVCN1 protein appears mainly in intracellular compartments [19]; whether this reflects a catabolic pathway or a function in organelles is unclear.

The current that flows through a single proton channel is quite small, compared to other ion channels. For a moderate driving force, a Na^+ or K^+ channel might conduct a few picoamperes (10^{-12} A) of current ($\sim 10^7$ ions/s), but a proton channel conducts only a few femtoamperes (10^{-15} A) of current ($\sim 10^4$ H⁺/s) [20]. The smaller conductance reflects the much lower permeant ion concentration; the K^+ concentration in cells is 10^6 times larger than the H^+ concentration. Nevertheless, macroscopic (collective) proton currents in many cells are as large or larger than K^+ currents [21], because nature is clever enough to express large numbers of H^+ channels. Having a tiny conductance is useful for proton channels in small organelles like phagosomes, because this enables a finely controlled response.

Discovery of the gene encoding the proton channel was hampered by a lack of selective inhibitors. The most potent inhibitor is Zn^{2+} , which although rather promiscuous, inhibits H^+ currents more effectively than Ca^{2+} currents, for example [22]. Unlike traditional ion channel blockers, Zn^{2+} does not occlude the channel, but instead binds to the external surface of the molecule where it slows channel opening and shifts the voltage dependence positively [23]. The effects of Zn^{2+} are strongly attenuated at low pH_o , reflecting competition between protons and Zn^{2+} for binding site(s). Modeling this competition suggested that Zn^{2+} binding is coordinated between 2 to 3 Histidine (His) residues [23]. Subsequently, mutation of the human proton channel confirmed that His¹⁴⁰ and His¹⁹³ (Figure 1) both contribute to Zn^{2+} effects [8]. Zn^{2+} appears to bind at the interface between monomers in the HVCN1 dimer (see below), where it prevents channel opening [24,25].

The HVCN1 protein and structure-function relationships

In 2006, human HVCN1 was cloned [5], as were homologous genes in mice and *Ciona intestinalis* [9]. The human HVCN1 (Figure 1) is expressed in two isoforms, the full-length protein (273 amino acids), and a short form, which lacks the first 20 amino acids, thus far documented only in B cells [26]. The latter protein isoform derives from translation from an alternative initiation site. One naturally occurring mutation has been identified, M91T, which reduces the probability of channel opening [27].

The protein encoded by the HVCN1 gene has two remarkable features. First, no explicit aqueous pore that might provide a conduction pathway similar to other ion channels is evident [8,9]. This feature was not altogether unexpected, because several properties of proton channels suggested the lack of a conventional aqueous pathway. That the deuterium conductance is only 50% that of H⁺ [28], conduction is strongly temperature-sensitive [29,30], and the channel is perfectly selective [31,32] suggested that protons permeate proton channels by a hydrogen-bonded chain mechanism [33], which does not require a continuous aqueous pathway. A recent study concludes that protons hop across frozen or non-exchangeable waters without displacing them [34]. Larger ions, in contrast, permeate ordinary ion channels by moving in single file along with water molecules. The second remarkable feature of HVCN1 is its astounding similarity to the voltage-sensing domain (VSD) of voltage-gated K⁺, Na⁺, and Ca²⁺ channels [8,9]. Most voltage-gated ion channels are tetramers of subunits that span the membrane 6 times (Figure 2). The first four domains (S1–S4) comprise the voltage sensing apparatus (VSD), and the remaining two domains (S5–S6) from each of the four monomers collectively form a single aqueous pathway across the membrane. Although using 24 membrane-spanning domains to form a single pore might seem needlessly baroque, the result is a four-fold amplification of the voltage sensitivity of a single VSD. For ion channels that mediate nerve impulses or trigger contraction of muscle fibers, extreme sensitivity to small changes in membrane potential is essential.

The discovery of the HVCN1 gene, and its homology with the VSDs of other, more widely studied ion channels, opened floodgates of interest in this molecule, with the result that new discoveries are being made rapidly. Diverse evidence indicates that the proton channel exists as a dimer, with a separate conduction pathway in each protomer [35–37]. Although most voltage-gated ion channels are tetramers with a single conduction pathway (Figure 2), the CIC (Cl⁻ channel) family of Cl⁻ channels and Cl⁻/H⁺ antiporters also are dimers with permeation pathways in each subunit [38–40]. Truncation of the HVCN1 carboxy terminus results in monomeric channels that retain most functions of the dimer [24,36,37]. The two protomers do not function independently in the dimer, however, but interact during gating [24,25,41,42]. One consequence of this cooperativity is that the dimer has twice the voltage sensitivity of the monomer [41], despite channel opening being slower [24,25,37]. Teleologically, strong voltage sensitivity may be more important to the cell than rapid opening kinetics [24]. For example, in phagocytes, proton channels counterbalance the effects of NADPH oxidase, which remains active for minutes (Box 1). Strong voltage sensitivity activates proton channels before excessive depolarization can occur, precluding self-inhibition of this electrogenic enzyme [10], which both produces and is inhibited by membrane depolarization [13].

Box 1

NADPH oxidase and reactive oxygen species (ROS)

A schematic representation of NADPH oxidase and HVCN1 function in phagocytes (figure I). The NADPH oxidase enzymatic complex assembles on the plasma (or phagosome) membrane of granulocytes, macrophages and B cells upon stimulation. The

membrane bound components, gp91^{phox} (*phox* refers to phagocyte oxidase) and p22^{phox}, bind the cytosolic components (p47^{phox}, p67^{phox}, p40^{phox} and Rac) that are recruited to the membrane, which in phagocytic cells invaginates to produce a phagosome. NADPH oxidase produces superoxide anion, O₂ •⁻, a precursor to other reactive oxygen species. Most O₂ •⁻ dismutates to hydrogen peroxide, H₂O₂, which is converted to HOCl by myeloperoxidase, MPO. Gp91^{phox}, also called Nox2, contains an electron transport chain comprising NADPH, FAD and two heme groups that pass electrons sequentially to O₂ at an external binding site, to produce O₂ •⁻. The enzyme is electrogenic, because electrons extracted from cytoplasmic NADPH are translocated to extracellular or intraphagosomal O₂ that is thereby reduced to O₂ •⁻ [10]. Without charge compensation, the membrane would depolarize to extreme positive voltages at which NADPH oxidase ceases to function [13]. Proton currents provide most of this charge compensation [49], and alleviate the cytosolic acidification that results from NADPH utilization and reconstitution by the hexose monophosphate shunt (HMS), which also inhibits NADPH oxidase [51]. Other transporters that may contribute to pH regulation include CIC-3 (a Cl⁻/H⁺ antiporter), H-ATPase, and the Na⁺/H⁺ antiporter. ROS enable clearance of engulfed bacteria, as shown by impaired immune responses in chronic granulomatous disease (CGD) patients [81]. CGD occurs when mutations to components of NADPH oxidase prevent its function. The disease affects phagocytes and, to a lesser extent, B cells [82]. The primary goal of the high levels of ROS produced by phagocytes is clearance of bacteria. In B cells, where ROS production is about 10 times smaller than in phagocytes, ROS have been implicated in BCR signaling [66]. ROS temporarily inhibit protein-tyrosine phosphatases to allow initiation and amplification of BCR downstream signaling pathways. ROS oxidize the cysteine residue in tyrosine phosphatases catalytic domain to a sulfenic acid (-SOH). The reaction is reversible and the active thiolate anion (-S⁻) can be quickly reformed by reduction, mediated by the many reducing agents present in the cytosol. In recent years the number of signaling pathways and phosphatases shown to be regulated by oxidation has increased considerably [83,84]. Figure reprinted from [21] with permission of the American Physiological Society.

Proton channels in phagocytes regulate NADPH oxidase activity

The best-established function of proton channels is in leukocytes that phagocytose and kill bacteria (Box 1). Proton channel properties change dramatically in activated leukocytes [43,44]. In the enhanced gating mode, they open faster and at less positive voltages and close more slowly, substantially increasing the proton current. Enhanced gating results primarily from phosphorylation of HVCN1 by PKC (protein kinase C) on Thr²⁹ of the intracellular amino terminus [45], and is prevented or reversed by PKC inhibitors [46,47]. Part of this response may involve arachidonic acid (AA) [6,48], produced locally by activated phagocytes, although it is unclear whether the effect is direct or via AA-mediated stimulation of PKC [47]. Without enhanced gating, proton channels would still open during NADPH oxidase activity, triggered by membrane depolarization, decreased intracellular pH (pH_i), and increased pH in the extracellular milieu or phagosome. However, in the enhanced gating mode, more proton channels open with smaller depolarization. This, in turn, improves the efficiency of NADPH oxidase by 15–20% by minimizing depolarization-induced self-inhibition [49].

At the same time that proton flux compensates charge, it also prevents large changes in both cytoplasmic and phagosomal pH. Regulation of pH_i is essential, because NADPH oxidase activity is optimal at pH_i 7.5 and decreases drastically at more acidic and more basic pH [50]. The importance of proton channels in minimizing pH_i changes during phagocytosis was demonstrated recently in a study using the pH-sensitive dye, SNARF

(seminaphthorhodafleur) [51]. When a neutrophil first engulfed an opsonized zymosan particle, there was a precipitous drop in pH_i , followed by an almost equally rapid recovery. This acidification reflected H^+ generation as a consequence of NADPH oxidase activity, since it was abolished by the oxidase inhibitor, diphenylene iodonium (DPI). Inhibiting either Na^+/H^+ antiport or proton channel activity prevented pH_i recovery. Acidification was more rapid in the presence of Zn^{2+} (but not inhibitors of other transporters) and in the HVCN1-deficient mouse [51], indicating that the proton channel is the first transporter to respond during phagocytosis. When proton channels were inhibited or absent, pH_i dropped to levels known to inhibit NADPH oxidase [50]. This raises the philosophical question of whether charge compensation or pH_i regulation is more important to the cell. Fortunately, proton channels perform both functions simultaneously and inseparably.

Using proton channels to compensate charge in phagocytes has two additional benefits [49]. Proton efflux minimizes osmotic effects that would occur if other ions performed this function. If all charge were compensated by K^+ , the phagosome would swell to ~20 times its original volume [49,52]. Finally, H^+ is required inside the phagosome as a substrate to form H_2O_2 and HOCl , the main products of NADPH oxidase, both of which are generated at a high rate [53].

The downstream effects of HVCN1 deficiency in neutrophils were investigated both in terms of ROS production and neutrophil function [17,51,54,55]. All studies support the concept that HVCN1 is required for optimal ROS production. Furthermore, NADPH oxidase-dependent bacterial killing was significantly reduced in HVCN1-deficient neutrophils *in vitro* [54]. Bacterial clearance in HVCN1-deficient mice *in vivo* after peritoneal injection of *Staphylococcus aureus* was slightly but not significantly impaired. Clearance of *Pseudomonas aeruginosa* and *Burkholderia cepacia* was not impaired. The residual ROS production in HVCN1-deficient cells may be sufficient to ensure bacterial clearance to a point where the infection can be resolved. Patients with variant forms of chronic granulomatous disease (CGD, Box 1) whose NADPH oxidase activity is reduced to 3–30% normal may have milder symptoms than CGD patients with complete absence of NADPH oxidase activity [56–60]. However, defects in HVCN1 activity might become more relevant in different settings of inflammation and/or infection. Further work may clarify this point. HVCN1-deficient neutrophils also had diminished chemotactic responses to the bacterial peptide *N*-formyl-Met-Ile-Val-Ile-Leu (fMIVIL) *in vitro* [55]. Ca^{2+} entry was reduced, which in turn impaired migration and actin depolymerization. It would be interesting to see whether *in vivo* defects in HVCN1-deficient mice are more apparent in neutrophil responses in which migration is critical.

HVCN1 in basophils facilitates histamine release

Another cell with abundant HVCN1 expression is the basophil [61], not surprisingly given its lineage relationship with eosinophils and neutrophils. On the other hand, basophils lack NADPH oxidase, which seems to be the *raison d'être* for proton channels in phagocytes. Agents that stimulate histamine release by basophils, including anti-IgE, the phorbol ester PMA (phorbol 12-myristate 13-acetate), and the chemotactic peptide fMLF (formyl-Met-Leu-Phe), all enhanced the gating of proton channels in human basophils [62]. Inhibiting proton channels with Zn^{2+} abolished histamine release, suggesting that proton channels in basophils might compensate charge or regulate pH. Measurement of pH_i during anti-IgE stimulation revealed acidification, which was exacerbated by Zn^{2+} . Thus, stabilization of pH_i is a likely function of HVCN1 in basophils. Histamine release is a clear example of a cellular process that is unrelated to NADPH oxidase activity, but is mediated by proton channels.

HVCN1 sustains optimal B cell receptor (BCR) signaling

Proton currents were discovered in human B cells in 2002 [63] and the HVCN1 protein was identified in a proteomic screen of plasma membrane proteins expressed by mantle cell lymphoma cells in the peripheral blood [64]. Protein expression levels in resting naïve and memory B cells were comparable to granulocytes; however, HVCN1 was down-regulated in proliferating B cells, such as cells in the germinal centre or primary cells stimulated *in vitro* via CD40 (a tumor necrosis factor receptor family member) in the presence of IL-4 (interleukin-4) [26]. Down-regulation may be mediated by BCL6 (B-cell lymphoma 6), since HVCN1 appears to be a direct target for BCL6-mediated transcriptional repression [65]. This pattern of expression suggests HVCN1 involvement in the initial phase of B-cell activation, which indeed was found impaired during *in vivo* and *in vitro* stimulation of HVCN1-deficient B cells. The impairment was mediated by diminished ROS production following BCR stimulation. ROS had been postulated to ensure correct BCR signal propagation by inhibiting protein tyrosine phosphatases, such as SHP-1 (Figure 3 and Box 1). This has been somewhat controversial [66] due to the possibility that reducing conditions in the cytosol would scavenge any ROS as soon as they were formed. Recent evidence, however, suggests that there are mechanisms to temporarily block the reducing action of cytosolic scavenging proteins such as peroxiredoxin [67]. The HVCN1-deficient model therefore provides a new way to illustrate how attenuation of ROS production in B cells has a detrimental effect on BCR downstream signaling. Diminished ROS correlates with diminished oxidation of the protein tyrosine phosphatase SHP-1, which dephosphorylates and therefore inhibits a crucial kinase in B-cell activation, spleen tyrosine kinase (Syk) [68]. Syk controls many downstream pathways such as MAP kinase activation, Ca²⁺ mobilization (from ER-stores as well as entry from the extracellular milieu) and PI3 kinase activation [69,70]. Syk activation was indeed impaired in BCR-stimulated HVCN1-deficient cells, but not in the initial phases of activation, only at later time-points. The defect could be “rescued” by treating the cells with low doses of a SHP-1 inhibitor, sodium stibogluconate [71]. Surprisingly, not all Syk downstream pathways were affected equally as neither ERK activation nor Ca²⁺ mobilization was impaired. This suggests that these events are controlled by the very early activation of Syk and are not affected in the absence of sustained Syk activation. On the other hand, the protein kinase Akt, which is downstream of PI3K [72], was impaired in HVCN1-deficient cells and this resulted in decreased cell metabolism, as both mitochondrial respiration and glycolysis were diminished following BCR stimulation (Figure 3). The defects in HVCN1-deficient B cells were specific for BCR stimulation as downstream signaling from TLR4 (Toll-like receptor 4) and CD40 were unimpaired.

Consistent with the BCR-specific defect in signaling, HVCN1 was found to be associated with the BCR complex and to colocalize with the receptor upon stimulation. This raises the possibility that close proximity of H⁺ transport to the BCR might be important. Whether proximity is necessary to support NADPH oxidase activity or for other reasons is unknown and requires further investigation. Intriguingly, Ca²⁺ entry is impaired in the absence of HVCN1 in neutrophils [55] but not in B cells [26], which might reflect different signal regulation of Ca²⁺ mobilization in neutrophils and B cells or differences in membrane depolarization. Ca²⁺ entry is impaired by the large depolarization attained by HVCN1-deficient neutrophils, which attenuates entry of positive ions [55]. In contrast with neutrophils, B cells have substantial K⁺ conductances [73] that would abrogate any depolarization due to NADPH oxidase activity. Consequently, the driving force for Ca²⁺ entry should be preserved.

HVCN1 regulates human spermatozoa activation

Gene expression analysis (<http://biogps.gnf.org/#goto=genereport&id=84329> and [9]) revealed HVCN1 expression in human testis and recently a role for HVCN1 in human spermatozoa was proposed [74]. Spermatozoa reside in the testis in a quiescent state that is maintained by low cytosolic pH, below 6.5. The abundant proton channels are thought to be inhibited by a high concentration of Zn^{2+} present in seminal fluid. Once the sperm enter the female reproductive tract, Zn^{2+} might be buffered by albumin and other proteins, relieving the inhibition. The resulting efflux of acid through proton channels increases pH_i , triggering capacitation (maturation) that is necessary for egg fertilization.

Other species may lack this mechanism. Zn^{2+} enhances sperm motility in the Japanese eel [75]. Mouse spermatozoa lack proton currents, suggesting a different mechanism of alkalization [74] although the murine cells investigated were less mature than the human counterparts [76]. Notably, HVCN1-deficient mice do not exhibit obvious fertility defects.

HVCN1 regulates pH in airway mucosa

A recent study by Iovannisci *et al.* [27] demonstrated HVCN1 involvement in establishing the pH in airway epithelia. Low pH is associated with diseases such as asthma and cystic fibrosis while alkaline pH is associated with rhinitis [77,78], which emphasizes that pH regulation of airway surface liquid (ASL) needs to be finely tuned. The pH in ASL is controlled by acid secretion from airway epithelial cells. As in most cells, multiple mechanisms contribute to acid secretion. In human nasal mucosa, proton channels secrete over half the acid measured in ASL; this fraction changes in chronic rhinosinusitis [78]. Iovannisci *et al.* [27] found that HVCN1 opens when extracellular pH exceeds 7. They emphasize that in airway epithelia, proton channel gating is modulated by the difference in pH across the membrane rather than depolarization, because the epithelial membrane potential is stable.

Interestingly, this group identified a human subject with a missense mutation that produced a substitution of Met→Thr at position 91. Mutation M91T inhibited channel opening, with the mutant requiring +20 mV additional depolarization or ~0.5 unit more alkaline mucosal pH to open the same number of channels. The mutation is likely to be heterozygous, which implies two possible scenarios: 1) the mutant has dominant expression, in which case the observed effect is mediated by M91T/M91T dimers; or 2) dimers with the altered phenotype are composed of one mutant and one wild-type copy, suggesting that M91T/M91T dimers might have an even stronger phenotype. Surprisingly, Met⁹¹ is not well conserved among species. *Macaca mulatta*, *Canis familiaris* and *Bos taurus* have Thr at position 91 and mice have Arg. It is not clear if this produces different characteristics for HVCN1 in different species.

Therapeutic implications of targeting HVCN1

As descriptions of HVCN1 involvement in diverse cellular processes proliferate, the potential of HVCN1 as a therapeutic target expands. Beyond the archetypal role of HVCN1 in supporting bactericidal ROS production by phagocytes, recent studies describe a role for HVCN1 in regulating production of ROS intended for signaling. Significantly, in basophils, spermatozoa and airway epithelia, HVCN1 serves functions altogether unrelated to ROS production. Its regulation of histamine release by basophils and the threshold of activation of autoreactive B cells make HVCN1 a desirable therapeutic target in allergic reactions and autoimmune diseases characterized by hyperreactive B cells, such as rheumatoid arthritis and systemic lupus erythematosus. For B cells in particular, the potential for HVCN1 inhibitors to impair optimal B-cell activation without complete abrogation of B-cell

responses should provide a useful therapeutic window, avoiding the increased susceptibility to infections risked by strong immunosuppression. Some B-cell malignancies have been shown to rely on BCR signaling [79,80]; thus, inhibiting HVCN1 in HVCN1-expressing tumors could help reduce the survival signal that cells derive *via* engagement of their BCR. An HVCN1 inhibitor introduced into the female reproductive system might block capacitation (activation) of spermatozoa and thus act as a contraceptive. In asthma and cystic fibrosis, inhibiting HVCN1 should ameliorate mucosal acidification, preventing epithelial injury that may contribute to asthma pathogenesis. But can HVCN1 inhibition be achieved? Its structural similarities to other voltage-gated cation channels raise the possibility that some side-targeting might occur. And even if side-targeting could be avoided, lack of a crystal structure makes it difficult to predict which residues mediate proton transport and how the conformation could be altered to achieve proton transport inhibition. Identifying an effective inhibitor might be a difficult task, but surely the therapeutic possibilities will be abundant.

Concluding remarks

Interest in voltage-gated proton channels mushroomed after identification of the gene in 2006. The molecule has its own unique charm, with perfect selectivity, tiny conductance, strong modulation by pH and unusual dimeric architecture. Its homology to the VSD of other voltage-gated ion channels makes it a unique model system in which voltage-gating mechanisms may be studied. Many questions remain unanswered regarding the structure of the channel (Box 2); surely a great boost to HVCN1 research would come from unveiling the crystal structure. In addition to helping to unravel the molecular mechanisms underlying proton conductance, structural information would likely prove invaluable in the design of HVCN1 inhibitors. These inhibitors, in turn, would help further understanding of HVCN1 function in cells.

Box 2

Major questions for future research

- What is the crystal structure?
- What interactions take place between monomers during gating of the dimeric proton channel complex?
- Where is the permeation pathway? How is selectivity achieved? What is the difference between the open and closed states?
- Which cells express proton channels, and for what purposes?
- Are there other naturally-occurring mutations in the HVCN1 sequence that have consequences for its function?
- Can selective inhibitors HVCN1 be identified? Can inhibitors be targeted to specific tissues? Will these inhibitors ameliorate diseases that might benefit from HVCN1 inhibition?

So far HVCN1 has been shown to regulate neutrophil, basophil and B-cell activation, human sperm capacitation and pH in mucosal airway epithelial cells. As expression in more cells is discovered, new functions will undoubtedly be identified. Furthermore, additional mutations or polymorphisms that alter channel properties might emerge. Exploration of what are now only putative proton channels in other species will provide clues to understanding differences in HVCN1 properties in diverse species. Beyond its intrinsic interest to

evolutionary biologists, comparison of a wide range of proteins will illuminate our understanding of how these extraordinary molecules work.

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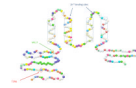


Figure 1. Sequence of human HVCN1 showing its arrangement in the membrane

Two sites on the intracellular amino terminus have been shown to influence channel opening: phosphorylation of Thr²⁹ strongly enhances opening in leukocytes [45], whereas the missense mutation M91T reduces it in airway epithelial cells [27]. Histidines constituting Zn²⁺ binding site(s) [8,24,25] are indicated, together with transmembrane domains (four rectangular boxes).

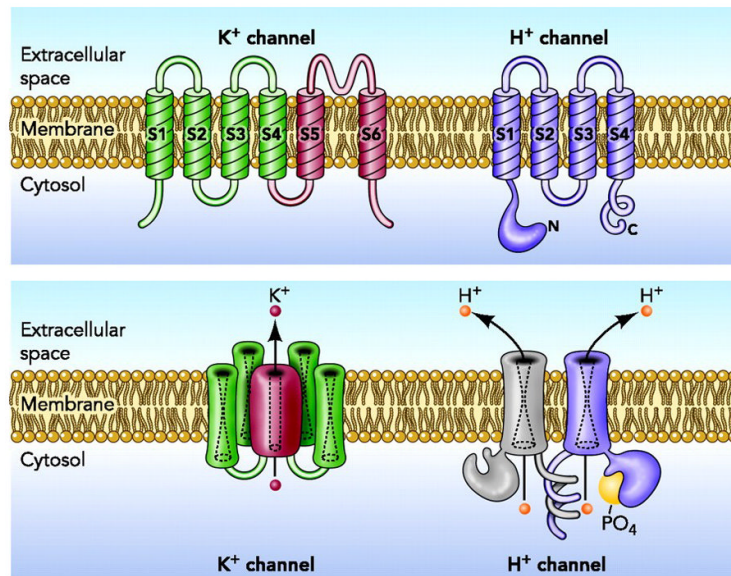


Figure 2. Architectural distinction between K^+ channels and proton channels

Voltage-gated K^+ channels are tetramers of the subunit shown in the top left panel. The four S4-S5 domains combine to create a single central pore in the assembled channel (lower panel). The voltage-gated H^+ channel (right, upper and lower panel) is a dimer, but each protomer contains its own conduction pathway and can function as a monomer. Proton channel activity is greatly enhanced by phosphorylation at Thr²⁹ (Figure 1). Figure modified from [21] with permission of the American Physiological Society.

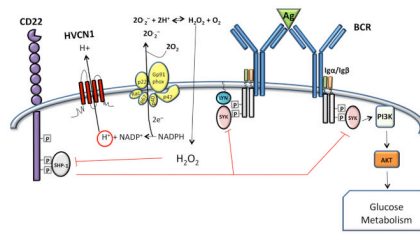


Figure 3. Schematic representation of HVCN1 in the context of B cell receptor (BCR) signaling Antigen (Ag) binding to the BCR results in phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the Ig α / β heterodimer by Lyn, a src-family tyrosine kinase, creating docking sites for Syk [85]. This serves to amplify BCR signaling by further recruitment and activation of Syk, which leads to PI3K activation, activation of Akt and increased glucose uptake and metabolism. Amplification of signaling is negatively regulated by CD22, which is also phosphorylated by Lyn, providing a docking site for protein tyrosine phosphatase (PTP) SHP-1 [86]. SHP-1 dephosphorylates Syk, counterbalancing ITAM-Syk mediated signal amplification. SHP-1 is inhibited by ROS, which oxidize a cysteine residue in the catalytic site of the enzyme. BCR stimulation results in ROS generated by the NADPH oxidase enzymatic complex, which transfers electrons across the plasma/endosome membrane to molecules of oxygen. The transfer of one electron results in the production of O_2^- that combines with protons to form H_2O_2 and O_2 , which freely diffuse through the membrane ($2O_2 + 2H^+ \rightleftharpoons H_2O_2 + O_2$). ROS generate a localized oxidizing environment causing inhibition of SHP-1, which results in amplification of BCR signal. HVCN1 sustains NADPH oxidase activity through charge compensation and intracellular pH regulation; therefore, in the absence of HVCN1, the oxidizing environment cannot be maintained and this results in SHP-1 remaining more active, diminishing BCR-signal strength. Figure adapted from [26].

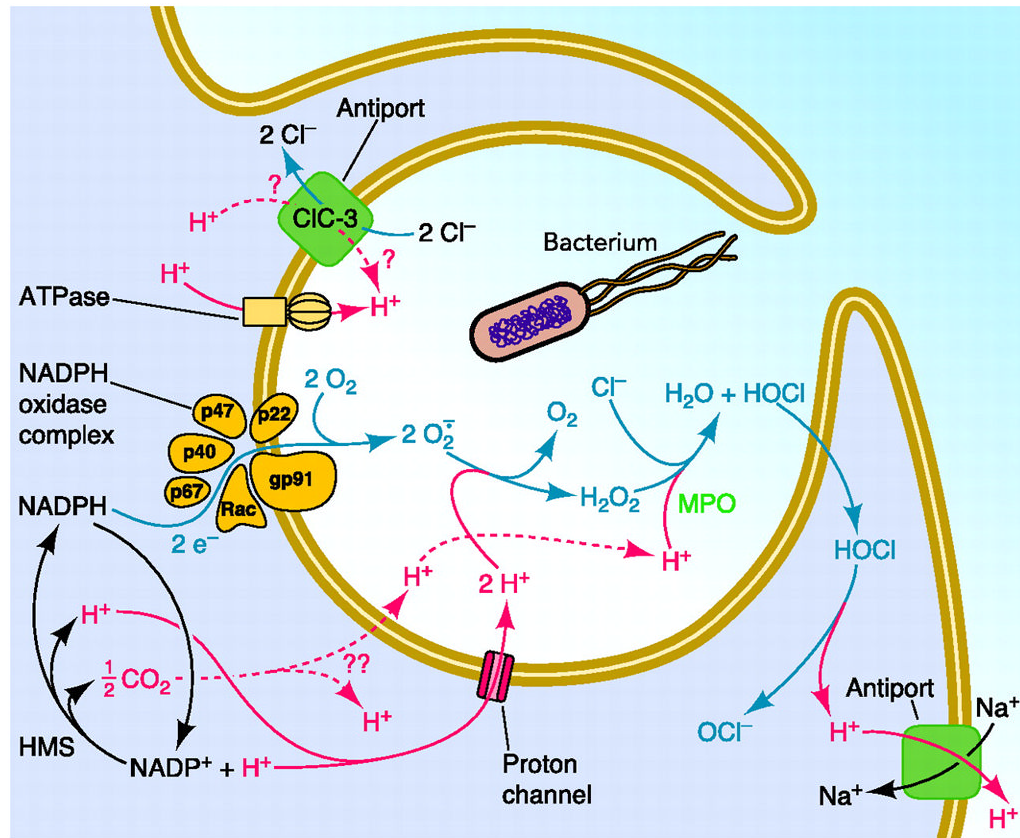


Figure I.
Schematic representation of NADPH oxidase and HVCN1 function in phagocytes.