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Voltage-gated proton channels exist in the plasma membrane of human oocytes

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STUDY QUESTION: Do human oocytes express voltage-gated proton channels?

SUMMARY ANSWER: Human oocytes exhibit voltage-gated proton currents.

WHAT IS KNOWN ALREADY: Voltage-gated proton currents have been reported in human sperm, where they contribute to capacitation and motility. No such studies of human oocytes exist.

STUDY DESIGN, SIZE, DURATION: Voltage-clamp studies were undertaken using entire oocytes and vesicles derived from oocytes and in excised patches of membrane from oocytes.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Frozen, thawed human metaphase II oocytes were obtained from material donated to the gamete repository at the Rush Center for Advanced Reproductive Care. Prior to patch clamping, oocytes were warmed and equilibrated. Formation of an electrically tight seal requires exposing bare oolemma. Sections of the zona pellucida (ZP) were removed using a laser, followed by repeated pipetting, to further separate the oocyte from the ZP. Patch-clamp studies were performed using the whole-cell configuration on oocytes or vesicles derived from oocytes, and using inside-out patches of membrane, under conditions optimized to detect voltage-gated proton currents.

MAIN RESULTS AND THE ROLE OF CHANCE: Proton currents are present at significant levels in human oocytes where they exhibit properties similar to those reported in other human cells, as well as those in heterologous expression systems transfected with the *HVCN1* gene that codes for the voltage-gated proton channel.

LARGE SCALE DATA: N/A

LIMITATIONS, REASONS FOR CAUTION: Human oocytes are large cells, which limits our ability to control the intracellular solution. Subtle effects of cryopreservation by vitrification and subsequent warming on properties of HVCN1, the *HVCN1* gene product, cannot be ruled out.

WIDER IMPLICATIONS OF THE FINDINGS: Possible functions for voltage-gated proton channels in human oocytes may now be contemplated.

STUDY FUNDING/COMPETING INTEREST(S): NIH R35GM126902 (TED), Bears Care (DM). No competing interests.

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Key words: HVCN1 / H_V1 / ion channels / metaphase II human oocytes / patch clamp / voltage clamp / assisted reproduction / IVF / gamete repository / vitrified oocytes

Introduction

Ion channels are membrane proteins that enable ions to cross cell membranes in a highly controlled manner, selectively, and at specific times. The voltage-gated proton channel is unique among ion channels in several respects. It is perfectly selective for protons (DeCoursey, 2003, Musset *et al.*, 2011). Like many other voltage-gated channels, it opens when the membrane potential is made more positive, but

in sharp contrast, the voltage at which proton channels open varies drastically when either external pH (pH_o) or internal pH (pH_i) is changed (Cherny *et al.*, 1995). This powerful regulation by pH results in the channel opening only under conditions that will produce proton extrusion from cells (Cherny *et al.*, 1995; DeCoursey, 2003). Proton channels have been identified in a wide range of species across the animal kingdom (DeCoursey, 2013) but also play critical roles in a number of human cells. They regulate the pH of fluid in airways (Iovannisci *et al.*, 2010) and B cell receptor signaling (Capasso *et al.*, 2010) and are perhaps best known for enabling sustained activity of NADPH oxidase by compensating for both its electrical and pH consequences (Henderson *et al.*, 1987; Henderson *et al.*, 1988; DeCoursey *et al.*, 2000; DeCoursey *et al.*, 2003; Murphy and DeCoursey, 2006; Morgan *et al.*, 2009; DeCoursey, 2010; El Chemaly *et al.*, 2010). On the other hand, proton channels have been implicated in contributing to the severity and metastasis of several human cancers (Wang *et al.*, 2012) and chronic lymphocytic leukemia (Hondares *et al.*, 2014), as well as exacerbating neurological damage in ischemic stroke (Wu *et al.*, 2012).

In the reproductive system, voltage-gated proton channels have been shown to play key roles in the capacitation (Lishko *et al.*, 2010) and motility (Musset *et al.*, 2012) of human sperm. We were interested in knowing whether proton channels are also expressed in human eggs. Amphibian oocytes have proton channels (Barish and Baud, 1984; Humez *et al.*, 1995), and there are some indications of possible functions (Baud and Barish, 1984; Humez *et al.*, 1996). A H^+ conductance appeared in response to acid loading in mouse trophoblast and pluriblast although it was Zn^{2+}-sensitive only in the latter (Harding *et al.*, 2002). We are not aware of any study identifying proton currents in human oocytes. Given that human but not mouse sperm express proton channels (Lishko *et al.*, 2010), it is essential to determine whether human oocytes express proton channels. Here, we present voltage-clamp evidence that this is the case.

Materials and Methods

Obtaining human oocytes

We obtained human oocytes from a gamete repository generated by the Rush Center for Advanced Reproductive Care. Oocytes were retrieved by transvaginal ovarian follicle aspiration following treatment with recombinant follicle stimulating hormone and purified urinary gonadotropins (HMG) with GnRH antagonist for controlled ovarian hyperstimulation. After an adequate number of follicles measuring 18–20 mm were visualized by transvaginal ultrasound, the patient was administered human chorionic gonadotropin (HCG) for initiation of the ovulatory cascade. Transvaginal aspiration of the follicles under ultrasound guidance was performed 36 h after the administration of HCG. Cumulus-oocyte complexes were subsequently denuded using HEPES-buffered human tubal fluid (HTF) medium containing 5 mg/ml hyaluronidase (Irvine Scientific, Santa Ana, California, USA). Upon removing cumulus cells, mature metaphase II (MII) oocytes were identified under the microscope by presence of the first polar body, and vitrified (SAGE Vitrification Kit, Cooper Surgical, Connecticut, USA). Briefly, MII oocytes were equilibrated 8–10 min in equilibration solution (7.5% v/v dimethylsulfoxide (DMSO), 7.5% v/v ethylene glycol in MOPS (3-(N-morpholino)propanesulfonic acid)-buffered HTF solution), followed by three consecutive rinses in vitrification solution for a total exposure of 1 min (15% v/v each of DMSO and ethylene glycol in 0.6 M sucrose MOPS-buffered solution). Oocytes were loaded in a small amount of vitrification solution in groups of three onto HSV™ devices (Irvine Scientific) before plunging into liquid nitrogen (LN_2) to be stored for future use.

Storage of samples and data and preparation of oocytes for recording

The oocytes were stored in LN_2 until the day of analysis at which point they were removed from the gamete repository and warmed using SAGE Vitrification Warming Kit (CooperSurgical, Connecticut, USA). The vitrification device was removed from LN_2 and quickly submerged into prewarmed (37°C) thaw solution (1.0 M sucrose in MOPS-buffered HTF) for 1 min during which the oocytes floated free of the device. The oocytes were subsequently transferred to 0.5 M sucrose solution for 3 min at room temperature (RT), followed by 5- and 1-min incubations in two separate drops of MOPS-buffered HTF solution at RT.

As the zona pellucida (ZP) interferes with membrane patch-clamping, we managed to completely remove it by using a laser-assisted hatcher (Zilos®, Hamilton Thorne, Massachusetts, USA). A fairly large hole of >20 µm was created using multiple 400- to 500-µs pulses at 1460 nm laser power. The oocytes were stripped free of the ZP by repeated pipetting with a 150-µm Stripper™ tip (CooperSurgical, Connecticut, USA). Samples were kept in HEPES-buffered HTF (CooperSurgical) supplemented with 20% synthetic serum substitute (Irvine Scientific) at 37°C until they were ready for patch-clamp recording. A total of 18 oocytes were warmed in batches of three each day that patch clamping was performed.

Electrophysiology

We used whole-cell or excised-patch configurations of the patch-voltage-clamp technique (Hamill *et al.*, 1981). In some cases, we recorded from the entire oocyte. This approach is 'expensive' because of the difficulty involved in obtaining these cells for research. A small piece of cytoplasm enclosed by the oolemma pinched off occasionally during the pipetting process. To maximize sample usage, we also recorded from this piece of pinched-off oocyte. For the purpose of this manuscript, we define these as vesicles derived from oocytes. Finally, when we observed proton currents in cell-attached patches, we excised the patch in the inside-out configuration. Our aim was to determine whether proton channels were present and define their key properties. Therefore, our pipette and bath solutions lacked most permeant ions. The predominant cation was tetramethylammonium, a large inert cation and the main anion was methanesulfonate, a large anion. We included 55–100 mM buffer in all solutions in order to maximize control of pH despite the challenge of large proton fluxes across the membrane. Buffers were selected with a pK_a near the final pH of each solution (Homopipes for pH 5, Mes for pH 6, BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid) or PIPES (1,4-piperazinediethanesulfonic acid) for pH 7 and Tricine for pH 8). We used PIPES for Zn^{2+} studies at pH 7, because its binding of Zn^{2+} is weaker than by BES (Cherny and DeCoursey, 1999).

Ethical approval for use of human tissues

Both the human oocyte research use and the human gamete tissue repository have been reviewed and approved by the Rush University Medical Center Institutional Review Board.

Results

Human oocytes are large 110–115-μm diameter spheres surrounded with a thick extracellular matrix (the ZP) composed of four glycoproteins (Wassarman, 2008). Their large size and ZP make oocytes difficult to study by conventional patch-clamp techniques. Obtaining a gigohm seal requires access to a clean membrane, in this instance the oolemma. Prior to developing a reliable method to obtain zona-free oocytes, we tried several approaches to expose the bare membrane needed for patch clamp studies. Initially, we tried to obtain a herniated area of oocyte by making a small laser-assisted opening through the ZP. Although this enabled obtaining gigohm seals and forming inside-out patches of membrane, progression to whole-cell configuration was compromised by the limited access to the membrane surface. In addition, the mere presence of the ZP made bath solution changes challenging, as oocytes did not adhere to the surface of the chamber well.

Once we removed the entire ZP, we were able to record from whole oocytes, vesicles derived from oocytes, and inside-out patches of the oocyte membrane. In all measurements, we observed evidence of voltage-gated proton currents. The properties of these currents appeared similar in these different preparations. Fig. 1 shows a family of proton currents in an excised inside-out patch of membrane. The currents recorded during voltage pulses spanning a range of voltages are superimposed. The *x*-axis is time, and the initial spike is a capacity transient reflecting current injected at the start of each voltage step in order to rapidly clamp the membrane to a new voltage. Immediately after the spike, the current rises with a sigmoidal or nearly exponential time course as proton channels open progressively during the depolarizing pulse. By convention, upward deflections of the record indicate outward flow of positive charge from the cell to its environment. At more positive voltages, more channels open and the rate of opening increases. Because we were interested in isolating proton currents, we used solutions lacking most permeant ions, composed mainly of tetramethylammonium methanesulfonate and high buffer concentrations (up to 100 mM). We held the membrane at a sufficiently large negative voltage that almost all proton channels were closed and then applied depolarizing voltage pulses in 10-mV increments. Small depolarizations elicited no time-dependent current, but with larger pulses a slowly activating current appeared. The current increases during each pulse as more channels open, which occurs slowly for proton currents in mammalian cells.

Fig. 2A illustrates proton currents in a human oocyte vesicle at three pH_o. As is characteristic of proton channels in all species, the voltage at which proton channels first open ($V_{threshold}$) is shifted to more positive voltages when pH_o is decreased. In this cell (Fig. 2A), the first distinct outward current appeared at −20 mV at pH_o 8 and +10 mV at pH_o 7. At pH_o 6, $V_{threshold}$ was near the reversal potential, V_{rev}, and consequently distinct currents were evident only during larger pulses. However, it is possible to deduce whether channels opened during the depolarizing pulse by examining the 'tail currents' seen upon repolarization. Channels that were opened at positive voltages remain

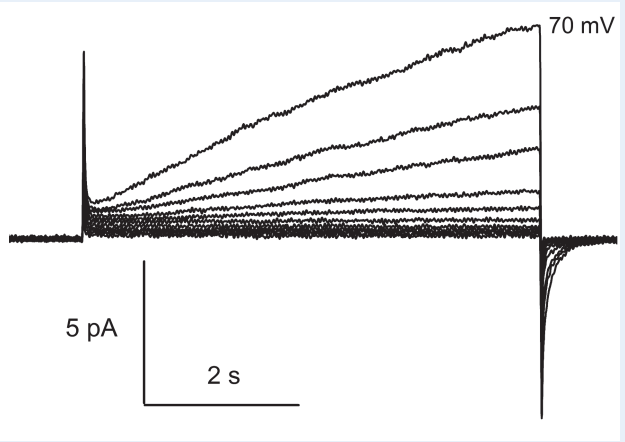

Figure 1 A family of proton currents in an inside-out patch of membrane excised from a human oocyte at pH_o 7 and pH_i 7. The membrane was held at −40 mV, and pulses were applied in 10-mV increments to −30 mV up to +70 mV.

open and conduct large inward currents upon return to the negative holding potential, because the driving force ($V - V_{rev}$) is large. The channels do not close instantly, but with a time course that depends on voltage and other factors. The effect of pH_o on the voltage range where H^+ currents open is more readily seen in Fig. 2B. The proton conductance, g_H, indicates in a general way the voltage dependence of channel opening at different pH_o (Fig. 1C).

To confirm that these currents are proton-selective, one must determine the reversal potential (V_{rev}) and compare it with the Nernst potential for H^+. Fig. 3 illustrates tail current measurements. A large prepulse opens many proton channels, followed by repolarization to various voltages, which allows determination of the reversal potential, V_{rev}, from the amplitude and direction of tail current decay as the channels close. In the experiment in Fig. 3, V_{rev} at pH_o//pH_i 7//7 was +4 mV (A) and at pH_o//pH_i 8//7 was –44 mV (B). Thus, V_{rev} changed by 48 mV, whereas the Nernst prediction is a 58-mV change. Such small deviations from ideal are typical and reflect incomplete control over pH, as well as pH_i changes during the prepulse, which itself extrude enough protons to increase pH_i slightly. It is clear that the conductance studied here is strongly selective for protons.

Proton currents in most species are inhibited by divalent cations (Mahaut-Smith, 1989; DeCoursey, 2003), with Zn^{2+} being especially potent in mammalian cells (Cherny and DeCoursey, 1999). The inhibition by Zn^{2+} is manifested as a positive shift of the g_H–V relationship and slower channel opening. Both effects are evident in Fig. 4. The g_H–V relationship is typically shifted by 30–40 mV at 10 μM Zn^{2+} in heterologously expressed human proton channels (Musset *et al.*, 2010), roughly comparable to that observed here. The slowing of activation is also evident, although noise in the data precludes quantification. Similar effects were observed in three whole oocytes and one vesicle. To firmly establish Zn^{2+} effects, we reversed the effects by bathing the cell in an EGTA-containing solution (Fig. 4).

One of the most unusual properties of proton channels is the shift of their voltage range of activation by the pH gradient, with increases in pH_o and decreases in pH_i producing identical 40-mV shifts (Cherny *et al.*, 1995). We quantified the position of the g_H–V relationship

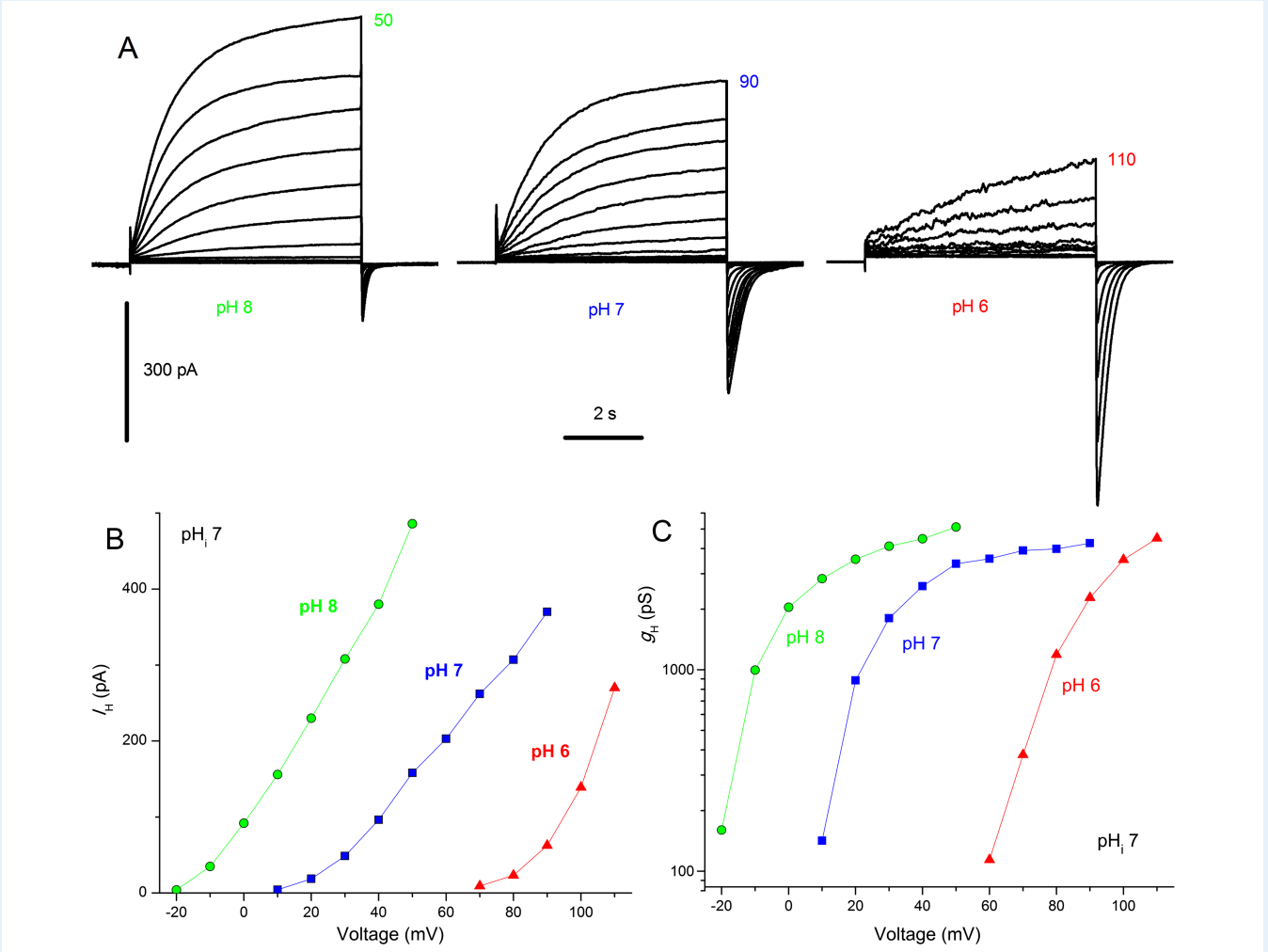

Figure 2 (**A**) **Families of proton currents in a human oocyte vesicle at pH$_o$ 8, 7 and 6, with a pH 7 pipette solution.** The capacitance was ~100 pF. Depolarizing pulses were applied in 10-mV increments from the holding potential of −70 mV (pH 8) or − 50 mV (pH 7 or 6) up to the voltage indicated on each family. (**B**) Current–voltage curves from the families of currents in A. Proton current amplitude (I_H) was obtained by fitting single exponentials to the currents and extrapolating to infinite time. (**C**) Proton conductance (g_H)–voltage curves from this vesicle, calculated using the reversal potential measured in each solution. The values at pH$_o$ 6 were determined from the tail current amplitude, scaled by the largest outward current measured.

by determining the voltage at which the g_H is 10% of its maximal value (Cherny *et al.*, 2015). Fig. 5 shows values reported in a previous study of heterologously expressed HVCN1 (the voltage-gated proton channel) measured at several pH$_i$, all with pH$_o$ 7. As pH$_i$ was increased, the voltage at which activation occurred shifted positively. Present oocyte data with pH$_o$ 7 are plotted as stars. Importantly, the slope of this relationship is the same 40 mV/unit. The position of the oocyte data also falls on the previous pH$_i$ 7 data. Because whole oocytes are too large for perfusion by the patch pipette to control pH$_i$ (Byerly and Moody, 1986), this result indicates that the actual pH$_i$ was quite near 7.0 in the cells studied.

Discussion

Here we present conclusive evidence that human oocytes consistently exhibit sizeable voltage-gated proton currents. Although proton currents are known to exist in amphibian oocytes, namely *Ambystoma* (Barish and Baud, 1984) and *Rana esculenta* (Humez *et al.*, 1995), and suggestive evidence exists in murine oocytes (Harding *et al.*, 2002), this is the first report of voltage-gated proton channels in human oocytes. The restricted quantities of material and initial technical difficulties with this preparation limited the types and extent of measurements that could be achieved. For example, the large intracellular volume of the oocytes prevented adequate control over pH$_i$. The near impossibility of controlling pH$_i$ in (comparably large) amphibian oocytes by diffusion of buffered solution from the pipette has been well documented (Byerly and Moody, 1986). Nevertheless, the practical result is that pH$_i$ is likely to remain fairly constant over the time course of an experiment. That the measured reversal potential, V_{rev}, with pH$_o$ 7 was near 0 mV indicates that pH$_i$ was near 7, because its extreme proton selectivity makes the proton channel a reliable pH sensor. In addition, the dependence of the voltage at which proton channels opened varied with pH$_o$ in a manner consistent with pH$_i$ being near 7 (Fig. 5). The oocytes studied were subjected to vitrification

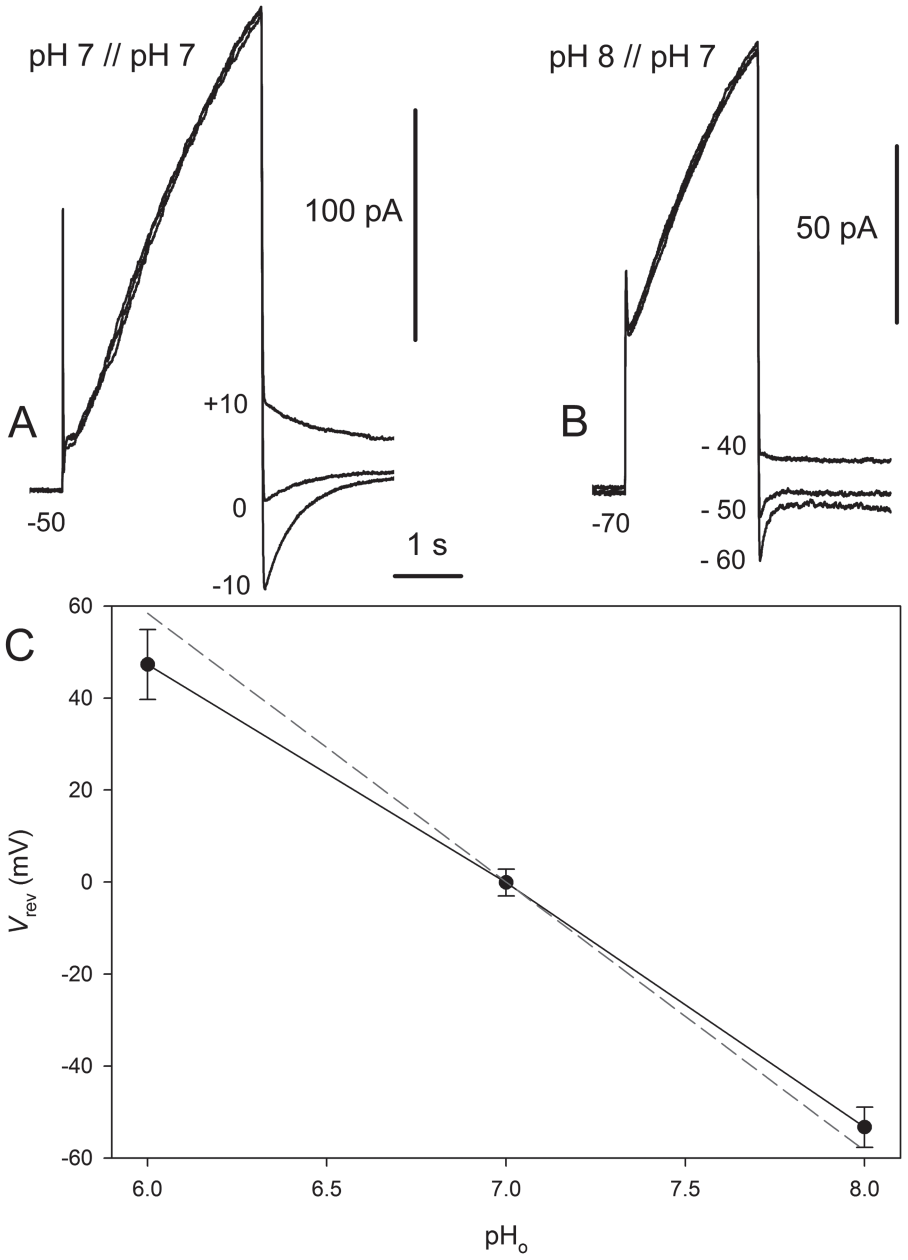

Figure 3 Reversal potentials confirm proton selectivity. Prepulses to +50 mV (A) or + 10 mV (B) opened proton channels, followed by repolarization to the indicated voltages where the channels closed. The reversal potential, V_{rev}, can be determined from the amplitude and direction of tail current decay. The Nernst potential for $pH_o//pH_i$ 7//7 (A) is 0 mV, and that for $pH_o//pH_i$ 8//7 (B) is −58 mV; in both cases, the observed V_{rev} was somewhat more positive. (**C**) Values of V_{rev} measured in three oocytes are plotted (mean ± SEM), with the gray dashed line showing the Nernst potential for H^+, assuming pH_i 7. Small deviations from the Nernst prediction most likely reflect imperfect control of pH.

and subsequent warming, which might affect properties of HVCN1. However, the existence of functional proton channels in the plasma membranes of the cells studied is evident from our studies. It is also conceivable that expression levels or HVCN1 properties might have been influenced by the hormonal treatment used for controlled ovarian hyperstimulation. On the other hand, these procedures reliably produce viable oocytes that are competent for IVF and subsequent live birth.

Although a rough estimate of membrane area can be obtained from the membrane capacity, this was larger than could be compensated by our patch-clamp circuitry. If a typical oocyte has a diameter of 100 μm, its capacity would be 314 pF, assuming a perfectly spherical surface with a specific capacitance of 1 μF/cm^2. Four whole oocytes generated proton currents ranging 1.5 to 7 nA, for an estimated current density of 5–22 pA/pF. This is quite typical of a large variety of cells from mammalian and other species (see Table 3 in DeCoursey, 2003). Amphibian

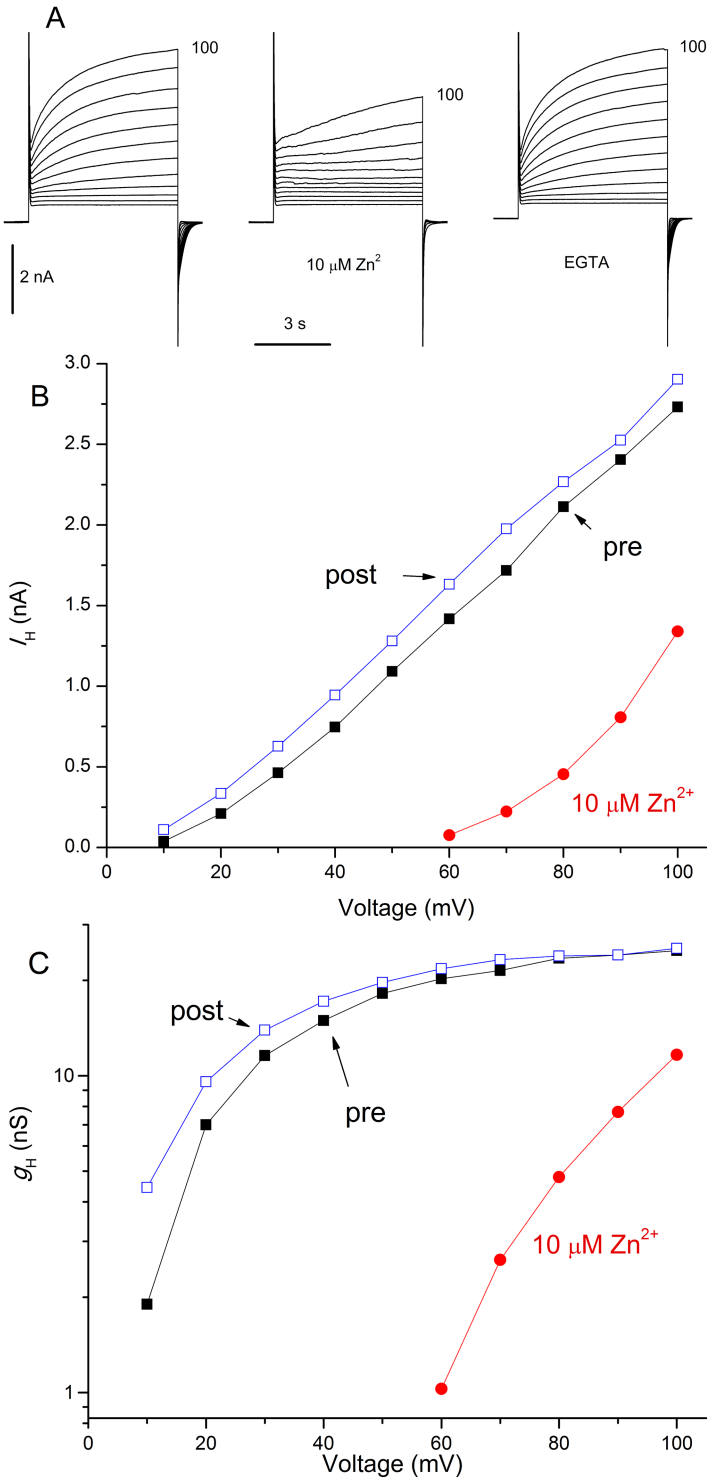

Figure 4 Inhibition of proton currents by Zn^{2+} in a whole oocyte. (**A**) The cell was held at −60 mV, and pulses were applied in 10-mV increments from −10 to +100 mV. The same pulses were applied in the presence of 10 μM Zn^{2+}, and then the bath was exchanged with EGTA-containing solution to remove Zn^{2+} which restored the currents. (**B**) Proton current–voltage curves from the current families in A. The time-dependent current at each voltage is plotted. The most distinct effect of Zn^{2+} was to shift the voltage range of activation positively. (**C**) Proton conductance calculated from the currents in B.

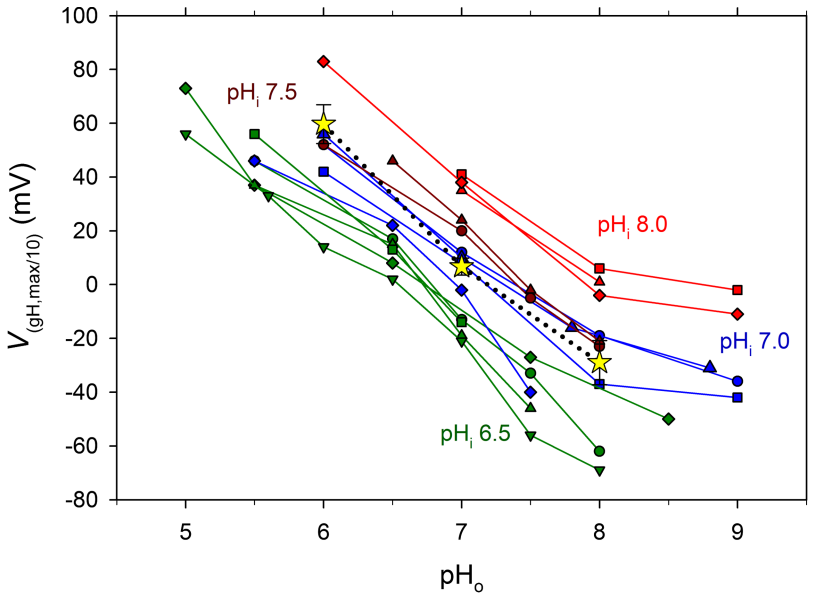

Figure 5 Dependence of the position of the g_H–V relationship on pH$_o$ in oocytes resembles that of the human proton channel gene product, HVCN1, expressed in HEK-293 cells. Oocyte data (mean ± SEM, $n = 3$) are shown as stars connected by a dotted line. Heterologously expressed HVCN1 measurements from a previous study (Cherny *et al.*, 2015) are shown color-coded for pH$_i$ (green = 6.5; blue = 7.0; red = 8.0) with measurements in each cell (symbol shapes indicate individual cells) connected by lines, in whole-cell configuration with controlled pH$_i$ and pH$_o$ 7. The results indicate that pH$_i$ was near 7.0 in the oocytes studied and that the dependence of voltage-gating on pH$_o$ was normal. The position of the g_H–V relationship is quantified by the voltage at which the g_H is 10% of its maximal value, V ($g_{H,max}/10$).

oocytes have proton currents of 8.4 (Barish and Baud, 1984) and 32 pA/pF (Humez *et al.*, 1995) respectively. Human sperm (Lishko *et al.*, 2010) and some phagocytes (Gordienko *et al.*, 1996; Musset *et al.*, 2008) have higher proton current density of up to 100 pA/pF or more. Intriguingly, a study using RNA sequencing reported that HVCN1 message increased by nearly an order of magnitude from oocyte to pronuclear and zygote stages, with levels dropping during subsequent cell division stages to low levels at the morula stage (Xue *et al.*, 2013).

Characteristics of proton currents in human oocytes

The properties of proton currents in human oocytes are quite similar to those reported in other human cells (Table I). Combined with the fact that only a single genetic isoform exists in humans, it can be concluded that these currents are generated by the *HVCN1* gene product. They are activated by membrane depolarization and, with sigmoidal kinetics, thought to reflect cooperative gating of the channel dimer (Gonzalez *et al.*, 2010; Musset *et al.*, 2010; Tombola *et al.*, 2010). The voltage at which depolarization first elicits proton current, $V_{threshold}$, is typically +20 mV at symmetrical pH in native cells (Cherny *et al.*, 1995; DeCoursey, 2003). $V_{threshold}$ appears to be slightly more negative in heterologous expression systems, in part due to overexpression. Proton current turns on notoriously slowly with a time constant, τ_{act}, of several seconds, but turns off somewhat more rapidly. Finally, the most potent inhibitor of voltage-gated proton channels, with the exception of a recently engineered peptide (Zhao *et al.*, 2018), is the divalent cation Zn^{2+} (Mahaut-Smith, 1989; Cherny and DeCoursey, 1999). Zn^{2+} exerts similar inhibitory effects on proton currents in oocytes as in other cells, slowing activation and shifting the g_H–V relationship positively, which reduces the current at any given voltage. In all of these properties, proton currents in oocytes closely resemble those in other human cells. Although this result may not appear surprising, there are precedents for altered properties of human proton channels in other human tissues: due to the absence of the first 20 amino acids in some B cells (Hondares *et al.*, 2014) and post-translational cleavage of a 68-amino-acid segment in sperm (Berger *et al.*, 2017). In addition, the properties of proton currents in phagocytes are altered radically during the respiratory burst (Schrenzel *et al.*, 1998; Bánfi *et al.*, 1999; DeCoursey *et al.*, 2000; DeCoursey *et al.*, 2001; DeCoursey, 2003; DeCoursey, 2010), as a result of protein kinase C-mediated phosphorylation of Thr^{29} in the intracellular N terminus (Musset *et al.*, 2010), when NADPH oxidase is activated and proton channels are needed to compensate both charge and pH (Henderson *et al.*, 1987; Henderson *et al.*, 1988; DeCoursey *et al.*, 2003; Murphy and DeCoursey, 2006; Morgan *et al.*, 2009; Ramsey *et al.*, 2009; DeCoursey, 2010).

Possible functions of proton currents in human oocytes

Possible functional roles for proton channels in human oocytes can only be speculated at present. The two main consequences of proton channel activation are electrical and chemical. Given the exquisite regulation of activation by the pH gradient ΔpH (pH$_o$ − pH$_i$) (Cherny *et al.*, 1995), the channel only opens when doing so will produce H^+ extrusion from cells. The membrane potential at which proton channels first open is roughly $V_{threshold} = -40$ mV × ΔpH + 20 mV (Cherny

Table I Comparison of properties of proton currents in human oocytes, human leukocytes, and heterologously expressed HVCN1 proton channels.

Property	Oocyte	Other human cells	Cell type	HVCN1
Activated by depolarization	Yes	Yes	Neutrophil[a]	Yes[b]
Sigmoidal kinetics	Yes	Yes	Neutrophil[a]	Yes[c]
$V_{threshold}$ (mV)	+20 mV	+20 mV	Eosinophil[d]	+7[e], −10[f]
τ_{act} (+60 mV) (s)	3.3 ± 0.6 (7)	3.0 ± 0.3 (11)	Eosinophil[d]	3.4 ± 1.2 (9)[g] 3.5 ± 0.6 (41)[h]
τ_{tail} (−40 mV) (ms)	209 ± 28 (6)	200 ms 200 ms	Eosinophil[d] B lymphocyte[i]	~220[j]
Inhibition by Zn^{2+}	Yes	Yes	Neutrophil[a]	Yes[b]

Properties of proton currents in human oocytes are compared with those reported in other human cells and for HVCN1, the product of the human gene, *HVCN1*, transfected into mammalian cell lines. $V_{threshold}$ is the voltage at which proton currents are first evident during depolarizing pulses; τ_{act} is the time constant of current turn-on, and τ_{tail} is the mean ± SEM (*n*) time constant of current turn-off after repolarization. Most measurements were performed at pH_o 7 and pH_i 7 at room temperature. References: [a](DeCoursey and Cherny, 1993), [b](Ramsey *et al.*, 2006), [c](Musset *et al.*, 2010), [d](DeCoursey *et al.*, 2001), [e](Ramsey *et al.*, 2010), [f](Musset *et al.*, 2008), [g](Cherny *et al.*, 2015), [h](Banh *et al.*, 2019), [i](Schilling *et al.*, 2002), [j](Villalba-Galea, 2014).

et al., 1995). H^+ efflux would tend to hyperpolarize the membrane potential. A classical function in phagocytes is charge compensation, in which H^+ efflux compensates for another electrogenic transporter, in this case, NADPH oxidase (Henderson *et al.*, 1987; Schrenzel *et al.*, 1998; DeCoursey *et al.*, 2003). H^+ efflux tends in increase pH_i and decrease pH_o. The volume of oocytes is large, which means its pH_i will change rather slowly. An advantage of proton channels is that they can change pH an order of magnitude faster than other pH-regulating transporters like Na^+-H^+ antiporters or Cl^-/HCO_3^- exchangers (DeCoursey and Cherny, 1994). If we assume a buffering capacity of 30.6 (Lane and Bavister, 1999) and an oocyte diameter of 100 µm, a 3-nA proton current (e.g. Fig. 4) would increase average pH_i by 0.12 units/minute, comparable to or faster than the rate of pH recovery from an acid load in bovine (Lane and Bavister, 1999) or murine oocytes (Fitzharris and Baltzs, 2006). As HVCN1 expression increases during progression from oocyte to pronuclear and zygote stages (Xue *et al.*, 2013), the rate of pH_i increase due to HVCN1 activation will increase proportionately.

Human sperm have large proton currents that play important roles in capacitation and motility (Lishko *et al.*, 2010; Musset *et al.*, 2012). It has been proposed that the proton channel plays a critical role in increasing sperm pH_i, thus triggering capacitation (Lishko *et al.*, 2010). In contrast, mouse sperm lack proton channels altogether (Lishko *et al.*, 2010) and a Na^+/H^+ antiporter is an important regulator of pH_i (Wang *et al.*, 2003). The zinc-rich seminal plasma is thought to inhibit proton channels, thus preventing premature sperm capacitation (Lishko *et al.*, 2010). As sperm travel through the female reproductive tract, seminal plasma and Zn^{2+} are left behind and there is a significant change in pH from acidic vaginal fluid to alkaline uterine and oviductal fluid (Ng *et al.*, 2018), which coincides with strong proton currents and sperm capacitation.

The most straightforward possibility is that proton channels are involved in pH_i regulation in human oocytes. Their unique pH-dependent gating mechanism (Byerly *et al.*, 1984; Cherny *et al.*, 1995) makes proton channels ideal acid extruders. Unlike other membrane transporters that regulate pH, proton channels extrude excess acid efficiently and at no metabolic cost to the cell (DeCoursey and Cherny, 1994). Because the resting membrane potential of human oocytes is −10 to −30 mV (Feichtinger *et al.*, 1988) and ovine oocytes −10 to −16 mV (Boni *et al.*, 2008), well positive to that of excitable cells that range −60 to −100 mV (Aidley, 1991), only slight acidification or depolarization would be required to activate proton channels. Human oocytes in metaphase II have a pH_i of 7.4 (Dale *et al.*, 1998), and the pH of human follicular fluid is reported to be between pH 7.2 and 7.3 (Shalgi *et al.*, 1972; Imoedemhe *et al.*, 1993); hence, proton channels are unlikely to open before ovulation. However, the pH of human oviductal fluid can be as high as pH 7.7 (David *et al.*, 1973) and in other mammals up to 8.4, reviewed in (Ng *et al.*, 2018) creating 0.3 to 1.0 unit pH gradient (pH_o—pH_i) that greatly enhances the probability of open proton channels. Furthermore, upon fertilization, the pH_i of cleavage stage human preimplantation embryos (two to eight cells) drops to 7.12 (Phillips *et al.*, 2000), further increasing the outward pH gradient and creating conditions quite likely to open proton channels. Mouse oocytes lack the ability to self-regulate pH during the phase of oocyte growth, leading to the suggestion that granulosa cells regulate oocyte pH_i (Fitzharris and Baltz, 2006). Regulation of pH_i is restored as mouse oocytes gain meiotic competence; however, this is inactivated when oocyte reaches the MII stage (FitzHarris and Baltz, 2009). Interestingly, oocytes from older mice had elevated pH_i that was associated with loss of cohesion (Cheng *et al.*, 2016). As the oocytes in our study were in the MII stage, proton channels may be responsible for pH_i regulation in human MII oocytes and are dynamically regulated during oocyte growth and maturation. The existence of proton channels in the human oocyte provides a mechanism of pH regulation that helps it adapt to the changing environmental conditions as it traverses the fallopian tube, is fertilized, and continues its journey into the uterus as an embryo. Finally, in light of the stark species differences in sperm, it was important to demonstrate unequivocally the existence of proton channels in human oocytes.

Authors' roles

The study was conceived by R.Y.S. Oocytes were retrieved and collected by L.S. and M.W.M. The human gamete repository was cre-

ated by L.S. Oocytes were prepared for patch-voltage-clamping by R.Y.S. and L.S. D.M. and V.V.C. conducted patch-clamp studies and together with T.E.D. analyzed the data. T.E.D., R.Y.S., L.S., D.M. and NT wrote the manuscript. All authors revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Conflict of interest

None to declare.

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