Voltage Dependence and pH Regulation of Human Polycystin-2-mediated Cation Channel Activity*

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Silvia González-Perrett‡, Marisa Batelli‡, Keetae Kim¶, Makram Essafi¶, Gustavo Timpanaro‡, Nicolás Moltabetti‡, Ignacio L. Reisin‡, M. Amin Arnaout¶, and Horacio F. Cantiello¶‡**

From the *Laboratorio de Canales Iónicos, Departamento de Fisicoquímica y Química Analítica, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina 1113, the ‡Renal Unit, Massachusetts General Hospital East, Charlestown, Massachusetts 02129, the §Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, and the ¶Departamento de Fisiología, Facultad de Medicina, Buenos Aires, Argentina 1121

Polycystin-2, the product of the human PKD2 gene, whose mutations cause autosomal dominant polycystic kidney disease, is a large conductance, Ca2+-permeable non-selective cation channel. Polycystin-2 is functionally expressed in the apical membrane of the human syncytiotrophoblast, where it may play a role in the control of fetal electrolyte homeostasis. Little is known, however, about the mechanisms that regulate polycystin-2 channel function. In this study, the role of pH in the regulation of polycystin-2 was assessed by ion channel reconstitution of both apical membranes of human syncytiotrophoblast and the purified FLAG-tagged protein from in vitro transcribed/translated material. A kinetic analysis of single channel currents, including dwell time histograms, confirmed two open and two close states for spontaneous channel behavior and a strong voltage dependence of the open probability of the channel (Po). A reduction of cis pH (pHcis) decreased Po and shifted the voltage dependence of channel function but had no effect on the single channel conductance. An increase in pHcis, in contrast, increased NPo (channel number times Po). Elimination of the II+ chemical gradient did not reverse the low pHcis inhibition of polycystin-2. Similar findings confirmed the pH effect on the in vitro transcribed, FLAG-tagged purified polycystin-2. The data indicate the presence of an H+ ion regulatory site in the channel protein, which is accessible from the cytoplasmic side of the protein. This protonation site controls polycystin-2 cation-selective channel activity.

Autosomal dominant polycystic kidney disease (ADPKD)† is a prevalent human genetic disorder affecting 1:400 to 1:1000 individuals worldwide. Mutations in at least two genes, PKD1 and PKD2, are responsible for more than 90% of all cases of the disease (1). Despite little knowledge concerning the role(s) of the gene product of PKD1, polycystin-1, recent studies determined that polycystin-2, the gene product of PKD-2, is a Ca2+-permeable, non-selective cation channel (2, 3). It is assumed that membrane-associated polycystin-1-polycystin-2 complexes may be part of a regulatory pathway involved in the control of membrane transport in target epithelia, including those of the kidney and the liver. Coiled-coil interactions between polycystin-1 and -2 are postulated to occur in vivo (4), and the two gene products, but neither one alone, were reported to increase the whole cell conductance when heterologously overexpressed in mammalian cells (5). Dysfunctional control of this transport-associated regulatory pathway has been implicated in cyst formation and cell proliferation. This may be partly explained by the cell location and developmental characteristics of the target tissue(s) in which polycystin-2 is expressed (6). Recent findings indicate, however, that polycystin-2 engages in ion channel activity in the absence of any other associated proteins (2). As wild type (2, 3) as well as ADPKD-causing mutated polycystin-2 (7) both behave as functional ion channels in plasma membranes, it seems likely that as yet largely unknown regulatory mechanisms may contribute to the activation/regulation of polycystin-2 channel function in vivo.

In this report, we investigated the regulatory role of pH in the control of polycystin-2 channel function. Human polycystin-2 from two different sources, including hST apical membranes and the in vitro transcribed/transduced human gene product, was functionally reconstituted in a lipid bilayer system where the effect(s) of pH on its cation channel activity was determined. The data indicate that polycystin-2 is strongly voltage-dependent and contains a regulatory site that is highly sensitive to H+ ions, enabling its regulation as an ion channel as a function of intracellular pH.

EXPERIMENTAL PROCEDURES

Human Placenta Membrane Preparation—Syncytiotrophoblast membrane vesicles were obtained from term human placenta as described (8) with minor modifications (2). Apical membrane enrichment was 26-fold from the initial homogenate, and the final pellet was resuspended in a buffer solution containing HEPES-KOH (10 mM), pH 7.14, sucrose (250 mM), and KCl (20 mM).

Solutions—Both sides of the lipid bilayer were bathed with solution containing 10–15 μM Ca2+, 10 mM MOPS-KOH, and 10 mM MES-KOH, pH 7.14. The final K+ concentration in the solution was ~15 mM. KCl was added to the cis side of the chamber to a final concentration of 150 mM. Whenever indicated, KCl was added to the trans compartment to a final concentration of 150 K+ and 135 Cl–, respectively.

In Vitro Transcription/Translation of FLAG-tagged Polycystin-2—In vitro translated FLAG-tagged polycystin-2 was obtained as recently described (2). Briefly, a 3.2-kb XhoI-PciI fragment encoding the protein from pVL1393-PKD2 was transferred to the XhoI-NsiI site of pGEM-7zf (+) to generate the plasmid pGEM-PKD2. This cDNA was transcribed and translated in vitro with the Tn7-T7-coupled reticulocyte lysate system (Promega) in the presence or absence of microsomal
Fig. 1. Single channel currents of hST polycystin-2. A, representative single channel currents at various holding potentials (indicated on the top of the tracings). Currents were obtained in asymmetrical KCl (150 and 15 mM, cis and trans compartments, respectively). The closed states are indicated by the line at the left of the tracings. Data are representative of five experiments. B, percent distribution (%) of single channel current experimental values (open circles, N) in all-point histograms obtained from single channel currents in panel A. The abscissa indicates the distribution of currents between minimum and maximum current values (as percentage), and the ordinate (N) is the number of events for each current value. The area underneath the fitted values (solid line) is the sum of open and closed probabilities. Closed and open states are indicated by the c and o on top of the histograms, respectively. The histograms show the increase in open probability as a function of reducing the holding potential.

Fig. 2. Single channel currents and voltage dependence of hST polycystin-2. A, representative single channel tracing in asymmetrical KCl (150 and 15 mM, cis and trans compartments, respectively). The holding potential was 20 mV. In B, the single channel amplitude histograms were obtained by fitting the single channel current values (open circles) in panel A with a two-Gaussian distribution (solid line). Closed and open states are indicated by the c and o, respectively. In C, closed and open dwell histograms (left and right, respectively) were obtained from the tracing in panel A, which was best fitted (solid lines) with two exponentials for both histograms. The corresponding time constants were 0.56 and 5.39 ms for the closed dwell times, and 3.55 and 14.5 ms for the open times, respectively. In D, open probability (mean ± S.E.) as a function of the various holding potentials (filled circles, n = 5) was fitted to the Boltzmann equation (solid line, see “Experimental Procedures”), indicating a strong voltage dependence of channel function.
membranes. Plasmid DNA (1 μg) and the in vitro reaction mixture (50 μl), including L-[35S]methionine (Amersham Biosciences), were incubated for 90 min at 30 °C. In vitro translated 35S-labeled polycystin-2 and luciferase (as control) were analyzed by SDS-PAGE and autoradiography.

Ion Channel Reconstitution—Lipid bilayers were formed with a mixture of synthetic phospholipids (Avanti Polar Lipids, Birmingham, AL) in N-decane following methods reported previously (2, 8). All phospholipids used were 1-palmitoyl-2-oleoyl-based, including 1-palmitoyl-2-oleoyl phosphatidylcholine and 1-palmitoyl-2-oleoylphosphatidylethanolamine. The lipid solution (~20–25 mg/ml) was spread over the diameter aperture (250 μm) of a polystyrene cuvette (CP13–150) with a thin glass rod. The cuvette was inserted into a polyvinyl chloride holder, thus defining two aqueous compartments of volumes 800 and 1,600 μl, respectively, and separated by a planar lipid film as originally described (9). Both sides of the lipid bilayer were bathed with a 10–15 μM Ca2+ solution, which was buffered at pH 7.14 with 10 mM MOPS-KOH and 10 mM MES-KOH. The final K+ concentration in the solution was ~15 mM. Unless otherwise stated, experiments were initiated by bathing the trans side of the bilayer with this solution. 135 mM KCl was added to the cis side of the chamber to generate a trans bilayer osmotic gradient that promoted the vesicle-planar bilayer membrane fusion (10).

Changes in pH—Changes in pH in the cis and trans hemi-chambers of the lipid reconstitution chamber were conducted by addition of small volumes (1–10 μl) of concentrated solutions of either HCl or KOH, ranging between 0.5 and 2.0 N. The final pH was calibrated with a pH mini-electrode, either by means of titration curves of comparable volumes and saline concentrations or directly from the final cis or trans solutions.

Electrical Recordings—Holding potentials (Vh) were applied from the trans chamber with either a DC voltage source or a wave function generator having the opposite, cis side defined as virtual ground. Unless otherwise stated, a cis minus trans voltage convention was utilized throughout the study. Bilayer formation was monitored by applying a 2.5-nV peak-to-peak 20-Hz triangular wave with a typical membrane capacitance of 100–200 pF. All the experiments were performed at room temperature (20–25 °C). Electrical signals were recorded using a current-to-voltage converter with a 10-gigaohm feedback resistor. Output (voltage) signals were low pass filtered at 700 Hz (~3 db) with an eight-pole Bessel type filter (Frequency Devices, Haverhill, MA). Signals were displayed on an oscilloscope, and channel recordings were simultaneously digitized with a pulse code modulator (Sony PCM-501 ES) and stored in videotapes with a VCR (Toshiba HQ). Data were later transferred to a personal computer for subsequent analysis at 4 kHz (unless otherwise stated). Whenever indicated, single channel current tracings were further filtered (see “Results”) for display purposes only.

Data Acquisition and Analysis—Unless otherwise stated, pCLAMP Version 5.5.1 (Axon Instruments, Foster City, CA) was used for data analysis, and Sigmaplot Version 2.0 (Jandel Scientific, Corte Madera, CA) was used for statistical analysis and graphics. Single channel conductances (γ) under asymmetrical conditions were calculated by the best fitting of current-to-voltage experimental data to either a straight line or the Goldman-Hodgkin-Katz equation, such that γ = I(Vh - E), was obtained from Eq. 1.

\[
I(V_h) = \frac{(z_i z_F P V_h / RT)}{(C_{in} / (1 - \exp(-\alpha))} - \frac{(z_i z_F P V_h / RT)}{(C_{out} / (1 - \exp(-\beta))}
\]

where \(i\) represents the cation species (either K⁺ or Na⁺) in the trans compartment, and \(j\) represents the cation species in the cis compartment. \(V_h\) is the holding electrical potential in mV; \(z_i\) and \(z_F\) are the charge for species \(i\) and \(j\), respectively. \(C_{in}\) and \(C_{out}\) are the cis and trans concentrations of \(i\) and \(j\), respectively, and \(\alpha = RTV_h / z_F\) and \(\beta = RTV_h / z_i F\). \(P_i\) and \(P_j\) represent the permeability coefficient for either species \(i\) or \(j\), respectively. \(F\) is the Faraday constant, 96,500 coulombs/ equivalent; \(R\) is the ideal gas constant, 0.082053 liter-atm/mol K) or...
single channel substates were observed that started at pH_{cis} (12), where variance deflections (calculations.
and cis data values were expressed as the mean paired substates are more frequent after lowering pH. Several single channel subconductance states was conducted as originally described (12), where variance deflections (\(\sigma^2, \text{pA}^2\)) were plotted versus mean values for sliding windows (abcissa, see “Experimental Procedures”). Various single channel substates were observed that started at \(-5 \text{ pA}\) under control conditions (top deflections histogram), which shifted to transitions starting at zero level after lowering pH_{trans} (middle deflections histogram). Channel resumed maximal conductance upon return to normal pH (bottom deflections histogram). Data are representative of four experiments.

\[ P_o = P_{\text{max}} / (1 - \exp(-kF(V_o - V_m)/RT)) \]  

where \(P_{\text{max}}\) is the maximal value for \(P_o\), \(kF/RT\) represents the slope, \(k\) is equal to the \(z\) times \(\delta\) product, \(z\) and \(\delta\) are the charge of, and the fractional voltage drop sensed by, the gating particle, respectively. \(V_m\) is the holding potential at which \(P_o = 0.5\). \(F, R, \text{ and } T\) have their usual meaning. Whenever indicated, statistical significance was obtained by paired t test comparison of sample groups of similar size (11). Average data values were expressed as the mean \(\pm\) S.E. under each condition, and \(n\) represents the number of experiments analyzed. Statistical significance was accepted at \(p < 0.05\).

Patlak’s mean versus variance analysis to enhance detection of brief subconductance states was conducted as originally described (12). Tracings of 12.5 s in duration (50,000 points) were used to perform this analysis. Five-point segments or “sliding windows” were considered where each window started one point later than the previous one. The mean versus variance of the windows was plotted by connecting lines following the temporal sequence in the original traces. Histograms were constructed for those mean current values whose variance was either less or equal to that corresponding to the closed state variance. Thus, mean current values corresponding to “in-between” substate transitions (large variance) were discarded (12).

Woodhull’s Model for the Calculation of the Equilibrium Constant for \(H^+\) Regulation of Polycystin-2—To calculate the dissociation constant for the \(H^+\) ion blocking site with the polycystin-2 channel, the model proposed by Woodhull (12) was used following the reaction shown in Eq. 3,

\[ \frac{[H^+]_{b_1}}{b_1} \times \frac{[H^+]_{b_2}}{b_2} \rightarrow \frac{[H^+]_{b_1} [H^+]_{b_2}}{b_1 b_2} \] (Eq. 3)

where \([H^+]_{b_1}, [H^+]_{b_2}\), and \([H^+]_{b_1} [H^+]_{b_2}\) represent the \(H^+\) ions in the cis and trans compartments and the protonation site, respectively. \([H^+]_{b_1}\) and \([H^+]_{b_2}\) represent the \(H^+\) concentrations in either compartment. Further, \(b_1, b_2, b_1 b_2\), and \(b_1 b_2\) are the voltage-independent \((V_k = 0 \text{ mV})\) components of the velocity constants for the reaction. Any departure from zero mV would entail a correction for each velocity constant, which is affected by an exponential factor taking into consideration the holding potential (13).

Following Woodhull’s interpretation of this model for the pH inhibition of macroscopic Na\(^+\) currents in nerve cells (13), several assumptions were made. The \(H^+\) association and dissociation reactions with the channel site change exponentially with respect to the \(V_c\) as in Eyring rate constant theory (14). Whenever \(H^+\) occupies the blocking site, polycystin-2-permeable ions, in this case K\(^+\), do not permeate the channel. Thus, ions other than \(H^+\) do not interfere with the regulatory (protonation) site, and in this sense, K\(^+\) and \(H^+\) ions do not compete with each other. This model is not concerned with polycystin-2 opening and closing mechanisms but rather with the fractional probability of open channel blockage by \(H^+\) occupation of the regulatory site. Another important assumption of the Woodhull’s model is that the current driven by \(H^+\) ions as they enter the channel is negligible as compared with that observed by K\(^+\) ions. Thus, \(H^+\) movement does not make a contribution to the open channel current. This is further supported by the fact that there is no change in the single channel conductance at different pHs. Finally, the \(H^+\)-ion-H\(^+\) site system is in steady state, namely, the time constants for the open channel state are long as compared with the time taken by the \(H^+\) ion to reach a steady state concentration inside the channel.

For holding potentials between \(-30\) and \(10 \text{ mV}\), for which \(P_o(V_o)\) is almost constant as a function of voltage, the equilibrium constant of the
blocking reaction $K(V_n)$ as a function of $V_n$ was calculated from experimental data with Eq. 4,

$$I_{\text{plut,40}} F_0^{\text{plut,40}} - 10^{-1.44 + K(V_n)}$$

where $I_{\text{plut,40}}$ and $I_{\text{plut,14}}$ are the mean currents obtained as $P_c \times I_c$, where $P_c(V_n)$ and $K(V_n)$ are the open probability and single channel currents obtained at different holding potentials, respectively. Values were obtained from fitted data of Figs. 2D and 3, D and E. The $F_0^{\text{plut,40}}$ and $F_0^{\text{plut,14}}$, which represented the fractional number of open (unblocked) channels at each pH in the original Woodhull’s model, indicates the fractional change in channel current as measured by the change in open probability for the single channel currents. The equilibrium constant for the reaction, $K(V_n)$, then takes the form shown in Eq. 5,

$$K(V_n) = \frac{b_{-1}}{b_{+1}} \exp \left( \frac{zFE}{2RT} \right) + \frac{b_{+1}}{b_{-1}} \exp \left( \frac{-zFE}{RT} \right) + \frac{1}{b_{+1}} \exp \left( - \frac{(25 - 12zFE)}{2RT} \right)$$

In our study, $b_{+1}$ and $b_{-1}$ are negligible as compared with $b_{-1}$ and $b_{+1}$ such that $K(V_n)$ can be reduced to Eq. 6,

$$K(V_n) = \frac{b_{-1}}{b_{+1}} \exp \left( \frac{zFE}{RT} \right)$$

where $z$ is the charge of the membrane potential sensor, $\delta$ is the electrical distance sensed from the cis (cytoplasmic) side of the channel, and $F$, $R$, and $T$ have their usual meaning. Fitting the values to this equation, the $z\delta$ product was obtained, as well as the value $K$ for $V_n = 0$ mV, representing the dissociation constant $K(0) = (b_{-1}/b_{+1})$.

## RESULTS

**Kinetic Analysis of Single Channel Currents of hST Polycystin-2**—Human syncytiotrophoblast apical membranes were reconstituted in the presence of asymmetrical KCl (150 mM) in the cis side and 15 mM KCl in the trans side. Polycystin-2 single channel currents were obtained at different holding potentials (Fig. 1A), and current amplitudes and open probabilities were calculated from all-point histograms (Fig. 1B). The mean open probability (area under open state) largely depended on the holding potential. The main single channel conductance was $157 \pm 4.90$ pS ($n = 7$), as recently reported (2). Kinetic analysis of single channel currents (Fig. 2) indicated that the channel had two open and closed states, as indicated by the closed and open dwell histograms (Fig. 2C, left and right, respectively), which were best fitted with two exponentials for each state. The open probability of the channel ($P_o$) was strongly dependent on the holding potential (Fig. 2D). The voltage dependence of the $P_o$ was further assessed by fitting experimental data to the Boltzmann equation (see “Experimental Procedures”). The fitted parameters indicated a $P_{\text{max}}$ of 0.72 $\pm$ 0.02 (cis) at $0$ mV, and a $V_{\text{m}}$ of $29.4 \pm 3.9$ mV ($n = 5$).

**Effect of $pH_{\text{cis}}$ on hST Polycystin-2 Channel Activity**—To assess the regulatory effect(s) of pH on polycystin-2 channel activity, the pH of the cis chamber was reduced by addition of a small volume of HCl (2.0%). Single channel currents were reduced after lowering $pH_{\text{cis}}$ (Fig. 3, A and B). The single channel current amplitude of hST polycystin-2 in symmetrical KCl (150 mM) did not change by reducing $pH_{\text{cis}}$ from 7.14 to 6.4 (Fig. 3B). The kinetic behavior of the channel did not change because the dwell histograms were still best fitted by two exponentials (Fig. 3C). However, a change in pH reduced the open probability of the channel, which decreased from 0.74 $\pm$ 0.11 (cis) at pH 7.14 to 0.24 $\pm$ 0.08 (cis) at pH 6.4 for a holding potential of 20 mV. This was a reflection of the increase in the dwell zero time (second rate constant), which increased as a function of lowered pH. The single channel conductance for these tracings was also similar between control and low pH $pH_{\text{cis}}$ as measured by K$^+$ currents in the presence of the KCl chemical gradient (Fig. 3D). The non-selective cation channel conductance of hST polycystin-2 in symmetrical KCl (150 mM) was $135 \pm 11.2$ pS ($n = 5$), and it did not change by reducing $pH_{\text{cis}}$ from 7.14 to 6.4 ($138 \pm 21$ pS, $n = 3, p < 0.3$). Fitting of the $P_o$ values as a function of the various holding potentials showed a displacement of the Boltzmann distribution determined after $pH_{\text{cis}}$ reduction (Fig. 3E). The $P_o$ versus voltage function shifted to the left, from $V_{m} = 29.4 \pm 3.9$ mV ($n = 5$) at $pH_{\text{cis}}$ 7.14 to $V_{m} = 14.7 \pm 2.6$ mV ($n = 3$) at $pH_{\text{cis}}$ 6.4, with a decrease in the slope $k$ from $-2.30 \pm 0.70$ (cis) at $pH_{\text{cis}}$ 7.14 to $k = -1.06 \pm 0.5$ (cis) at $pH_{\text{cis}}$ 6.4. Further, the reduction in $pH_{\text{cis}}$ also increased the number of single channel subunits (Fig. 4), which were further manifested by the transitions observed in the mean $versus$ variance plots (Fig. 4, right) (12). Thus, an increase in H$^+$ concentration induced more fluctuations between the channel substates, but the current amplitudes themselves did not change. Conversely, an increase in $pH_{\text{cis}}$ increased the mean current by $25\%$ ($n = 3, p < 0.045$, Fig. 5) by increasing the open probability of the high conductance state of the channel but not the single channel conductance of hST polycystin-2 (Fig. 5B, histograms). Elimination of the pH gradient ($pH_{\text{cis}} = pH_{\text{trans}} = 6.4$) at low pH, however, was without effect in reversing the inhibitory effect of $pH_{\text{cis}}$ reduction alone ($n = 7$, data not shown).

**Effect of Changes in $pH_{\text{cis}}$ on the Purified Polycystin-2**—To confirm that a regulatory site for H$^+$ ions indeed exists in the polycystin-2 ion channel, the effect of changes in $pH_{\text{cis}}$ was
FIG. 6. Effect of pH$_{cis}$ on in vitro translated purified polycystin-2 channel function. A, left, effect of lowering pH$_{cis}$ on the channel currents of FLAG-tagged human polycystin-2 in vitro transcribed/translated product. Representative single channel currents at various pH$_{cis}$ values. Data were collected in asymmetrical KCl. The main single channel conductance at pH 7.14 was 172 pS with a substate of 141 pS. No remaining single channel activity was observed at pH 6.0. The closed state is indicated by a solid line, and substates are indicated with dashed lines. The holding potential was 20 mV for all tracings. Data are representative of six experiments. A, right, log-linear all-point histograms indicating the change in single channel activity as a function of lowering pH$_{cis}$. Channel activity decreased in substates, indicated with arrows, to complete inhibition. B, representative tracings of at least two channel units of the in vitro translated polycystin-2 where the inhibitory effect of lowering pH$_{cis}$ was reversible (left). Changes in pH$_{cis}$ from a control value of 7.15 were accomplished by addition of HCl acid and reversed by addition of KOH. Channel activity was completely inhibited at pH 4.5 and restored by raising pH$_{cis}$ to 7.15, respectively. Data were obtained at a holding potential of 40 mV, and dashed lines indicate channel closings. Amplitude histograms are indicated on the right of each tracing. Data are representative of five experiments.
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Fig. 7. Effect of pH\textsubscript{cis} on polycystin-2 open probability of the channel. Left, the effect of pH\textsubscript{cis} on the hST polycystin-2 ion channel was evaluated by plotting the open probability, P\textsubscript{o}, as a function of pH in the cis compartment. All data were obtained with a pH\textsubscript{trans} of 7.14. Single channel currents were obtained at various pH\textsubscript{cis} values in asymmetrical KCl. Experimental values were pooled from five different experiments. The open probability was calculated as described in the legend for Fig. 2, and the P\textsubscript{o} versus pH data (filled circles) for V\textsubscript{h} = 20 mV were fitted to the equation P\textsubscript{o}(pH\textsubscript{cis}) = 10\textsuperscript{\rho p \cdot \text{K} / 10^{pH} + 10^{pH}}. Best fitting is shown as a solid line. Right, the equilibrium constant (K) as a function of holding potential (filled circles) was obtained by applying Woodhull’s model (see “Experimental Procedures”) to the mean single current values (inset). The results were fitted with the corresponding exponential function (K(V\textsubscript{h})), solid line), which yielded a p\textsubscript{K} = -6.5 (V\textsubscript{h} = 0 mV) and a z\textsubscript{K} = 0.67 (see “Experimental Procedures”). Inset, mean single channel currents were obtained as the product of the theoretical values of single channel current amplitude (i, pA), and P\textsubscript{o} values were obtained by fitting experimental data to the Goldman-Hodgkin-Katz and Boltzmann equations, respectively. Original data were obtained from Figs. 2D and 3, D and E. Data for pH\textsubscript{cis} 7.14 are shown in filled circles, and data at pH\textsubscript{cis} 6.4 are shown in filled triangles. pH\textsubscript{trans} was 7.14 in both cases.

Further assessed on the ion channel activity of purified FLAG-tagged human polycystin-2, obtained from the in vitro transcription/translated material (Fig. 6). A reduction of pH\textsubscript{cis} decreased the channel activity, including an increase in the subconductance state level of the channel (Fig. 6). A completely inhibited channel reactivated upon reinstating a higher pH in the cis side of the chamber (Fig. 6B). The data are in agreement with the effect of an H\textsuperscript{+} ion titration site, which is likely intrinsic to the channel protein by comparison of the similar effect in the hST and in vitro preparations.

Effect of pH\textsubscript{cis} on Polycystin-2 Open Probability—To further assess the nature of the regulation by pH on hST polycystin-2 single channel tracings, the mean open probability (P\textsubscript{o}) was assessed as a function of the pH in the cis compartment (Fig. 7). Experimental values were fitted to Eq. 7,

\[ P_o(pH) = \frac{10^{\rho p \cdot \text{K} / pH}}{10^{\rho p \cdot \text{K} / pH} + 10^{pH}} \]  

which is based on the assumption that polycystin-2 presents a single H\textsuperscript{+} titration site accessible from the cytoplasmic domain. Orientation of the channel protein was confirmed in our original studies (2). The experimental data were best fitted with this equation, indicating that a regulatory site indeed exists for the regulation by pH\textsubscript{cis} of polycystin-2 ion channel activity, which has an equilibrium constant p\textsubscript{K} of -6.4.

The equilibrium constant for the H\textsuperscript{+} regulatory site was validated by assessing the effect of pH on the single channel currents following a modification of the model described by Woodhull to assess the blocking effect of low pH on nerve Na\textsuperscript{+} currents (13). Theoretical current-to-voltage (IV) relationships (Fig. 7) were obtained for single channel currents at two different pHs, namely, 7.14 and 6.40, for which single channel currents and open probabilities were fitted to the Goldman-Hodgkin-Katz and Boltzmann equations, respectively (Figs. 2D and 3, D and E). The IV plots, where the single channel currents are shown as i \times P\textsubscript{o} versus holding potentials in the range of -20 to 10 mV showed a strong voltage dependence of the pH effect on the mean channel currents (Fig. 7). By applying the Woodhull’s model (13) to the pH 7.14 versus 6.40 substracted currents (Fig. 7), the product z\textsubscript{K} was calculated as 0.67, and the p\textsubscript{K} was calculated at -6.5, where p\textsubscript{K} = -log K for the protonation reaction.

DISCUSSION

The present study demonstrates that polycystin-2 cation channel function is voltage-dependent and highly sensitive to changes in pH. The data are most consistent with the assumption that polycystin-2 contains a highly sensitive pH site, which regulates its cation-selective channel activity by controlling the voltage dependence of the single channel kinetics. Dwell time histograms confirmed a two-open, two-closed state for the spontaneous channel at normal pH and the strong voltage dependence of the open probability. Although the single channel conductance did not change by modifying pH\textsubscript{cis}, the change in pH largely decreased the open probability of the channel. This effect was also voltage-dependent, being greater at a more positive V\textsubscript{h} such that the Boltzmann distribution of P\textsubscript{o}(V\textsubscript{h}) was displaced to the left, as indicated by the decreased slope as a function of reducing pH\textsubscript{cis}. This suggests a placement of the titration site in the conductance pore of the channel. Conversely, an increase in pH\textsubscript{cis} increased the polycystin-2-mediated K\textsuperscript{+} currents also associated with an increased NP\textsubscript{o}, but not the single channel conductance. The data further indicate that the changes induced by pH on polycystin-2 ion channel activity were not elicited by modifying the H\textsuperscript{+} chemical gradient but instead by a regulatory site in the channel protein because a decrease in pH such that the pH gradient was eliminated (pH\textsubscript{cis} = pH\textsubscript{trans} = 6.4) was without effect in reversing the inhibitory effect of reducing the cis pH alone. An effect of pH\textsubscript{cis} on polycystin-2 channel regulation was also observed with the purified protein from the in vitro translated material, confirming a pH-sensitive site in the channel protein itself. Taken together, these data suggest that an H\textsuperscript{+} ion (protonation) regulatory site, only accessible from the intracellular (cis) side of the channel where changes were made, controls the polycystin-2 cation channel from human placenta. The orientation of the reconstituted polycystin-2 channel was confirmed previously (2) and further assessed in experiments where either addition of anti-
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body to the cis side or amiloride to the trans side of the reconstitution chamber induced the expected blocking effect on either side of the channel (2).

To assess the affinity of the protonation site for pH regulation of polycystin-2, \( P_o \) versus \( \text{pH}_{cis} \) data were fitted to a Henderson-Hasselbach type equation (Fig. 7), indicating the presence of an equilibrium constant (\( pK_a \)) of \(~6.4\), which was confirmed independently by applying a kinetic model for the protonation reaction on the single channel currents at different pHs (Woodhull model, Fig. 7). The data are in agreement with the presence of a regulatory site in the channel protein, which is only accessible from the cytoplasmic side of the channel, thus suggesting that pH regulation of polycystin-2 is largely intracellular. This is supported by the fact that changes in cis, but not trans, pH modified the polycystin-2 channel currents. Only one of five experiments where pH was lowered from the trans side showed a detectable change in channel function (data not shown).

Thus, although the extracellular domain of the channel may also be sensitive to pH (which will require further experimentation), it is unlikely that this effect is associated with the same site described in this report, and it may be speculated that it is instead associated with changes in surface potential as reported previously for other channels (15, 16). Interestingly, polycystin-L, a channel homolog of polycystin-2 (17), and the ADPKD-causing truncated R742X-polycystin-2 (7) were also blocked by a reduction in cytoplasmic pH, further suggesting a homologous topology in the ion channel, consistent with a pH regulatory site most likely present in the pore conduction structure of the channel protein. However, the L-type calcium channel, which shares homology with polycystin-2 (18) and is also sensitive to changes in pH (15), may have a regulatory site external to the conduction pore of the channel (16).

The human syncytiotrophoblast, the most apical membrane barrier of the human placenta, provides the only natural electrolytic homeostasis of both the maternal blood and the fetal environments. The pH regulation of the polycystin-2 channel activity suggests the importance of \( \text{H}^+ \) ions as second messengers whose presence may contribute to the control of hydroelectrolytic homeostasis. Further studies will be required to assess the metabolic role of this novel regulatory pathway in vivo and its role in the dysfunctional aspects associated with ADPKD.

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